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Plant calorimetry: A window to plant physiology and ecology

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Abstract

The use of calorespirometry in developing models for biological growth is discussed. The relationships between respiratory heat, CO_2, and O_2 rates and physiological parameters such as substrate carbon conversion efficiency, and substrate and biomass carbon oxidation states are presented and discussed. A biochemical model relating ratios of the respiratory rates to the physiological parameters and biochemical pathways in plants is presented. Examples of applications of these models and equations to plant physiology and ecology are given. © 1997 Elsevier Science B.V.

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1. Introduction

In 1783, Antoine Lavoisier showed in a presentation to the French Academy of Sciences that oxidation of organic compounds by O_2 to produce CO_2 was the source of metabolic heat, the fire of life. This demonstration made it clear that chemical reactions observable in the laboratory also take place in living organisms. In the same lecture, Lavoisier and la Place also proposed the laws of conservation of mass and energy. It has since been accepted that all living organisms obey the same laws of thermodynamics that govern nonliving systems [1]. Morowitz [2] showed that, from considerations of nonequilibrium thermodynamics, the organization of living systems arises spontaneously from the presence of energy gradients. Contemporary concepts of energy transformations in living systems are derived from these fundamental principles. Application of these principles to measurements of mass and energy changes in living systems enables understanding of the functioning of living systems on a scale from cells to ecological systems.

Adult homeotherms such as the guinea pig used in Lavoisier's experiments are open, steady-state systems with time-independent biomass. Oxygen and food enter the system (the animal) and CO_2 leaves the system. If the environment, physical activity and inputs are kept constant, the rate of heat production remains constant. Under these conditions the respiratory quotient, R_{CO_2}/R_{O_2}, and the ratios of heat-rate, ϕ, to R_{O_2} and R_{CO_2} are constant and depend only on the oxidation state of the substrate carbon. This situation gives rise to concepts of oxycaloric and calorespirometric constants and indirect calorimetry. The calorespirometric constant is defined as the ratio ϕ/R_{CO_2} and the oxycaloric constant as ϕ/R_{O_2}. In such a system

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an enthalpy balance is simple to define and use, i.e. the heat of combustion of the food intake (minus any undigested waste) equals the total metabolic heat out [1].

The oxycaloric and calorespirometric ratios are closely related to Thornton's rule. In 1917, Thornton [3] published a collection of data showing that the heat of combustion of many organic compounds was approximately constant when expressed as heat per mole of oxygen, ΔH_{O_2}. Although ΔH_{O_2} varies slightly with class of compound, it averages $-455 \pm 15\,\mathrm{kJ\,mole^{-1}}$ of O_2 for the compounds in living systems. Thornton's constant is identical with the ratio ϕ/R_{O_2} for steady-state systems. The ratio ϕ/R_{CO_2} is related to Thornton's constant by $(1-\gamma_p/4)$, where γ_p is the chemical oxidation state of the carbon compound. The basis of indirect calorimetry is that metabolic heat rate can be calculated by multiplying R_{CO_2} or R_{O_2}, respectively, by the calorespirometric or oxycaloric constant. Note that the oxycaloric and the calorespirometric ratios are not equal to Thornton's constant when metabolism produces carbon products other than CO_2, for example lactate or new biomass.

When an organism is growing, the enthalpy balance is more complex because biosynthetic reactions must be taken into account. In a steady-state system metabolism provides only maintenance energy. During growth, metabolism must also provide the energy needed to drive biosynthesis. Biological organisms differ, both within and among species, in terms of the efficiency with which they use metabolic energy for biosynthesis and maintenance. The energy-use efficiency of a given organism also varies with environmental conditions. The relation between growth rate, metabolic rate and efficiency is thus central to any description of living, growing systems.

Growth rate models commonly include some measure of the rate of substrate use (input) and rate of biomass production (output). Energy supplied by oxidation of a portion of the substrate through respiration is often conceptually divided into the fraction used for maintenance and that used for growth. This division has led to development of compartment models for growth that have been applied to animals, microorganisms, and plants. The origins of many such models apparently trace back to Pirt's [4,5] analysis of the relation between bacterial growth and respiration. These models have further led to discussions of metabolic efficiency – variously based on enthalpy, free energy, and mass [6]. Such models have been successfully applied to animals for obtaining the efficiency of feed conversion and to microorganisms for describing both mass and energy efficiencies. However, because plants are autotrophs and ectotherms living in a variable environment, application of compartment models to green plants requires incorporation of nontestable assumptions. Compartment models have therefore not proven useful in plant selection programs.

Rapidly selecting high yielding biomass crop plants and matching them to a particular environment requires some means for predicting growth rate from measurements of physiological properties as a function of environmental variables. Plant physiologists seeking means of relating plant growth to metabolism have focused on photosynthesis because it is unique to plant metabolism. However, efforts to link growth rate and photosynthesis have only resulted in finding tautological correlations. Such a statement draws strong criticisms, but nonetheless is correct. None but tautological correlations have been shown to exist between growth and photosynthesis rates. Missing the point that *tautological* correlations must exist, but are not useful as predictive tools, one anonymous critic of this conclusion recently wrote in a proposal review, "hundreds of studies show that plant community photosynthetic rate is tightly coupled to plant community growth rate." The tautological nature of such statements was pointed out by Demetriades-Shah et al. [7] who showed that just as larger trees cast larger shadows than smaller trees in intercepting light for photosynthesis, larger chickens also cast larger shadows than smaller chickens. This then leads to the absurd question, is chicken growth rate also caused by their ability to intercept light? In 1985 J.H. Cherry [8] stated, "The use of physiological and biochemical knowledge to suggest selection criteria or chemical targets for improvement in genetic yield potential has been one of disappointment." During the decade since, we have developed calorimetric techniques for studying plants and plant tissues that we believe will, in part, relieve that disappointment. The intent of the present paper is to show that calorespirometric measurements of heat, O_2 and CO_2 rates of plants and plant tissues provides a means to relate plant growth to plant metabolic physiology and biochemistry, and

thereby provide a means for rapid selection of plants to optimize productivity in a given environment.

2. Attempts to model plant growth energetics without metabolic heat rate measurements

It has long been recognized that plant growth rate is correlated to respiration rate, and there is a sizeable body of literature on the relation between plant growth rate and short term measures of respiration rate, mainly R_{O_2} and R_{CO_2} [9,10]. Discussion of this relationship has been couched mostly in terms of compartment models. The compartment model "was extended to plants, principally by Thornley" during the 1970s [10] on the basis of an empirical relation derived by McCree [11]. In Thornley's model, respiration rate is equated to a maintenance rate plus the product of a growth coefficient and the growth rate as shown in Eq. (1).

$$R_{CO_2} = m + gR_{SG} \quad (1)$$

The growth coefficient (g, dimensionless) is a function of the fraction of respiration used for growth. The maintenance rate (m, mass/time) is defined as the respiration rate used to support all physiological functions except growth. The major evidence cited to support the validity of compartment models represented by Eq. (1) is that plots of R_{CO_2} vs. some measure of growth rate appear to be linear (when analyzed uncritically) and generally have positive slopes and intercepts, properties consistent with Eq. (1).

Major uncertainties limit the application of compartment models to plants [9]. The parameters are difficult (and may be impossible) to meaningfully quantify because the separation of respiration into growth and maintenance components is a conceptual, not a physical separation. Because plants are autotrophs and plant tissue compositions are diverse, both the substrate carbon source for biomass synthesis and the composition of the biomass are ambiguous. As a consequence, there are no unambiguous methods for direct, dynamic measurement of either the growth or maintenance component of plant respiration [9,10].

Penning de Vries et al. [12] developed a method to calculate the maximum possible value of the growth coefficient based on detailed knowledge of the biochemistry of the plant. A value of 0.88 carbon atoms incorporated into biomass per carbon in substrate glucose was obtained for maize embryos. The number obtained, though complex to calculate, simply represents the maximum ratio possible from reaction stoichiometries. Values of the growth coefficient obtained in this way are upper limits that implicitly assume 100% energy-use efficiency in violation of the second law of thermodynamics. The Penning de Vries method does not allow calculation of the actual value of the growth coefficient.

Recognition that the Penning de Vries method is only a statement of the overall stoichiometry of the reaction substrate → biomass has inspired numerous attempts by others to develop simpler methods based on elemental and molecular composition and heats of combustion of plant tissue to approximate growth coefficients for other plants (e.g. [13–15]). The methods based on tissue composition are related to Penning de Vries model in an obvious way, i.e. if the composition of the substrate and the biomass are known, the fraction of the substrate carbon retained in the biomass is easily calculated. Those based on measurement of heat of combustion simply rely on some form of Thornton's rule to obtain the oxidation state of the biomass which is equivalent to knowing the composition. However, all of these stoichiometric growth coefficients may be seriously misleading because the biomass composition of plants probably varies with environmental conditions as indicated by studies showing elemental composition of microorganisms to vary with growth conditions [16–18].

When plant composition varies with growth conditions, growth coefficients and substrate carbon conversion efficiencies will also vary with conditions and thus be different from those calculated from average or assumed composition. Furthermore, because of the difficulty of obtaining even approximate growth coefficient values as a function of environmental conditions, physiological state, etc., many workers have simply adopted the Penning de Vries value of 0.88 for corn embryos as a near universal constant to be applied to all plants and growth conditions. Widespread acceptance of this value led Thornley and Johnson [10] to conclude "there is a reasonable consensus concerning the representation of growth respiration", but such consensus is an illusion.

Simultaneous experimental determination of values of both the growth coefficient and maintenance rate, using the linear Thornley equation describing the compartment model, requires changing growth rate and respiration rate without changing the growth coefficient or maintenance rate. This is an improbable, if not impossible, physiological requirement, particularly in view of observations like those of Duboc et al. [16]. The finding of an approximately linear relation between respiration rate and growth rate in most studies simply reflects the fact that young tissues respire and grow faster than old tissues. The approximate linearity only reflects the insensitivity of the plot to the factors that actually cause growth rate differences among plants.

Further problems exist in evaluating temperature and other environmental effects on growth, using compartment models. Because the growth coefficient is a stoichiometric ratio, it is 'expected to be independent of temperature', but only so long as 'temperature does not influence the use of different metabolic pathways' [10]. However, plants are known to alter metabolism in response to changes in temperature and in other environmental conditions via changes in pathways and in the balance of pathways that result in altered composition and/or carbon use efficiency. The growth coefficient is thus a function of temperature and other environmental variables and is not constant as commonly assumed.

Quantification of the maintenance rate by measuring the respiration rate of non-growing tissues has also been criticized extensively [9]. This method incorrectly assumes that maintenance processes are both qualitatively and quantitatively the same in growing and non-growing tissues. Thornley and Johnson [10] further state that "maintenance respiration is an area of great uncertainty", and "the factors affecting them (maintenance processes) are largely obscure."

Because the rates of degradation of enzymes, membranes, and ion gradients all increase with temperature, maintenance rate is assumed to increase with increasing temperature [9,10], another aspect of compartment models not dealt with by Eq. (1). While this assumption is probably correct, the consequences are neither simple nor obvious. If maintenance rate increases more slowly with temperature than growth rate, the total efficiency of converting substrate carbon to structural biomass must increase with increasing temperature. On the other hand, if maintenance rate increases faster than growth rate, the carbon conversion efficiency must decrease because the fraction of respiratory energy required for maintenance increases. Understanding the effects of environmental variables on the relation between plant respiration and growth with a compartment model thus requires quantitative measurement of the (unmeasurable) maintenance rate as a function of environmental variables.

Compartment models are thus of little value for quantifying the energetics of plant growth or interpreting respiration-growth correlations. Even empirical application of such models for prediction of growth properties is limited because use of the model requires measurement of growth rates to obtain values of the model parameters, rather than allowing prediction of growth from measured parameters. The uncertainties in growth coefficients and maintenance rates and the effort and time required to obtain these values precludes ready application of these models to selection and breeding programs. Despite many efforts, compartment models have not served as the basis for achievement of any improvement in any crop. This lack of methods to rapidly assess growth rates and efficiencies of plants as a function of temperature and other environmental variables led to calorimetric measurements of metabolic heat rates in plant tissues as a possible means of resolving these problems.

3. Inclusion of metabolic heat rate measurements in models of plant growth energetics

A useful model relating plant metabolism to growth and environmental responses should be capable of describing both resource-limited and genetically-limited growth in terms of measurable, unambiguous variables. Plant growth rate may depend on the rate of acquisition of carbon or of other resources, or it may depend on the rate and efficiency of processing of those resources into structural biomass, all of which are functions of the environmental conditions and the genetically determined abilities of the plant. This expansion on Liebig's law of the minimum [19] is shown schematically in Fig. 1 [20]. The model described by Fig. 1 has been compared by analogy to a bicycle factory [21]. The production rate of bicycles may depend on the rate of acquisition of

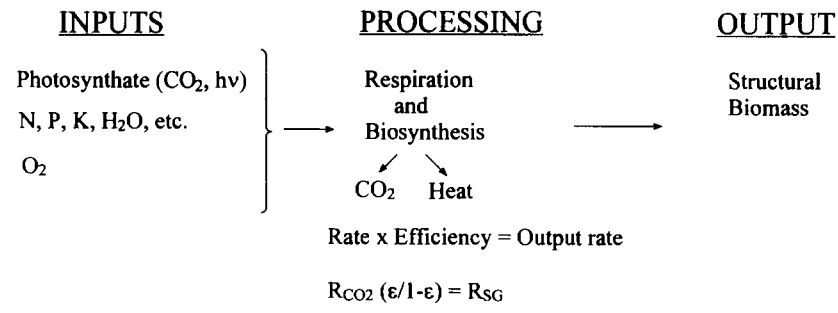

Fig. 1. Conceptual model for plant growth. Growth rate (output, R_{SG}) is given by the product of rate (R_{CO2}) and efficiency ($\epsilon/1-\epsilon$) whether limited by availability of an input or by the rate of respiration and/or biosynthesis.

parts or it may depend on the rate and efficiency with which the workers can assemble the parts into bicycles. Enhancement of the bicycle production rate (or plant biomass production rate) can only be done by improving the rate limiting process, whether it be rate of acquisition of parts (or nutrients including photosynthate) or the rate of assembly of the finished product.

Based on the model in Fig. 1, growth rate (R_{SG}) can be shown to be equal to the rate of respiratory CO_2 production (R_{CO_2}) multiplied by a function of the substrate carbon conversion efficiency (ϵ), Eq. (2)[22].

$$R_{SG} = R_{CO_2}(\epsilon/1-\epsilon) \tag{2}$$

The specific growth rate, R_{SG}, has units of moles of C in new structural biomass per unit time per unit tissue mass, R_{CO_2} has units of moles per unit time per unit tissue mass, and ϵ the ratio of moles of carbon retained in new structural biomass to total moles of carbon, i.e. moles of respiratory CO_2 plus moles of C in new structural biomass. Eq. (2) is valid under all reasonable conditions, including photosynthate and nutrient limitations.

Respiratory CO_2 rates have routinely been measured on plants and plant tissues for many years, but evaluation of ϵ has been difficult. In the only study to measure ϵ as a function of temperature, Yamaguchi [23] determined substrate carbon conversion efficiency of rice, soybean, and maize seedlings at several temperatures by measuring the mass of carbon converted to structural biomass and the mass of carbon lost as CO_2 during growth of the seedling. Although this method appears to give accurate results [22], it is slow, lacks sensitivity, is labor-intensive, and requires total destruction of the plant. In contrast, relative values for the ϵ function in Eq. (2) can be rapidly obtained from R_{CO_2} and metabolic heat rates measured simultaneously on the same plant or tissue sample. For comparison and selection of plants, relative values of performance are often all that are needed.

The ratio of metabolic heat rate ϕ to the CO_2 rate is a linear function of $(\epsilon/1-\epsilon)$ as shown in Eq. (3)[22]

$$(\epsilon/1-\epsilon)\Delta H_B = -\phi/R_{CO_2} - (1-\gamma_P/4)\Delta H_{O_2} \tag{3}$$

where ΔH_B is the total enthalpy change for incorporation of one mole of substrate carbon into one mole of biomass carbon, including enthalpy effects from all elements, and γ_P is the mean oxidation state of the substrate carbon. If the reasonable assumption is made that the composition of the biomass formed and the substrate carbon source remain constant over the duration of the measurements, then ΔH_B and γ_P are constant, and changes in ϕ/R_{CO_2} are caused by changes in the efficiency function $(\epsilon/1-\epsilon)$. Furthermore, because ϕ and R_{CO_2} are both readily measured as functions of environmental variables, ϵ can be expressed as a function of environmental variables.

Combination of Eqs. (2) and (3) gives the equation for specific growth rate in terms of ϕ and R_{CO_2}.

$$R_{SG}\Delta H_B = -R_{CO_2}(1-\gamma_P/4)\Delta H_{O_2} - \phi \tag{4}$$

We note in passing that Eqs. (2)–(4) apply to animals and microorganisms as well as plants.

In Eq. (4), $R_{SG}\Delta H_B$ is growth rate, given as the rate of storage of chemical energy in structural biomass. The rate of energy storage is expressed as the difference between the rate of energy produced by respiration, $R_{CO_2}(1-\gamma_P/4)\Delta H_{O_2}$, and the rate of energy lost

to the surroundings, ϕ. Again, because ϕ and R_{CO_2} are both readily measured as functions of environmental variables, growth rate can also be expressed as a function of environmental variables.

Definition of growth rate as the rate of accumulation of potential energy is a novel idea that allows direct comparison of plants producing biomass with different composition, e.g. starch and lipids, and thereby increases our understanding of what is meant by 'growth'. By this definition, growth rate is measured as the rate of increase in heat of combustion of the organism, but in doing so, only 'structural' biomass must be included. No one would include the stomach contents of a shark as part of the shark's biomass, but plant scientists have traditionally used the rate of increase in total dry mass of a plant, including photosynthate, as a measure of growth rate. We hasten to add that over a long time period, or in large plants, the amount of photosynthate is negligible and mass measurements do give accurate indication of growth rate. The other common means of measuring plant growth is by measuring 'elongation' which can occur by cell division or by cell expansion. The difficulty of defining exactly what is meant by these mass and length measures of growth is exemplified by the growth of a maize plant through a day-night cycle (i.e. does maize grow at night?) The plant elongates faster at night than in the day, but in doing so loses mass through respiration. During the day the plant gains mass through photosynthesis, but production of structural biomass is probably slower as indicated by slower elongation.

Eqs. (3) and (4) may be simplified by incorporating Thornton's constant and by assuming that in plants substrate carbon is carbohydrate which has a value of γ_P equal to zero, i.e.

$$(\epsilon/1-\epsilon)\Delta H_B = -\phi/R_{CO_2} + 455 \tag{5}$$

$$R_{SG}\Delta H_B = 455R_{CO_2} - \phi \tag{6}$$

Because of the simplifying assumptions, Eqs. (5) and (6) must be altered accordingly to accurately describe systems which are catabolizing significant amounts of lipid or producing significant amounts of fermentation products. Such situations are evident because ϕ becomes greater than $455R_{CO_2}$ and $(\phi/R_{CO_2}) > 455\text{kJmol}^{-1}$ thus indicating the more complete Eqs. (3) and (4) must be used.

The relations between the three ratios of respiratory measures and the physiological parameters in a growing system are shown in Eqs. (7)–(9).

$$\phi/R_{CO_2} = -(1-\gamma_P/4)\Delta H_{O_2} - (\epsilon/1-\epsilon)\Delta H_B \tag{7}$$

$$\phi/R_{O_2} = [-(1-\gamma_P/4)\Delta H_{O_2} - (\epsilon/1-\epsilon)\Delta H_B]/[(1-\gamma_P/4) + (\epsilon/1-\epsilon)(\gamma_B-\gamma_P)/4] \tag{8}$$

$$R_{CO_2}/R_{O_2} = [(1-\gamma_P/4)+(\epsilon/1-\epsilon)(\gamma_B-\gamma_P)/4]^{-1} \tag{9}$$

In Eq. (8), γ_B is the mean oxidation state of carbon in structural biomass. Determination of all three experimental parameters thus provides three equations, but still leaves four unknowns, namely γ_P, γ_B, ΔH_B, and ϵ; parameters that are very informative about the physiology if they can be quantified. Further progress requires assuming or obtaining by an independent method a value for at least one of these physiological parameters.

4. Effects of environmental variables

Measurements of R_{CO_2} and ϕ as functions of environmental variables often provide significant insights into the physiological response of the plant to the environmental variables [24]. For example, Fig. 2 shows growth rates of two maize cultivars calculated as functions of temperature from ϕ and R_{CO_2} values measured at only two temperatures, 15° and 25°C. The growth curves were calculated from Eq. (6) by assuming the heat rate and R_{CO_2} are described as functions of temperature by Arrhenius functions, Eqs. (10) and (11)

$$\phi = A_\phi e^{-\mu\phi/T} \tag{10}$$

$$R_{CO_2} = A_{CO_2} e^{-\mu CO_2/T} \tag{11}$$

where μ is the temperature coefficient (best expressed in kK, kiloKelvin) and A, a constant. The results correctly predict, from measurements made far from any temperature that would damage the tissues, that the Jubilee cultivar grows best in a hot climate while the Squaw cultivar grows best in a cool climate. Furthermore, the study of maize cultivars [25]

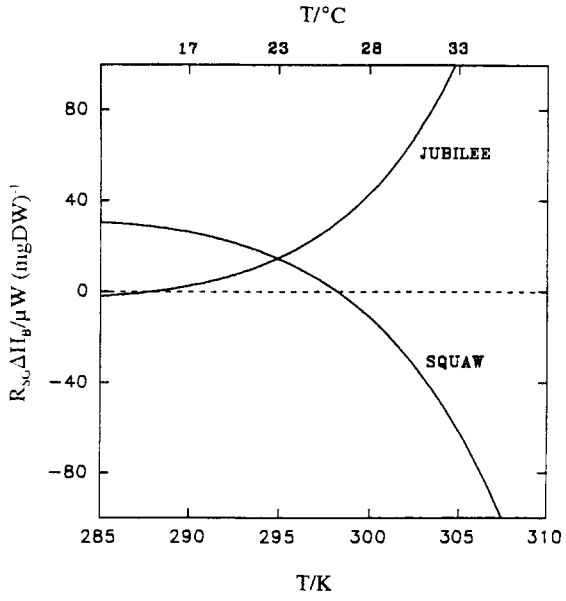

Fig. 2. Growth rates of two maize cultivars calculated Eq. (6) as functions of temperature Eqs. (7) and (8) from ϕ and R_{CO_2} values measured at only two temperatures, 15° and 25°C.

clearly showed that μ_ϕ was not correlated with μ_{CO_2}. Similar results obtained for poplar, sagebrush, yarrow, cheatgrass and several other species indicate that, in general, μ_ϕ is not correlated with or equal to μ_{CO_2}.

Growth–temperature curves calculated from simultaneous measurements of ϕ and R_{CO_2}, at several temperatures covering the growth range (which thus avoid the assumption of Arrhenius behavior) provide an even better description of and insights into the physiology of plant response to temperature [26], but very few data have as yet been collected in this manner.

Probably even more could be learned by making simultaneous measurements of all three respiratory rates, i.e. ϕ, R_{CO_2}, and R_{O_2}, as functions of environmental variables such as temperature. However, no one has yet devised a convenient, general, and accurate method for simultaneously measuring ϕ, R_{CO_2}, and R_{O_2} on the same tissue sample and no such data are available.

R_{CO_2} and ϕ data collected as functions of environmental variables such as nutrient concentrations [27], toxins (e.g. salt, heavy metals, ozone), and water activity should rapidly provide data similar to that for temperature on the mechanisms of the effects of these environmental variables on plant growth.

Responses of plants to environmental variation have traditionally been measured by following the growth of plants under the various conditions of interest. Such studies are sometimes simple and inexpensive, sometimes complex, costly and difficult, but only provide empirical data on growth with little or no information on the mechanism of action of environmental variables. For example, hundreds of studies have examined the effects of elevated atmospheric CO_2 on plant growth. Depending on conditions and species, some studies show increased growth, some no effect, and a few, decreased growth. Neither the combined data nor any model in the literature except that in Fig. 1 adequately explains these variable results [28]. Calorimetric measurements of ϕ and R_{CO_2} on plants grown under varying CO_2 concentrations would provide a physiological understanding of how CO_2 affects plant growth.

To determine the effects of an environmental variable on the metabolic physiology that result in changes in plant growth from calorimetric measurements, it is necessary to understand how changes in metabolic pathways affect the observable respiratory variables, ϕ, R_{CO_2}, and R_{O_2} and their ratios. For example, which metabolic processes are responsible for different values of μ_ϕ and μ_{CO_2} in the same maize plant? More than 90% of the metabolic heat from a cell is generated from reduction of oxygen in the mitochondria [29]. CO_2 is generated by the Krebs cycle, the pentose phosphate pathway, and by fermentation reactions when present. For ϕ and R_{CO_2} to have different temperature dependencies, these processes cannot be absolutely coupled. Thus, either the ratio of anaerobic to aerobic products or the substrate must change with temperature. Changing substrate and significant levels of fermentation are both unlikely in the maize seedling tissues studied. Therefore, a change in the ratio of glycolytic products going into the Krebs cycle versus into biomass synthesis as temperature changes is the most likely explanation of the observed difference in temperature dependencies of ϕ and R_{CO_2}. The ratio of carbon going into the Krebs cycle relative to that going into new structural biomass is approximately equal to ϵ^{-1}. Thus, differing temperature dependencies of ϕ and R_{CO_2} requires that ϵ also change with temperature

(see Eq. (3)), further invalidating the assumption of a constant growth coefficient in Eq. (1).

The value of ϵ has been reported to be constant for maize seedlings from 15° to 35°C [23], however careful examination of Yamaguchi's data shows that ϵ values change systematically with temperature. The changes are small, but small changes in ϵ result in large changes in growth rate because of the functional dependence shown in Eq. (1), i.e. the ratio $\epsilon/1-\epsilon$ changes rapidly with changing ϵ.

The foregoing sections discuss how measurements of plant respiration rates (ϕ, R_{CO_2}, and R_{O_2}) can be used to relate plant growth to metabolic physiology. The next goal, in relating plant growth to measures of plant respiration rate, is concerned with explaining changes or differences in the measured rates and their ratios in terms of changes or differences in the biochemistry of metabolic pathways.

5. Relations between respiratory variables and biochemistry

Equations can be derived to relate the physiological parameters γ_P, γ_B, ΔH_B, and ϵ to known biochemistry reactions. Eqs. (2)–(4)–(9) can then be used to relate the physiological parameters back to the measured respiration rates and their ratios. This approach is similar to the biochemical modeling method applied to plants by Penning de Vries [12] and others [13–15]. The results of these earlier workers were criticized earlier in this paper for failing to calculate and treat ϵ as a variable. This was a consequence of not understanding Eqs. (2)–(4) and Eqs. (7)–(9), which were developed at a later time [22]. The Penning de Vries method was also criticized as being too complex to be generally useful. A simpler model can be derived through thermodynamic rather than just stoichiometric consideration of metabolic processes. This allows examination of efficiency from a knowledge of only initial and final states without the detailed metabolic pathway analysis used by Penning de Vries. Simplification is achieved with little loss in accuracy by a minimal model including only those metabolic pathways expected to make major contributions to heat exchange, and grouping the remaining pathways expected to make only minor contributions. We have thus developed a simplified, but biochemically accurate, thermodynamic model of metabolic heat production based on a knowledge of biochemical pathways in plants. The model includes enthalpy changes as well as mass flow and is capable of examining the responses of ϕ/R_{CO_2}, ϕ/R_{O_2}, R_{CO_2}/R_{O_2} and ϵ to changes in γ_P, γ_B, biomass composition, the coupling of ATP synthesis to oxidative reactions, and the requirements for ATP in biosynthesis.

Our model includes the oxidative pathways of mitochondria, glycolysis, and the pentose phosphate pathway as the major contributors to metabolic heat rates in plants under aerobic conditions. Biosynthetic pathways (with smaller enthalpy changes) are grouped in a process we call 'biosynthesis'. For simplicity, biosynthesis is defined to begin with pyruvate and to form the product 'biomass'. This allows an estimate of the enthalpy of biomass production from enthalpies of combustion of pyruvate and the final product biomass. The enthalpy of combustion of the biomass is estimated from Thornton's rule and the average carbon oxidation state of the biomass.

The general, biochemical, and thermogenic processes used in our minimal model of metabolism are:

$$\text{glucose} \rightarrow \text{cellulose} \tag{12}$$

$$\text{glucose} \rightarrow \text{pyruvate} \tag{13}$$

$$\text{glucose} \rightarrow CO_2 + \text{reducing equivalents} \tag{14}$$

$$\text{pyruvate} \rightarrow \text{structural biomass (other than cellulose)}$$

$$\text{pyruvate} \rightarrow CO_2 + \text{ATP} \tag{16}$$

More complete description of the model and its derivation are given in Appendix A and in [29].

6. Applications of the thermogenic model of plant metabolic pathways

The purpose of the biochemical model is to relate observed changes in directly measurable respiratory variables (i.e. ϕ, R_{CO_2}, and R_{O_2}) to changes in biochemical pathways. The measured respiratory variables change in response to changes in environmental variables, age of the plant tissue, and with genotype. But what is the biochemical basis of these changes? Answering this question connects readily measured properties of plants to molecular events and thence

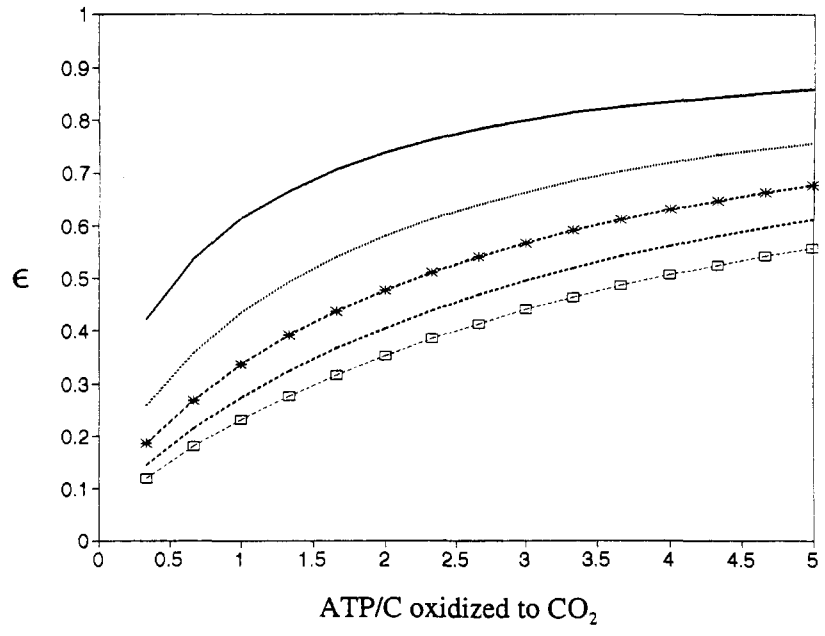

Fig. 3. Variation of substrate carbon conversion efficiency, ϵ, with coupling of oxidation to ATP synthesis. The different curves in descending order are based on requirement of 1, 2, 3, 4, or 5 moles of ATP per mole of C in new biomass.

begins to open the way for faster genetic engineering of plants for specific purposes.

The thermogenic model based on metabolic pathways relates observed changes in ratios of the respiratory variables to changes in biochemical pathways. Eq. (9) relates the respiratory quotient R_{CO_2}/R_{O_2} to ϵ and the oxidation states of the substrate carbon and biomass carbon. Eq. (3) relates ϵ to ϕ/R_{CO_2}. And since the biochemical model relates ϵ to biochemical pathways, we can obtain a relation between biochemistry and the respiratory variables.

The relationships between the biochemical reactions and ϵ are not obvious. Fig. 3 shows how ϵ varies with the efficiency with which the Krebs cycle produces ATP. As expected ϵ decreases as efficiency of ATP production decreases. Also, as shown in Fig. 4, ϵ decreases as ATP requirement per C in biosynthesis increases. Thus, ϕ/R_{CO_2} increases without change in R_{CO_2}/R_{O_2} as coupling of oxidative phosphorylation decreases and as the ATP requirement for biosynthesis increases. Eqs. (3) and (9) show, however, that both ϕ/R_{CO_2} and R_{CO_2}/R_{O_2} increase when the oxidation state of the substrate carbon decreases. Measurements of the absolute values and trends in ϕ/R_{CO_2} and R_{CO_2}/R_{O_2} can be used, together with the model, to quantify biochemical changes. As yet, insufficient data are available to test this conclusion.

Fig. 4 shows how ϵ varies with the number of ATP molecules required to transfer one carbon atom from substrate to structural biomass. The inefficiency in ATP production results from slippage in the coupling of the redox or ion gradient across cell membranes to the ATP synthesis reaction and from ATP hydrolysis not linked to biosynthetic pathways [30]. Variation in ATP per C incorporated into biomass occurs because synthetic pathways, e.g. lipids, proteins, starch or cellulose, vary in ATP requirements.

Fig. 4 is of particular interest to plant physiology because plants have both the cytochrome oxidase used in the Krebs cycle and an alternative oxidase unique to plants, with variable activity depending on environment, that results in production of only one-third the amount of ATP per O_2 reduced as the cytochrome pathway. Plants thus vary ATP/CO_2 produced in response to changes in environmental conditions.

The relation between the enthalpy changes for the reactions involved in respiration and ϕ/R_{CO_2} and ϕ/R_{O_2} are given in Eqs. (7) and (8). These equations show that values of ϕ/R_{CO_2} and ϕ/R_{O_2} for respiration can be changed by large amounts only by changing

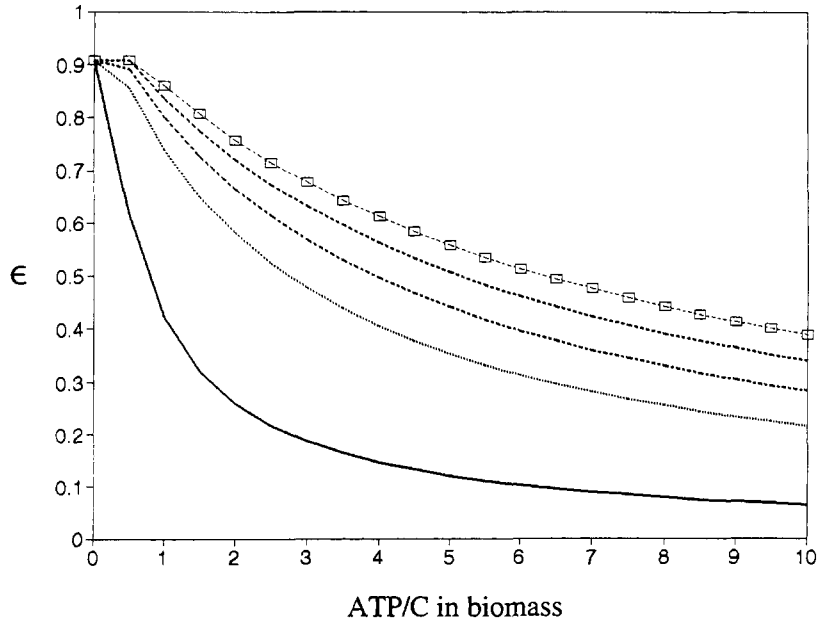

Fig. 4. Variation of substrate carbon conversion efficiency, ϵ, with requirement for ATP in biosynthesis of one carbon mole of biomass. The different curves in descending order are based on 5, 4, 3, 2, and 0.33 moles of ATP synthesized per C oxidized to CO_2 in the Krebs cycle.

oxidation states of substrate or biomass. Changing efficiency by such means as changing the ratio of the cytochrome to alternative oxidase pathways has only a small effect on the ratios. For example, in young growing plants typical values are $\gamma_P = 0 \pm 0.5$, $\gamma_B = -0.5 \pm 0.5$, $\Delta H_B = 25 \pm 25$kJ/mol, $\epsilon = 0.55 \pm 0.2$, and ΔH_{O_2} is -455 kJ/mol. A complete shift from the cytochrome pathway to the alternative pathway would give a change in ϕ/R_{CO_2} from 424 to 449 kJ/mol, or ~6%. The corresponding change in ϕ/R_{O_2} would be from 368 to 437 kJ/mol or 17%. A partial shift to the alternative pathway would give correspondingly smaller heat rate increases. Thus, although plants using the alternative pathway are more thermogenic than plants using the cytochrome oxidase pathway, it is the increased *rate* of carbon flow through the alternative pathway and not a difference in heat per unit of oxygen (or per electron) that gives rise to the increase in metabolic heat rate. These results show that extremely accurate calorimetric measurements would have to be made to characterize the alternative pathway, and thus calorimetry is not a good method for such studies.

The biochemical model presented here eliminates a commonly held misconception that changes in metabolic pathways with different enthalpy changes for the reactions, without changes in metabolic rate, are the cause of large changes observed in metabolic heat rate. Large changes in metabolic heat rates are observed, e.g. during flowering of *Arum* lilies as well as wound healing in potato tubers, and in response to exposure to extremes of temperature or toxins. The rate of heat production ϕ is equal to the rate of a process times the enthalpy change for the process. Thus, there are two ways to change ϕ, by increasing the rate or by altering the process to one with a larger, exothermic ΔH. Changing the respiration substrate from carbohydrate to lipid does cause readily measurable changes in ϕ/R_{CO_2} (approximately 50% increase), but there is no change in ϕ/R_{O_2} with substrate change. Because both ϕ/R_{CO_2} and ϕ/R_{O_2} change with ϵ but only ϕ/R_{CO_2} changes with substrate, measurements of these ratios may be used to distinguish metabolic pathway changes accompanying substrate changes.

7. Summary

This paper traces our understanding of the energetics of growth, particularly of plants, from its beginnings with Lavoisier to modern biochemistry.

Consideration of the relations between metabolic reaction pathways and respiratory quantities easily measured in the lab, i.e. ϕ, R_{CO_2}, and R_{O_2}, have led to the ability to predict temperature limits to growth of plants from measurements made within and far from those limits. Our considerations and measurements have led us clearly away from the assumption of any kind of constant efficiency for growth, either among genotypes of a species or with changes in the environment. Plants, and most likely other organisms, regulate both the rates and efficiencies of metabolic processes in order to cope with a changing environment. These new paradigms will lead not only to more productive crop plants, but to a better understanding of the biochemical changes that accompany evolution by selection.

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Appendix A

A minimal thermogenic model of plant metabolism

The heat calculated from this model is the total heat for conversion of one mole of carbon into non-cellulose biomass. Calculations based on the model can be run on a simple spreadsheet. The model allows calculation of the substrate carbon conversion efficiency, ϵ, by dividing the sum of carbon in the biosynthetic pathways plus carbon in cellulose (total retained carbon) by the total carbon metabolized. Ratios of heat produced per mole of carbon dioxide released, heat per mole oxygen consumed, and the ratio of carbon dioxide released to oxygen consumed (the respiratory quotient) can also be calculated. These ratios are directly comparable to experimental values. The effects of varying model parameters such as the need for ATP in biosynthesis, the oxidation state of the biomass, the coupling of respiration to ATP synthesis, and the cellulose content on values of ϵ and the three ratios of respiratory measures can be assessed through the model.

Enthalpy change data used in the following analysis were measured at temperatures from 18° to 37°C, but for our purposes the small effects of temperature on ΔH values can be ignored. ATP concentration in either steady-state or growing cells is essentially constant (i.e. $d[ATP]/dt = 0$). Therefore, it is also unnecessary to include enthalpy changes due to ATP synthesis and hydrolysis in calculation of metabolic heat rate. The nitrogen source is not considered for two reasons: first, nitrogen constitutes only about 14% of growing plant tissues, and second, heat effects due to incorporation of N are not much different from those of carbon [31]. The heat effect from incorporation of ammonia is small and the reduction of nitrate to ammonia can be treated much as another source of oxygen.

A.1 Cellulose synthesis

The synthesis of cellulose from glucose is included as a significant component in this plant metabolism model because conversion of glucose into cellulose requires ATP although the reaction has no direct requirement for reducing equivalents. A more complete version of Eq. (12) is

$$\text{cellulose}(n) + \text{glucose} + \text{ATP} \rightarrow \text{cellulose}(n+1) + \text{ADP, etc.} \quad (17)$$

The enthalpy change for this reaction can be calculated from the heats of combustion of cellulose and glucose and the heat of hydrolysis of ATP. The major contribution to the enthalpy change of this reaction comes from the hydrolysis of ATP which is neglected in calculating metabolic heat. The contribution of Eq. (17) to metabolic heat production in cells is therefore close to zero.

A.2 Glycolysis

Eq. (13) is a very abbreviated description of glycolysis. The net reaction for glycolytic production of pyruvate from glucose is given in Eq. (18).

$$\text{glucose} + 2\text{ADP} + 2\text{P}^{\text{i}} + 2\text{NAD} \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} \quad (18)$$

Minakami and de Verdier [32] arrived at an enthalpy change for Eq. (18) of 89.2 kJ/mol in red blood cells. The enthalpy change of hydrolysis of ATP is −20.9 kJ/mol [32,33]. Thus, the enthalpy change for Eq. (19) is 41 kJ/mol.

$$\text{glucose} + 2\text{NAD} \rightarrow 2\text{pyruvate} + 2\text{NADH} \quad (19)$$

For comparison, the heat of Eq. (19) was also calculated from heats of combustion for glucose (−2811 kJ/mol, [34]) and pyruvate (−1166.4 kJ/mol, [34]) and the heat of oxidation of NADH by molecular oxygen (−259 kJ/mol [35,36]). This gives a value of 42 kJ/mol for Eq. (19). Thus, estimates of the heat of reaction for this pathway based on heat of combustion data agree well with enthalpy changes measured directly in solution. Directly measured heats of reaction are not available for reactions in Eqs. (14)–(16) in our model, so this agreement between heat of combustion data and direct enthalpy measurements is essential.

A.3 Reducing equivalents and the pentose phosphate pathway

Eq. (14) represents the pentose phosphate pathway, included here to account for carbon consumed to provide reducing equivalents in biosynthesis. Need for activity of this pathway is determined by the difference between the oxidation state of pyruvate, the starting point for biosynthesis in our model, and the final oxidation state of the biomass produced. This pathway is considered here to account for carbon released as carbon dioxide and for the heat effect of the process. Carbon intermediates from this path reenter other pathways, but without major effect on the enthalpy change in the cell. Eq. (20) represents this pathway for our calculations.

$$\text{glucose} + 12\text{NADP} \rightarrow 6\text{CO}_2 + 12\text{NADPH} \quad (20)$$

The NADPH produced is assumed to be used to reduce carbon for biosynthesis. Since both production and use of NADPH involve oxidation-reduction reactions with carbon, the enthalpy effects are expected to nearly balance. The overall process is oxidation of some carbon to reduce other carbon (i.e. disproportionation), a process that is approximately enthalpically neutral. In our model, this pathway therefore has no effect on heat production, and is needed only to calculate substrate carbon conversion efficiency.

The pentose phosphate pathway may, in fact, make a substantial contribution to heat production in some cells. Studies of heat production in red blood cells showed that half of the heat produced comes from glycolysis [32,37,38]. Monti et al. [38] estimate that most of the other 50% is due to the pentose phosphate pathway. Because no data are available to estimate the magnitude of the heat effect of the pentose phosphate pathway in plants, heat from this source is attributed to other pathways by our model.

A.4 Biosynthesis

Eq. (15) represents the combined biosynthetic pathways. We start with pyruvate because it is a starting substrate for many biosynthetic pathways. The oxidation state of the final biomass, γ_B must be assigned to calculate the need for reducing equivalents. The number of electrons needed for reductive synthesis must include the difference between the oxidation state of glucose, i.e. $\gamma_P = 0$, and the oxidation state of carbon in pyruvate, 2/3. Each carbon consumed in the pentose phosphate pathway gives two molecules of NADPH, or four electrons. Therefore, the number of carbons needed to produce the reducing equivalents for synthesis of structural biomass is given by Eq. (21).

$$\text{Number of pentose carbons oxidized to } \text{CO}_2 = (2/3 - \gamma_B)/4 \quad (21)$$

This equation can be used to tie the activity of the pentose phosphate pathway to the rate of growth, or biomass production.The requirement for ATP in biosynthesis is treated as a variable in our model. Calculation of ATP production considers both glycolysis and the oxidative pathways of mitochondrial respiration.

A.5 The Krebs cycle and oxidative phosphorylation

In our model, the function of the Krebs cycle and oxidative phosphorylation is to produce ATP for biosynthesis. This is given in abbreviated form in Eq. (16). In fully coupled metabolism, the overall

reaction for these oxidative processes is given in Eq. (22).

$$\text{pyruvate} + 2.5O_2 + 15\text{ADP} + 15P_i \rightarrow 3CO_2 + 2H_2O + 15\text{ATP} \quad (22)$$

Removing ATP from the heat calculations because the concentration does not change leaves Eq. (23),

$$\text{pyruvate} + 2.5O_2 \rightarrow 3CO_2 + 2H_2O \quad (23)$$

the reaction for combustion of pyruvate. The heat of combustion of pyruvate is −1166.4 kJ/mol [34]. The contribution of this pathway to the heat production of cells is calculated from the moles of carbon entering the pathway which, in turn, is calculated from the need for ATP in biosynthesis. The need for ATP is thus assumed to control the rate of mitochondrial respiration.

ATP is produced in glycolysis and in the oxidative pathways. ATP production from glycolysis gives 2 ATP/glucose (Eq. (18)), plus ATP derived from the NADH produced as a product of glycolysis. When calculated per mole of carbon, 2/3 mol ATP is produced per mole C by glycolysis.

ATP production in the oxidative pathways varies according to the coupling between Krebs cycle oxidation and phosphorylation. One mole of ATP is produced for each mole of pyruvate by substrate level phosphorylation. In fully coupled phosphorylation, 15 mol ATP are produced per mole of pyruvate. Because phosphorylation may be partially or fully uncoupled, our model includes a coupling constant that varies the ATP production of the oxidative pathways from 1 ATP/mol to 15 ATP/mol pyruvate, or (1/3–5) mol ATP/mol C.

This same coupling constant applies to ATP production from the two moles of NADH produced per mole glucose by glycolysis (Eq. (18)). Each mole of NADH can produce 3 mol of ATP (or 2 ATP, depending on which shuttle system is used) in fully coupled respiration. When respiration is completely uncoupled, no ATP is produced from oxidation of NADH. We calculate ATP produced from glycolytic NADH as

$$\text{ATP/C from glycolytic NADH} = (\text{ATP/C from oxidative phosphorylation} - 1/3)/(5 - 1/3) \quad (24)$$

This equation incorporates the stoichiometry of four NADH and one FADH2 produced for each pyruvate in the oxidative pathways, while only one is produced in glycolysis. The value of 1/3 corrects for one substrate level phosphorylation in the Krebs cycle.

Combining the three sources of ATP: glycolysis, glycolytic NADH, and the oxidative pathways, gives Eq. (25) for ATP production per C-mole of biomass produced.

$$\text{ATP/C} \equiv b = 2/3(C_{\text{glycolysis}}) + ((c - 1/3)/(5 - 1/3)(C_{\text{glycolysis}})) + c(C_{\text{Krebs}}) \quad (25)$$

$C_{\text{glycolysis}}$ is moles of C passing through glycolysis and c is the coupling constant for oxidative phosphorylation, i.e. moles ATP per mole of C oxidized to CO_2 in the Krebs cycle.

A.6 Application of the model

In this model, each reaction is tied to growth rate. All reactions are written on the basis of one mole of carbon incorporated into product biomass other than cellulose. All biomass carbon is assumed to pass through glycolysis to pyruvate. Then, a fraction of pyruvate goes through oxidative phosphorylation to produce ATP to drive synthesis of biomass from the balance of the pyruvate. To obtain the equation used to calculate the moles of carbon needed to produce ATP, we substitute into Eq. (25) the moles of carbon passing through glycolysis for biosynthesis per C-mole biomass produced, i.e. 1, plus the moles of carbon oxidized to CO_2 per C-mole biomass produced. Then we solve for carbon oxidized to CO_2 in terms of the number of ATP needed in biosynthesis and the assigned coupling constant for ATP synthesis. This gives Eq. (26), which is used to calculate carbon flow through the oxidative pathways.

$$\text{Moles carbon oxidized to } CO_2 = (42b - 9c - 25)/(51c + 25)$$

The variable b is the ratio of ATP/C in biosynthesis. The variable c is the coupling constant (i.e. ATP/C) for production of ATP in the oxidative pathways. This equation must be modified if the less efficient shuttle system is used to transport cytosolic NADH into mitochondria.

Assigning values to b and c is equivalent to assigning a value to the efficiency in other models. Eq. (26) allows calculation of the carbon dioxide released, oxygen consumed, and heat produced per mole of non-cellulose biomass carbon, ratios of these parameters, and substrate carbon conversion efficiency. CO_2 is produced from both the pentose phosphate pathway and the oxidative pathways. Total CO_2 release is the sum of the rates of C flows through these two pathways.

Oxygen is consumed during oxidation of electron carriers in oxidative phosphorylation, with water being the major product. Each mole of pyruvate entering the oxidative pathways results in consumption of 2.5 mol O_2. This equals 5/6 mol O_2/mol C in the oxidative pathways. In addition, 1/6 mol O_2 is consumed for each mole of C in glycolysis. Total oxygen is the sum of these two processes.

Heat produced per mole of carbon is calculated from the sum of the heat from each pathway. Cellulose production and the pentose phosphate pathway have negligible heat effects. The heat from glycolysis (Eq. (19)) is +42 kJ/mol glucose and the heat of oxidation of the NADH produced is −516 kJ/mol-glucose. Dividing by six C/glucose gives +7 kJ/mol C for the first step and −86 kJ/mol C for the oxidation, or a net heat effect from glycolysis of −79 kJ/mol C passing through glycolysis. The oxidative pathways produce −1166.4 kJ/mol pyruvate, or −388.8 kJ/mol C.

The enthalpy change for the biosynthetic pathways is difficult to assess because the overall reaction is not defined stoichiometrically. The enthalpy change for the sum of Eqs. (12),(13) and (15) is equal to ΔH_B defined in Eq. (3) and expanded in Eq. (27)

$$\Delta H_B = (\gamma_B - \gamma_P)\Delta H_{O_2}/4 + \Delta H_{CB} + \Sigma r_E \Delta H_E \quad (27)$$

where γ_B is the mean oxidation state of biomass carbon, ΔH_{CB} includes the heat of such reactions as polymerization, transport, and other miscellaneous processes, and $\Sigma r_E \Delta H_E$ accounts for the effects of incorporating elements other than carbon in the biomass. The first term in Eq. (27) accounts for differences in oxidation state between the biomass and glucose. The heat effects accounted for in ΔH_{CB} and $\Sigma r_E \Delta H_E$, though minor under most circumstances, are not known in cells. If we ignore these effects, i.e. set $\Delta H_{CB} + \Sigma r_E \Delta H_E = 0$, we find it limits the model too much, and does not account for all available data. Thus, we believe there is a significant, though small, contribution to the overall enthalpy change for metabolism from the processes of biosynthesis. Others [18] have also noted a nonzero ΔH value for anabolism. In our model, we account for these by assigning nonzero values to $\Delta H_{CB} + \Sigma r_E \Delta H_E$.

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