review

Respiratory efficiency and metabolite partitioning as regulatory phenomena in yeasts

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Studies of metabolic regulation in yeasts have a long history. Yeasts have served as models for the regulation of fermentative metabolism. Yeasts differ in their partitioning of metabolism between respiration and fermentation, that is, between their use of oxygen and organic compounds as terminal electron acceptors. The respirative route is assumed to yield more ATP. Many yeasts carry out metabolism solely by the respirative route. Others show a predominantly fermentative metabolism, even when oxygen is freely available. Fermentation under aerobic conditions, that is, when respiration should be possible, was believed to result from repression of respiration by fermentation. This phenomenon is known as the Crabtree effect. However, recent work has shown that aerobic fermentation results from an inherently-limited respiratory capacity of some yeasts, rather than from a specific repression of respiration. Nevertheless, considerable enzymatic evidence exists that suggests that a Crabtree effect does indeed operate in some yeasts, specifically Saccharomyces cerevisiae. *Because multiple electron transport systems are known to exist in yeasts, repression of the normal ATP-producing system can be accompanied by the induction of an alternate pathway. No decrease in the overall rate of oxygen utilization would then be apparent. Repression would, however, affect the yield of ATP from oxidative metabolism. This effect should be detectable using a suitable analysis of growth energetics. To this end, a model has been developed and applied to a variety of yeasts in order to examine them for changes in respiratory efficiency indicative of a Crabtree effect. A Crabtree effect consistent with previous enzymatic findings was detected in* S. cerevisiae *and* S. uvarum, *but not in* Schizosaccharomyces pombe. *New regulatory classifications based on model findings are proposed and methods for independently verifying these findings are outlined.*

Keywords: Crabtree effect; alternate respiration; P:O; model; respiratory capacity

Regulation of sugar metabolism in growing yeasts

Early work: the Pasteur and Crabtree effects

During the era of classical biochemistry, the metabolism of sugars by yeasts was investigated and described in terms of regulatory effects of sugar and oxygen. One of these effects is named after Louis Pasteur, who, more than a century ago, observed that fermentation by Brewers' yeast (*Saccharomyces cerevisiae*) decreased in the presence of oxygen.[1] A second effect, essentially antithetical to the Pasteur effect, is named after Herbert Crabtree, who noted in 1929 that "the glycolytic activity of tumor cells exerts a checking effect on their respiration."[2]

Although the observations of Pasteur were made with yeasts, while those of Crabtree were made with mammalian tumor cells, the metabolism of glucose by growing yeast is more consistent with a Crabtree effect than a Pasteur effect. In 1948, Swanson and Clifton first demonstrated that glucose utilization by growing *S. cerevisiae* in aerated cultures proceeded almost completely via fermentation.[3] Fermentative assimilation dominated over aerobic oxidation as long as

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there was an appreciable amount of glucose present. This phenomenon of "aerobic fermentation" by growing yeasts is contrary to the Pasteur effect (inhibition of fermentation by respiration) but consistent with the Crabtree effect (inhibition of respiration by fermentation).

A mechanism for the Pasteur effect in terms of allosteric effects of ATP and citrate on phosphofructokinase (PFK) has been advanced by Sols.[4] The citric acid cycle is feedback controlled at the level of isocitrate dehydrogenase, the activity of which is adjusted to the ATP requirements of the cell by its allosteric dependence on AMP. Increasing the rate of oxygen delivery will produce an increase in the energy charge, resulting in a decrease in citric acid cycle activity and an increase in citrate levels. Increased levels of citrate allosterically inhibit PFK, restricting glycolytic activity.[4] Note that the operation of the Pasteur effect requires that the ATP requirements of the cell (i.e. growth) do not increase with the introduction of oxygen. If they increased by a sufficient amount, no change in the energy charge would occur and the citric acid cycle activity and citrate levels would be unaffected. Thus, the Pasteur effect is expected to be most pronounced in nongrowing cultures. In addition, PFK is stimulated by the presence of ammonium, which is present under growing conditions.[4] Indeed, most observations of the Pasteur effect have typically been made with nongrowing cells.[5] Experimental assessment of the Pasteur effect in growing cultures has shown it to be less pronounced[6] or totally absent.[5,7]

It is the Crabtree effect and not the Pasteur effect that appears to be the relevant regulatory phenomenon for growing yeast. Growing yeast are, moreover, of greater commercial interest than resting yeast. De Deken[8] examined glucose metabolism during aerobic growth of 25 yeasts to determine if excess CO_2 formation occurred relative to oxygen utilization. Excess CO_2 (i.e. aerobic fermentation) indicated operation of a Crabtree effect, which was defined as a repression of one energy source (respiration) by another (fermentation), and was found in about half of the yeasts tested.[8] Thus, the occurrence of aerobic fermentation was considered equivalent to the existence of repression of respiration by glycolysis.

There are actually two definitions of the Crabtree effect used implicitly by De Deken and subsequent workers. One is purely phenomenological: "ethanol production under fully aerobic conditions;" the other is mechanistic: "the repression of respiration by glycolysis." These two definitions can become blurred in practice, leading to circular reasoning. For example, a yeast is first identified as Crabtree positive by its display of aerobic fermentation. Later, it is assumed that respiratory repression occurs in this yeast simply because it is Crabtree positive.

For the present, we will use the occurrence of ethanol production under fully aerobic conditions to classify yeasts as "aerobic-fermenting" (AF) yeasts. By fully aerobic conditions it is meant that the dissolved oxygen tension (DOT) is above the critical oxygen concentration everywhere in the fermentation vessel. The critical oxygen concentration is that concentration at which the oxygen uptake system of the cell is saturated. When the DOT exceeds the critical concentration, uptake is independent of DOT. Those yeasts that do not show ethanol production under fully aerobic conditions will be termed "aerobic-respiring" (AR) yeasts. Later, a more satisfactory classification will be proposed. The term "Crabtree effect" will be reserved exclusively for the repression of respiration. For a yeast to show repression of respiration (i.e. a Crabtree effect), it is necessary (but not sufficient) that this yeast show aerobic fermentation. Thus, all Crabtree-positive yeasts are also AF yeasts, but the reverse is not necessarily true. One of the objectives of this paper will be to identify AF yeasts that also show a Crabtree effect. We do not consider strictly respirative yeasts, that is yeasts that do not exhibit fermentation under any circumstances.

Relation of the Crabtree effect with the petite mutation and anaerobic growth

In a companion paper, De Deken identified a correlation between aerobic fermentation (which was presumed to reflect a Crabtree effect) and the occurrence of the *petite* mutation.[9] The *petite* mutation was first described in 1949 and involves a loss of capacity for respiration.[10,11] By growing *S. cerevisiae* in the presence of acriflavin, yeasts are obtained that lack several components of the respiratory chain[12] and so carry out little respiration. Upon plating onto acriflavin-free plates, the acriflavin-treated cells give rise to dwarf colonies (*petites*) which retain respiratory deficiency over the course of vegetative growth.[10–12] The *petite* mutation involves an extrachromosomal factor[13,14] and is specifically induced[15] by acridines, such as euflavin or acriflavin, that inhibit the synthesis of respiratory enzymes.[12]

De Deken treated with euflavin the same 25 yeasts studied in his other paper. With some of the yeasts, variants were obtained that were unable to grow on glycerol and so were considered respiratorily deficient. These yeasts were considered *petite*-positive. With two exceptions, *petite*-positive yeasts were also AF yeasts.[9]

Bulder[16] noted that the failure of some yeasts to give rise to *petite* mutants did not result from the failure of the respiratory inhibitor to produce the mutation, but rather from the lethality of the *petite* mutation. Plating acriflavin-treated cells onto acriflavin-free plates gave rise to microcolonies that were presumably composed of *petite* mutants that had failed to grow beyond a few generations. Apparently, the loss of respiratory competence was lethal in these yeasts. A correlation between the ability of the parent strain to grow under anaerobic conditions and the occurrence of the *petite* mutation was noted, implying that the *petite* mutation is lethal for those yeasts

unable to grow in the absence of oxygen (i.e. that require respiration for growth).

Comparisons of the ability of yeasts to grow anaerobically are complicated by the requirement for certain growth factors in the absence of oxygen. Cochin, a pupil of Pasteur, first demonstrated that malt extract does not support batch growth of *S. cerevisiae* after repeated serial transfers under strictly anaerobic conditions, whereas a medium containing yeast extract does.[17] Andreasen and Stier[18,19] identified ergosterol and unsaturated fatty acids as the factors required for extended anaerobic growth. Certain steps in the biosynthesis of these compounds are mediated by cytochrome P-450 and involve molecular oxygen.[20] Thus, these compounds cannot be formed under strict anaerobic conditions.

Unlike *S. cerevisiae*, the failure of some yeasts to grow without oxygen is not due to nutritional factors. Even when placed in a glucose medium containing an extract of aerobically-grown *Candida utilis*, this AR yeast fails to grow more than two or three generations in the absence of oxygen.[17] The same thing is observed for a number of other AR yeasts: *Candida shehatae, Pichia stipitis, Candida tenuis, Pichia segobiensis,* and *Pachysolen tannophilus,* pointing to a correlation between the AR classification and the absence of anaerobic growth.[17] These yeasts ferment anaerobically,[17,21–24] so their failure to grow probably does not result from a lack of ATP.

Inability to grow in the absence of oxygen is further evidenced by the failure of *P. tannophilus* to incorporate radiolabeled glucose or xylose into trichloroacetic acid-insoluble cellular material, even though both sugars are utilized for ethanol production under such conditions.[24] The absolute requirement for oxygen typical of AR yeasts is well demonstrated by the observation that shifting a continuous culture of *C. shehatae* from oxygen-limited growth to anaerobic conditions results in the immediate cessation of growth, although fermentation continues unabated.[25]

Thus far, a correlation between the AF classification (which presumably reflects respiratory repression), the *petite* mutation, and the ability to grow anoxically has been described. This correlation can be explained in terms of the varying importance of respiration in the metabolism of different yeasts. For AF yeasts, such as *S. cerevisiae*, respiration seems to be optional; they can grow in the absence of oxygen, produce viable mutants that lack respiratory ability, and presumably repress their respiration in favor of fermentation when in the presence of large amounts of a fermentable sugar. Aerobic respiring yeasts, such as *C. utilis,* appear to have a fundamental requirement for respiration: they do not grow in the absence of oxygen, do not produce viable respiration-deficient mutants, and do not repress their respiration.

This simple picture is disrupted by the observation that the AF yeast *Schizosaccharomyces pombe* does not grow under anaerobic conditions,[26] nor does it give rise to *petite* mutants.[27] De Deken had classified this yeast as *petite*-positive, but did not characterize the mutants further. In a later, more thorough study, Heslot and co-workers clearly demonstrated that *S. pombe* is *petite*-negative.[27] Thus, *S. pombe* resembles the AR yeasts in its requirement of oxygen for growth. The picture can be restored by asserting that it is respiratory repression (i.e. the Crabtree effect), and not aerobic fermentation, that is correlated with the *petite* mutation and anaerobic growth. This concept implies that repression of respiration does not occur in *S. pombe*, but does occur in *S. cerevisiae*. Testing this idea would require detection in *S. cerevisiae*, but not *S. pombe*, of some characteristic that is more closely associated with respiratory repression than is aerobic fermentation.

Enzymatic and further physiological evidence for respiratory repression in yeast

The observation of aerobic fermentation *per se* does not necessarily require that repression of respiration actually occurs. The existence of repression is supported by numerous observations of decreased levels of respiratory enzymes in fermentative or "glucose-repressed" cells, versus those in depressed cells.[6] Most of these studies have been hampered by experimental difficulties arising from reliance on batch culture techniques.[17] For example, cells from batch growth on high glucose concentrations are considered "glucose-repressed," while those grown on low concentrations are "derepressed." Batch growth of *S. cerevisiae* on glucose is diauxic: ethanol production by aerobic fermentation occurs until glucose is exhausted, after which the accumulated ethanol is metabolized.[28] Thus, a comparison of cells harvested at the same time after growth on high versus low initial concentrations of glucose is really a comparison of glucose-fermenting versus ethanol-oxidizing cells.[17] Apparent differences between the cells may simply reflect the different substrates and not a specific effect of repression.

It was not until continuous culture techniques were used that a satisfactory analysis of the nature of the Crabtree effect could be made. When *S. cerevisiae* is grown on glucose in continuous culture, two distinct metabolic regimes are seen.[29] At low dilution rates, metabolism is completely respirative: cell yields are close to 0.5 g g^{-1} and no ethanol is formed. When the dilution rate is raised beyond a critical value (D_{CRIT}), respirofermentative metabolism is observed. Such metabolism is characterized by lower growth yields and ethanol formation. With continuous culture, cells showing both respirative (derepressed) and respirofermentative (repressed) metabolism can be obtained on the same substrate.

In his pioneering study of glucose metabolism by *S. cerevisiae* H1022 in continuous culture, von Meyenberg[29] noted that the specific rate of oxygen utilization (Q_{O_2}) increased linearly with dilution rate, reaching a maximum value at D_{CRIT}. Q_{O_2} declined with increasing dilution rate for $D > D_{CRIT}$ and ethanol production

increased.[29] This decline in Q_{O_2} associated with the onset of ethanol production was taken as evidence for repression of respiration. A similar decrease in Q_{O_2} was subsequently observed for another strain of *S. cerevisiae* also.[30] Beck and von Meyenberg[28] measured the titers of malate dehydrogenase and NADH-linked glutamate dehydrogenase as a function of dilution rate. They found a sharp decrease in the levels of both enzymes that was associated with the transition from respirative to respirofermentative metabolism at D_{CRIT}. Similar observations have been made for TCA cycle and respiratory chain enzymes.[6] In aggregate, these observations provide substantial evidence for repression of respiration in aerobic fermentation (at least for *S. cerevisiae*).

Aerobic fermentation is the result of respiratory limitation, not repression

A decade after von Meyenberg's study, Barford and Hall[31] reported the results of continuous culture experiments with *S. cerevisiae* UNSW 703100, in which they found no evidence for respiratory repression. Like von Meyenberg, they observed increasing Q_{O_2} up to D_{CRIT}. For dilution rates above D_{CRIT}, however, Q_{O_2} remained constant at the value attained at D_{CRIT} ($Q_{O_{2CRIT}}$). Identical behavior was obtained with glucose or galactose as carbon source.

The cultures used by Barford and Hall had been adapted to respirofermentative metabolism. For glucose, this was accomplished by prolonged culture at a dilution rate of 0.45 h^{-1}, which is equal to the maximum specific growth rate (μ_{MAX}) of the unadapted yeast on glucose. Adaptation was accompanied by an increase in μ_{MAX} from 0.45 to 0.60 h^{-1}. For other sugars and ethanol, adaptation could be achieved by serial batch cultivation on the desired substrate, and was usually accompanied by an increase in μ_{MAX} of 0.14–0.15 h^{-1}. The adapted cultures displayed either constant Q_{O_2} in batch culture, or constant Q_{O_2} for $D > D_{CRIT}$ in continuous culture, leading Barford and Hall to suggest that repression did not occur in yeasts adapted to a fully respirative condition.

In a subsequent paper,[32] Barford and co-workers tested this idea by evaluating the effects of adaptation on the Q_{O_2} profile in continuous culture using *S. cerevisiae* H1022 (the same strain used by von Meyenberg). Above D_{CRIT}, Q_{O_2} remained constant at $Q_{O_{2CRIT}}$ for this yeast also. In a third paper,[33] Barford and Hall advanced the idea that the respiratory capacity of *S. cerevisiae* may become saturated and exhibit a maximum specific oxygen uptake rate after suitable adaptation. In this case, aerobic fermentation occurs, not because respiration is repressed, but because even the fully-developed respiratory capacity of *S. cerevisiae* is too small to utilize oxidatively all the sugar taken up.

Glucose metabolism by *S. cerevisiae* H1022 was evaluated yet again by Rieger and co-workers in 1983.[34] They found invariant Q_{O_2} values for $D > D_{CRIT}$, just as did Barford and co-workers. Furthermore, they obtained evidence that a nutritional limitation in the medium used by von Meyenberg may have produced his findings of decreased Q_{O_2}.[34] Constant Q_{O_2} values for $D > D_{CRIT}$ have also been observed with *Saccharomyces uvarum*[35] and *S. pombe*.[36] These results and the analysis of the results of shifts in dilution rate[35,37] and co-metabolism of glucose-ethanol mixtures[34,38] have established that aerobic fermentation is a consequence of the limited respiratory capacity of AF yeasts.[39–41] Having a mechanistic explanation for aerobic fermentation, we can now refer to AF yeasts as respiration-limited and to AR yeasts as respiration-unlimited.

Is repression of respiration a relevant factor in yeast metabolism?

With the adoption of the new concept of respiratory limitation, the old concept of respiratory repression has been largely discarded as an important factor in sugar metabolism by yeasts. Repression certainly occurs. When a continuous culture of *S. uvarum* is shifted from a dilution rate lower than D_{CRIT} to one higher, decreases in titers of malate dehydrogenase and cytochromes *a*, *b*, and *c* occur over 16–24 h, yet Q_{O_2} does not change.[35,37] The lack of correlation between Q_{O_2} and the titers of respiratory enzymes suggests that although repression occurs, it is not relevant to the observed metabolic behavior. Käppeli[37] has suggested that changes in titers of respiratory enzymes reflect adaptation to the new respirofermentative physiological state caused by saturation of the limited respiratory capacity of *Saccharomyces*-type yeasts. Conversely, Barford and Hall[31–33] suggest that repression of respiration results from a failure to achieve a complete adaptation to respirofermentative metabolism.

The key observation that has dispelled respiratory repression as a relevant regulatory system is the lack of change in Q_{O_2} associated with the onset of respirofermentative metabolism. For example, if repression were a relevant factor in *S. uvarum*, it should give rise to a decline in Q_{O_2} corresponding to the decline in enzyme titers, yet none is observed. This argument is valid provided that oxygen is utilized solely by the normal, cytochrome-containing respiratory system. If an alternate respiratory system were present along with the normal one, this system could increase its activity in response to repression of the normal one. The result would be a decrease in the rate of normal respiration without a corresponding decrease in Q_{O_2}. Such a change should have detectable physiological and metabolic effects. This idea will be explored following a review of respiratory systems in yeasts.

Respiratory systems in yeasts

Normal respiration

The primary respiration in yeasts is the cytochrome-containing electron transport chain, which consists of

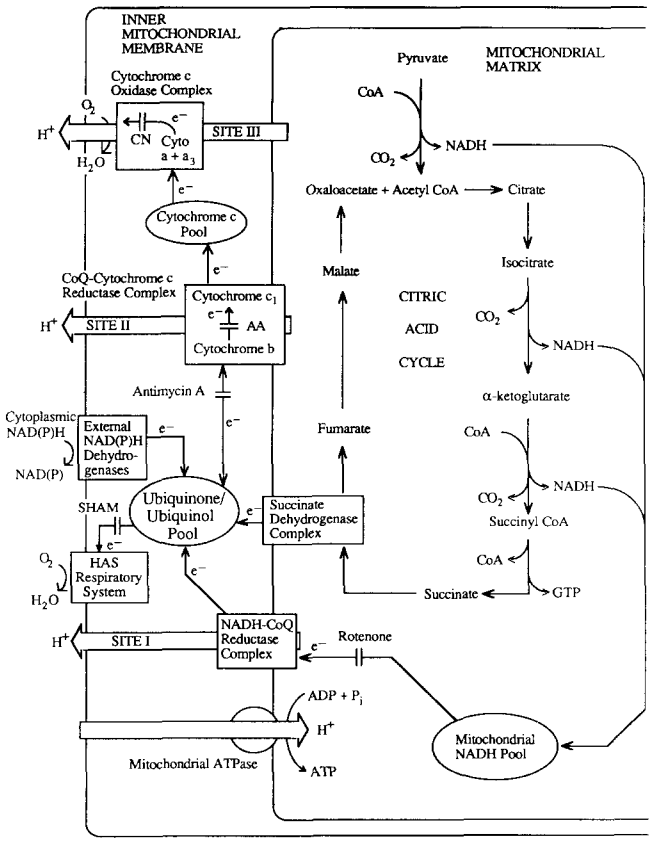

Figure 1 Diagram of generalized yeast mitochondria. Abbreviations are as follows: CN (cyanide), HAS (hydroxamate-sensitive), SHAM (salicyl hydroxamate), AA (antimycin A).

four complexes located in the inner mitochondrial membrane (*Figure 1*). Electron transfer between these complexes is mediated by two mobile carriers: the ubiquinone/ubiquinol redox couple (also called coenzyme Q) and cytochrome *c*. The first complex transfers electrons from NADH to ubiquinone and is known as the NADH–CoQ reductase complex. This transfer is coupled to phosphorylation via proton transport at site I and is inhibited by rotenone or piericidin.[42]

The second complex is succinate dehydrogenase, which carries out the oxidation of succinate to fumarate, transferring the electrons to ubiquinone. It is not coupled to phosphorylation. The third complex, CoQ–cytochrome *c* reductase, oxidizes ubiquinol back to ubiquinone, passing the electrons to cytochrome *c*. Electron transfer is coupled to phosphorylation via proton transport at site II. The complex contains cytochromes *b* and c_1 in a 2 : 1 ratio.[42] Electron transfer from cytochrome *b* to c_1 and the reverse transfer to ubiquinol is blocked by antimycin.[43] The last complex transfers electrons from cytochrome *c* to oxygen and is called cytochrome *c* oxidase. Electron transfer is coupled to phosphorylation via proton transport at site III and is blocked by cyanide or carbon monoxide. The cytochrome *c* oxidase complex contains cytochromes $a + a_3$ and a copper-containing protein.[42]

In yeasts, fungi, and plants, but not in animals, NADH dehydrogenases exist which are located on the outer surface of the inner mitochondrial membrane.[44] These dehydrogenases can accept electrons from NADH or NADPH[45] located outside the mitochondrion (*Figure 1*). Because NAD(H) does not permeate the inner mitochondrial membrane, special mechanisms that shuttle NAD(H) across this membrane are necessary in animal cells.[42] Yeasts can oxidize cytoplasmic NAD(P)H directly and so do not require them. Electrons accepted by these external dehydrogenases are passed to ubiquinone, and so bypass site I-driven phosphorylation. Thus, oxidation of exogenous NAD(P)H by intact mitochondria proceeds with an apparent P : O of 2 and is insensitive to rotenone.[45,46] In contrast, provided site I is active, oxidation of malate + pyruvate, which produces intramitochondrial NADH, proceeds with an apparent P : O of 3 and is sensitive to rotenone.[45,46]

Alternative forms of respiration

The existence of respiration that is resistant to cyanide has been known in yeasts for a long time.[47] Cyanide-insensitive respiration is widespread in other fungi and plants.[48] It occurs in protozoa and even in some lower animals.[48] This common cyanide-insensitive respiration is specifically inhibited by hydroxamates.[49] Another cyanide-insensitive respiration that is hydroxamate-insensitive has been detected in the yeasts *S. pombe*,[50–52] *S. cerevisiae*,[53] *Kluyveromyces lactis*,[54] *Hanseula saturnus*,[55] and *Endomycopsis capsularis*.[56] It is susceptible to high concentrations of azide, however. Thus, following Edwards,[57] we will refer to the first type of alternate respiration as hydroxamate-sensitive (HAS) and to the second as azide-sensitive (AZS).

HAS respiration branches off from the normal respiratory chain at ubiquinone (*Figure 1*).[43,58,59] Electron transfer between ubiquinone and oxygen via the HAS pathway does not generate a proton gradient and so does not drive ATP production.[60–64] Because it bypasses sites II and III, HAS respiration is insensitive to either cyanide or antimycin. Electrons flowing through the HAS pathway do not produce ATP after site I proton translocation at ubiquinone and so produce, at most, one ATP per electron pair (see *Figure 1*).

AZS respiration is believed to be mitochondrial and nonelectrogenic. Succinate is oxidized by normal respiration, but not by AZS respiration, whereas exogenous NAD(P)H is oxidized by both,[56] implying that electrons from NAD(P)H, but not succinate, have access to AZS respiration. Apparently, the AZS pathway does not communicate with the normal respiratory chain at ubiquinone. The position of the AZS pathway relative to the others is not known at present and so this pathway does not appear in *Figure 1*.

Occurrence of alternate respirations

The appearance of HAS respiration in the stationary phase of batch culture is frequently observed.[62] Condi-

tions similar to stationary phase have been artificially produced with *Saccharomycopsis lipolytica* by shaking dense suspensions of exponential-phase cells in medium lacking nitrogen for 90 min, during which time an HAS respiration develops that roughly equals respiration in the absence of cyanide.[59] The development of alternate respiration in stationary phase may reflect a change in the purpose of respiration from energy production to redox-balance maintenance as growth declines.

Alternate respiratory systems often appear when the normal pathway is blocked or limited in some way. This has been accomplished by use of respiratory inhibitors such as antimycin for both HAS and AZS respiration.[54,64–66] Drugs such as chloramphenicol that impair mitochondrial protein synthesis have also been used to obtain HAS respiration.[58,65–67]

Sustained growth in the absence of an element required for the synthesis of a key component of the normal respiratory chain (e.g. copper) can serve to artificially "repress" normal respiration, permitting the development of alternate respiration. Copper is an essential constituent of the cytochrome *c* oxidase complex. Growth of *C. utilis* in continuous culture on a copper-limited medium gives rise to a variant strain possessing HAS in lieu of normal respiration.[60] This strain lacks cytochromes $a + a_3$, which are components of the cytochrome *c* oxidase complex. Addition of copper under nongrowing conditions does not restore the activity of cytochromes $a + a_3$, indicating that the cytochrome *c* complex is absent and not merely inactive in the variant strain. Growth in copper-containing medium regenerates normal respiration and cytochromes $a + a_3$, without loss of HAS respiration. Apparently, HAS becomes derepressed in response to the artificially-imposed repression of cytochrome *c* oxidase produced by copper deficiency.

Development of alternate respiration usually occurs in response to limitation of growth or to repression/inhibition of normal respiration. For yeasts having a limited capacity for oxygen utilization, Q_{O_2} cannot exceed its maximum value of $Q_{O_{2CRIT}}$. For such yeasts, a repression of normal respiration accompanied by the development of alternate respiration would result in the replacement of normal with alternate respiration at constant Q_{O_2}. This replacement would lead to a drop in the ATP yield resulting from oxygen utilization and should be detectable by examination of growth energetics. This idea is explored in the next section.

Effect of respiratory efficiency (P : O) on growth yields

By growing *C. utilis* in continuous culture under various limitations, mitochondria with different P : O ratios (ATP generated per oxygen atom) can be isolated.[68] Mitochondria isolated from cells grown under glycerol, ammonium or magnesium limitation possess site I and have a P : O of three.[69] Those from cells grown under iron[70] or sulfur[71] limitation lack site I and have a P : O of two.[69] As described above, cultivation with copper limitation gives rise to variant cells possessing HAS, but lacking normal respiration. Site I is active in the mitochondria from these cells, giving them a P : O of one.[60] When cell yields from oxygen (Y_{O_2}) were calculated for glycerol-limited (P : O = 3), iron-limited (P : O = 2), and copper-limited variant cells (P : O = 1), respective values of 47.6, 30, and 15.8 g cells (mol oxygen)$^{-1}$ were obtained.[60,72] These values fall in a 3 : 1.9 : 1 ratio, almost exactly as expected from the corresponding P : O ratios. This correspondence implies that the growth yield from ATP (Y_{ATP}) is unaffected by changing mitochondrial efficiency, allowing mitochondrial P : O to be inferred from an analysis of growth energetics.

When the *petite*-negative yeast *Endomyces magnusi* is grown in the presence of antimycin, HAS respiration is observed.[64] In the absence of antimycin, normal respiration is observed. The growth rate of cells showing normal respiration is 2.6-fold greater than those showing HAS respiration, yet the rate of oxygen uptake is 20% less.[64] Thus, the Y_{O_2} for growth employing normal respiration is about three times greater than that employing HAS respiration.

A similar result is obtained with *Sporobolomyces ruberrimus*.[73] Cultures grown in the presence of antimycin produce only about 70% of the biomass of those grown in the absence of antimycin, and they exhibit HAS respiration. Assessment of the respiration rates of cells from both cultures shows that the antimycin-grown cells utilize oxygen at nearly twice the rate of the control. Once again, the Y_{O_2} of growth mediated by normal respiration is about three times greater than that mediated by HAS respiration.

The finding of a threefold greater Y_{O_2} for growth using normal versus HAS respiration suggests that the P : O of the former is 3, whereas that of the latter is 1. This inference assumes that Y_{ATP} is unaffected by the changes in respiratory P : O produced when respiratory systems are interchanged. Provided site I is present, electron transport via the normal respiratory chain does indeed proceed with a P : O of 3, whereas that proceeding by the HAS route proceeds with a P : O of 1 (*Figure 1*). The values inferred from growth energetics correspond well with the correct values. Thus, growth energetics can be used to infer changes in the relative amounts of different respirations.

Analysis of growth energetics can give evidence for respiratory repression

An analysis of growth energetics can indicate changes in respiratory efficiency, from which changes in the relative amounts of normal versus alternate respiration can be inferred. Specifically, by analyzing the energetics associated with respirofermentative metabolism by respiration-limited yeasts, we can infer whether normal respiration remains unchanged or is being replaced by an alternate form. In the latter case, a repression of (normal) respiration (i.e. a Crabtree effect) would be identified.

In this paper, we develop a model that can be applied to a variety of yeasts, both respiration-limited and respiration-unlimited. The model is based on that of Sonnleitner and Käppeli,[39] which explicitly uses the concept of limited respiratory capacity to account for aerobic fermentation. Respirative and fermentative metabolism are considered as a pair of parallel reactions. The former is favored over the latter, reflecting the lower K_m for pyruvate of pyruvate dehydrogenase versus pyruvate decarboxylase.[74] Respirative metabolism is limited to a maximum rate by the inherently limited respiratory capacity of *S. cerevisiae*. Thus, metabolism proceeds solely by the respirative route until its maximum rate is reached at D_{CRIT}. This rate is equal to the specific rate of sugar utilization (Q_S) at D_{CRIT} ($Q_{S_{CRIT}}$). For dilution rates greater than D_{CRIT}, Q_S exceeds $Q_{S_{CRIT}}$, and the excess is utilized via fermentative metabolism.

This model is extended to respiration-unlimited yeasts in this paper. A simple analytical format is used that is amenable to graphical analysis, eliminating the need for numerical solution by computer. More importantly, the key parameters of Sonnleitner and Käppeli's model are interpreted in terms of a respiratory efficiency parameter (β) that can be thought of as the weighted average of the P:O values of all active respiratory pathways. Thus, by employing a simple graphical analysis of metabolic data from continuous culture, changes in respiratory efficiency can be inferred, even when Q_{O_2} data are not available. This analysis is then used to provide a new operational definition for the Crabtree effect that is closely related to (normal) respiratory repression.

The Model

In the following section, expressions are developed relating the specific rates of sugar utilization, oxygen utilization, and ethanol production to the specific growth rate. The specific growth rate (μ) was chosen as the independent variable because it is equal to dilution rate (D) in continuous culture at steady state and so is under experimental control. When discussing steady-state continuous cultures, the parameters μ and D will be used interchangeably.

Conceptual ideas on which the model is based: metabolic stoichiometry

We start with the concept formulated by Sonnleitner and Käppeli.[39] Metabolism is considered as a pair of parallel processes: respirative and fermentative metabolism:

$$1 \text{ gram sugar} + \text{oxygen} \rightarrow Y_{S_R} \text{ grams biomass} \quad (1)$$

$$1 \text{ gram sugar} \rightarrow Y_E \text{ grams ethanol} + Y_{S_F} \text{ grams biomass} \quad (2)$$

Y_{S_R} and Y_{S_F} refer to the yield (g g^{-1}) of biomass from sugar for respirative and fermentative metabolism, respectively. Y_E refers to the effective yield of ethanol from sugar in g g^{-1}, which is not necessarily equal to the theoretical yield coefficient (Ψ_E) of 0.51 g g^{-1}. Unlike Sonnleitner and Käppeli, an explicit stoichiometric parameter for oxygen is not employed in equation (1). Rather, the quantity of oxygen used to metabolize 1 g of sugar via respirative metabolism is determined by energetics, which will be discussed later. The rates of respirative metabolism (equation 1) and fermentative metabolism (equation 2) are denoted by Q_R and Q_F. Their units are expressed as specific rates: g sugar (g dry wt)$^{-1}$ h^{-1}.

We can write an expression for the overall specific growth rate by summing the contributions from equations (1) and (2):

$$\mu = Y_{S_R}Q_R + Y_{S_F}Q_F = \mu_R + \mu_F \quad (3)$$

We define μ_R and μ_F as the specific rates of biomass production (i.e. growth) resulting from respirative and fermentative metabolism. The overall specific rate of sugar utilization is obtained by a similar sum:

$$Q_S = Q_R + Q_F = \mu_R/Y_{S_R} + \mu_F/Y_{S_F} \quad (4)$$

Metabolic energetics

We use an energetics analysis similar to that used by Barford and Hall,[33] except that it is expressed in differential form. Growth is assumed to be energy limited. That is, a differential increase in μ requires a proportional differential increase in the specific rate of ATP production (Q_{ATP}):

$$d\mu = Y_{ATP}dQ_{ATP} \quad (5)$$

The proportionality coefficient (Y_{ATP}) is the yield of biomass from ATP in g mol^{-1}. Y_{ATP} is assumed constant in the model because Y_{ATP} and β cannot be determined independently for respirative metabolism and the purpose of the model is to detect changes in β. We have already seen that the assumption of constant Y_{ATP} allowed correct inferences to be made concerning relative growth yields with *C. utilis*, *E. magnusi*, and *S. ruberrimus* having variable respiratory efficiencies.

ATP production occurs as a result of respiration and glycolysis. Each gram of sugar consumed is assumed to undergo glycolysis with the concomitant production of 1/90 mole of ATP. That is, all carbon assimilated into biomass is assumed to be derived from glycolytic pyruvate. Each mole of oxygen consumed is assumed to give, on average, 2β moles of ATP. As mentioned earlier, β is a parameter reflecting respiratory efficiency and represents the effective P:O for respiration as a whole. It will be treated as a function of μ. Thus, Q_{ATP} is given by

$$Q_{ATP} = Q_S/90 + 2\beta Q_{O_2} \quad (6)$$

Equation (6) is clearly an oversimplification. All sugar utilized does not undergo glycolysis; a fraction bypasses glycolysis to make anabolic precursors and NADPH via the pentose shunt. The effect of this simplification will be to overestimate glycolytic ATP

production, thus underestimating respiratory ATP production and respiratory efficiency (i.e. β). The effect on β will be small, however.

Differentiation of equation (6) and substitution into equation (5) yields a differential relation between substrate uptake and growth rate in terms of energetics parameters:

$$d\mu = (Y_{ATP}/90)dQ_S + 2Y_{ATP}d(\beta Q_{O_2}) \quad (7)$$

An expression for β in terms of Q_{O_2}, Q_S, and μ can be obtained by integrating equation (7) subject to the following condition: at $\mu = 0$, $Q_S = 0$, $\beta = \beta_0$ and $Q_{O_2} = Q_{O_{2_0}}$:

$$\mu = (Y_{ATP}/90)Q_S + (2Y_{ATP})(\beta Q_{O_2} - \beta_0 Q_{O_{2_0}}) \quad (8)$$

following rearrangement:

$$\beta = [\mu/(2Y_{ATP}) - Q_S/180 + \beta_0 Q_{O_{2_0}}]/Q_{O_2} \quad (9)$$

Equation (9) is valid for both respirative and respirofermentative metabolism. It is useful for analyzing metabolic rates in terms of changing respiratory efficiency.

Metabolic kinetics

Sugar uptake is assumed to follow saturation kinetics:

$$Q_S = Q_{S_{MAX}}S/(K_S + S) \quad (10)$$

Here $Q_{S_{MAX}}$ is the maximum value of Q_S and K_S is the half saturation constant (in g l^{-1}). Both of these parameters are assumed to be constant under all cultural conditions.

Development of the model

The concepts presented in the previous section will be used to develop expressions relating Q_S, Q_{O_2}, and Q_E to μ for both respirative and respirofermentative metabolism of both respiration-limited and respiration-unlimited yeasts.

1. Respirative metabolism of both respiration-limited and respiration-unlimited yeasts. Under fully aerobic conditions, purely respirative metabolism occurs at all values of D with respiration-unlimited yeasts, but only for $D < D_{CRIT}$ with respiration-limited yeasts. For purely respirative metabolism, μ_F and Q_F are zero and equations (3) and (4) become

$$\mu = \mu_R \quad (11)$$

$$Q_S = Q_R = \mu_R/Y_{S_R} = \mu/Y_{S_R} \quad (12)$$

Differentiation of equation (12) and substitution into equation (7) yields, upon rearrangement:

$$d[\beta Q_{O_2}] = \{1/(2Y_{ATP}) - 1/(180Y_{S_R})\}d\mu \quad (13)$$

When Y_{S_R} is a constant, Q_S will be linearly related to μ by equation (12), in which case we can write:

$$d(\beta Q_{O_2})/d\mu = \text{constant} \quad (14)$$

For constant β, equation (14) gives $dQ_{O_2}/d\mu$ equal to a constant, implying a linear relation between Q_{O_2} and μ. From this it follows that if both Q_S and Q_{O_2} are linear functions of μ, then β is constant. For this case (constant β and Y_{S_R}) equation (13) can be integrated using the following initial condition: at $\mu = 0$, $Q_{O_2} = Q_{O_{2_0}}$.

$$Q_{O_2} = \{(1/(2\beta Y_{ATP}) - 1/(180\beta Y_{S_R})\}\mu + Q_{O_{2_0}} \quad (15a)$$

$$= (1/{}^{M}Y_{O_2})\mu + Q_{O_{2_0}} \quad (15b)$$

Here $Q_{O_{2_0}}$ is simply the intercept of the linear relation between Q_{O_2} and μ that is seen when both β and Y_{S_R} are constants. ${}^{M}Y_{O_2}$ can be interpreted as the marginal yield of biomass from oxygen in respirative metabolism, that is, the additional quantity of biomass (in grams) produced by the input of an additional quantity of oxygen (in moles). The relation of ${}^{M}Y_{O_2}$ to β is given by

$$\beta = {}^{M}Y_{O_2}/2Y_{ATP} - {}^{M}Y_{O_2}/180Y_{S_R} \quad (16)$$

In practice, ${}^{M}Y_{O_2}$ is obtained from the reciprocal of the slope of a plot of experimental Q_{O_2} values versus μ as shown in *Figure 2*. Y_{S_R} is obtained as the reciprocal of the slope of a plot of Q_S versus μ for $\mu < \mu_{CRIT}$. Given ${}^{M}Y_{O_2}$ and Y_{S_R}, β is obtained from equation (16). $Q_{S_{CRIT}}$ and $Q_{O_{2_0}}$ are identified as shown in *Figure 2*.

2. Respirofermentative metabolism by respiration-limited yeasts. Respirative metabolism gives way to respirofermentative metabolism at μ_{CRIT} for respiration-limited yeasts. This occurs because these yeasts have a limited respiratory capacity characterized by a maximum oxygen utilization rate equal to $Q_{O_{2_{CRIT}}}$.[39] Thus, the maximum values of Q_R and μ_R are reached at μ_{CRIT} and so are equal to $Q_{S_{CRIT}}$ and μ_{CRIT}. With this, equations (3) and (4) yield, upon rearrangement:

$$\mu_F = \mu - \mu_{CRIT} \quad (17)$$

$$Q_S = Q_{S_{CRIT}} + (\mu - \mu_{CRIT})/Y_{S_F} \quad (18)$$

An alternate expression for Q_S can be obtained for $\mu > \mu_{CRIT}$ by substituting $Q_{O_{2_{CRIT}}}$ for Q_{O_2} in equation (7):

$$dQ_S = (90/Y_{ATP})d\mu - 180d(Q_{O_{2_{CRIT}}}\beta) \quad (19)$$

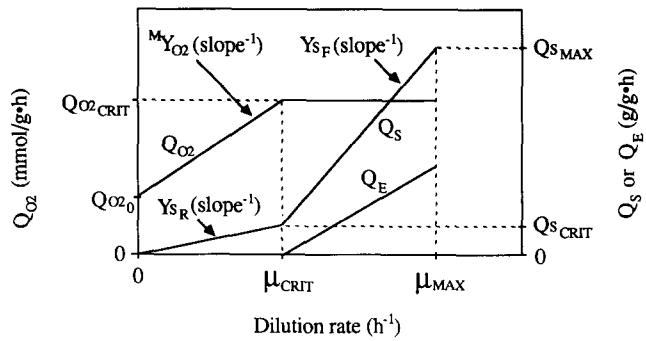

Figure 2 Graphical determination of model parameters for a respiration-limited yeast. ${}^{M}Y_{O_2}$, Y_{S_R}, and Y_{S_F} are determined from the slopes of plots of Q_{O_2} and Q_S versus μ as shown in the figure. Respirative metabolism of respiration-unlimited yeasts is analyzed in the same way as that of respiration-limited yeasts.

When the product of β and $Q_{O_{2CRIT}}$ is constant, the second term on the right-hand side of equation (19) vanishes and equation (19) can be integrated subject to the initial condition: at $\mu = \mu_{CRIT}$, $Q_S = Q_{S_{CRIT}}$.

$$Q_S = Q_{S_{CRIT}} + (90/Y_{ATP})(\mu - \mu_{CRIT}) \quad (20)$$

Comparison of equation (20) with equation (18) shows that Y_{S_F} is equal to $Y_{ATP}/90$, provided the product of β and $Q_{O_{2CRIT}}$ does not change with μ. The assumption of invariant $\beta Q_{O_{2CRIT}}$ implies that ATP production by respiration is unaffected by fermentative metabolism, that is, no repression of respiration by fermentation occurs. When this is true, Y_{S_F} can be interpreted as the actual cell yield resulting from fermentative metabolism. In anaerobic metabolism, respiration efficiency cannot affect the cell yield and Y_{S_F} is necessarily equal to $Y_{ATP}/90$. This result forms the basis for direct experimental assessment of Y_{ATP} values. Only for anaerobic conditions can the ATP produced by sugar catabolism be known with any certainty. Thus, Y_{ATP} values can be obtained only for anaerobic growth, and are typically close to 10 g mol^{-1} for a wide range of substrates and microbes.[75,76] We will use this value throughout this paper.

If normal respiration were repressed, the product $\beta Q_{O_{2CRIT}}$ would decrease and Y_{S_F} would not be equal to $Y_{ATP}/90$. In particular, Y_{S_F} would not have the same value as the anaerobic cell yield; it would be lower because fermentation-derived ATP must be used to make up for the loss in respiration-derived ATP caused by repression.

From equation (18) it is evident that

$$Q_F = (\mu - \mu_{CRIT})/Y_{S_F} \quad (21)$$

An expression for Q_E can be obtained from equations (2) and (21):

$$Q_E = Y_E Q_F = (Y_E/Y_{S_F})(\mu - \mu_{CRIT}) \quad (22)$$

Y_E must be less than its theoretical value Ψ_E of 0.51 g g^{-1} for respiration-limited yeasts because some of the sugar metabolized fermentatively is assimilated into biomass and so is unavailable for fermentation. As a first approximation, each gram of biomass formed represents one gram of sugar lost to fermentation, in which case we can write:

$$Y_E = \Psi_E(1 - Y_{S_F}) \quad (23)$$

Equation (23) is reasonably accurate for small Y_{S_F}.

3. Respirofermentative metabolism of respiration-unlimited yeasts. Respiration-unlimited yeasts have no respiratory limitations and so display respirative metabolism for all dilution rates under fully aerobic conditions. Respirofermentative metabolism is observed only when the supply of oxygen is limited externally. This class of yeasts is also different from respiration-limited yeasts in that they do not exhibit growth as a consequence of fermentative metabolism. That is, μ_F is always zero and $\mu_R = \mu$ under all conditions. With this, equation (4) gives:

$$Q_S = Q_F + Q_R = Q_F + \mu/Y_{S_R} \quad (24)$$

Under semiaerobic conditions, growth in continuous culture is oxygen-limited and high concentrations of sugar appear in the chemostat.[25] Thus, $S \gg K_S$ in equation (10) and Q_S is equal to its maximum value $Q_{S_{MAX}}$ for all μ. Substituting $Q_{S_{MAX}}$ for Q_S in equation (24) and solving for Q_F gives:

$$Q_F = Q_{S_{MAX}} - \mu/Y_{S_R} \quad (25)$$

Since fermentative growth does not occur with respiration-unlimited yeasts, Y_{S_F} is zero and Y_E is equal to Ψ_E by equation (23). Thus, with equations (2) and (25) we have

$$Q_F = \Psi_E[Q_{S_{MAX}} - \mu/Y_{S_R}] \quad (26)$$

Recall that for respirative metabolism, $Q_S = \mu/Y_{S_R}$, from which we obtain $Q_{S_{MAX}} = \mu_{MAX}/Y_{S_R}$. Substitution of this result into equation (26) gives:

$$Q_E = (\Psi_E/Y_{S_R})[\mu_{MAX} - \mu] \quad (27)$$

Since Y_{S_R} values are typically close to 0.5[6,17,25] and Ψ_E is equal to 0.51 g g^{-1}, the first term in equation (27) is approximately equal to unity and we have

$$Q_E = \mu_{MAX} - \mu \quad (28)$$

Substitution of $Q_S = Q_{S_{MAX}}$ into equation (7) gives the energetics that applies to oxygen-limited growth:

$$d(\beta_{OL} Q_{O_2}) = d\mu/2Y_{ATP} \quad (29)$$

The subscript OL refers to oxygen-limited conditions. A proportional relation between growth and oxygen uptake is often observed for low-to-moderate oxygen-limited growth rates,[25,77] in which case equation (29) can be integrated to give:

$$Q_{O_2} = \mu/(2Y_{ATP}\beta_{OL}) = \mu/{}^{OL}Y_{O_2} \quad (30)$$

Here ${}^{OL}Y_{O_2}$ (g mol^{-1}) is the yield of biomass from oxygen for oxygen-limited growth.

A graphical interpretation of respirofermentative metabolism of respiration-unlimited yeasts in terms of equations (28) and (30) is shown in *Figure 3*. Values for $Q_{S_{MAX}}$ and ${}^{OL}Y_{O_2}$ are found as shown in the figure.

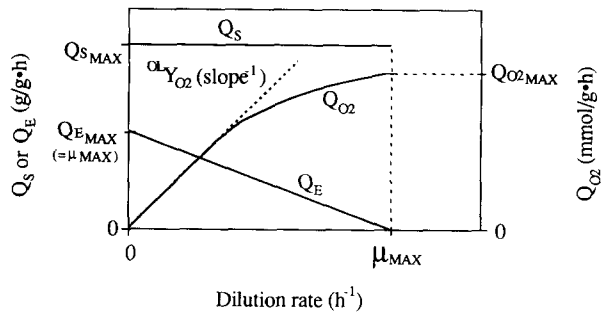

Figure 3 Graphical determination of model parameters for semiaerobic metabolism of a respiration-unlimited yeast

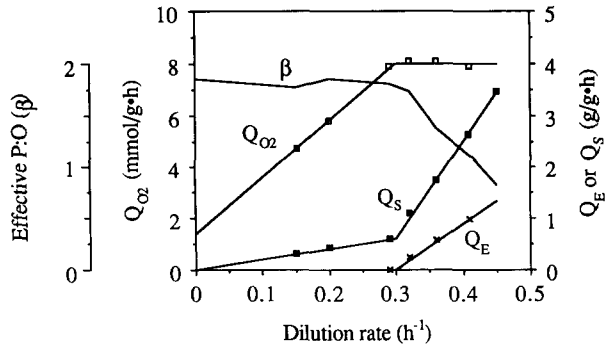

Figure 4 Glucose metabolism by *S. cerevisiae* H1022. Shown are Q_S (■), Q_{O_2} (□), and Q_E (×) as a function of dilution rate (equal to μ). The value of β was calculated by equation (9) and is shown by the broken line. Data are from Rieger and co-workers (ref. 34)

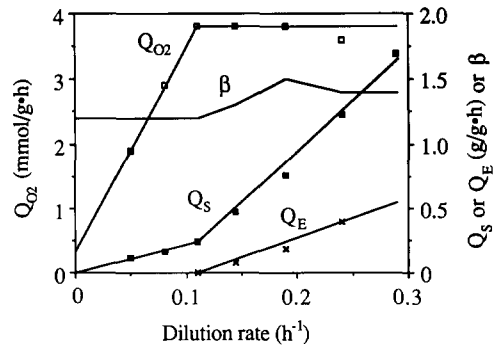

Figure 5 Glucose metabolism by *S. pombe* UNSW 707100. Symbols same as *Figure 4*. Data from Barford *et al.* (refs. 36, 80)

Application of the model

Parameter evaluation

Figure 4 shows plots of Q_S and Q_{O_2} data versus μ from continuous culture of *S. cerevisiae* H1022 on glucose.[34,39] The experimentally observed values of μ_{CRIT} and μ_{MAX} are 0.3 h^{-1} and 0.45 h^{-1}, respectively. Clearly, the graphical interpretation shown in *Figure 2* was valid for this yeast. A value for $Q_{S_{MAX}}$ of 3.5 g g^{-1} h^{-1} was given by Sonnleitner and Käppeli[39] and appears in *Table 1*. Other parameter values were obtained graphically as shown in *Figure 2* and appear in *Table 1*. This procedure was applied to data from a variety of respiration-limited yeasts in *Figures 5–8*. The results appear in *Table 1*.

The data for *S. pombe* ATCC 26189 were incomplete: respirative metabolism was not studied (*Figure 7*). Thus, a value for $Q_{O_{20}}$ could not be obtained and it was assumed to be zero. Reliable values for Q_{O_2} were not obtained at the higher dilution rates because of the very low cell densities present.[8] In experiments with mixed sugars, in which cell densities were higher, Q_{O_2} was constant and independent of D. When measured in cultures having sufficiently high cell densities, Q_{O_2} values were largely independent of carbon source and were consistent with an average value of 3.7 ± 0.3

Table 1 Model parameters for various respiration-limited yeasts

	S. cerevisiae				*S. pombe*		
Yeast:			UNSW		ATCC	ATCC	UNSW
Parameter	H1022	H1022	703100	*S. uvarum*	26189	26189	707100
Reference	29	34	33	35	80	80	36
Sugar	Glucose	Glucose	Glucose	Glucose	Glucose	Xylulose	Glucose
μ_{MAX}	0.45	0.45	0.60 (0.45)[a]	0.26	0.27	0.23	0.29
μ_{CRIT}	0.25	0.30	0.30	0.16	0.065[b]	0.065[b]	0.11
$Q_{S_{MAX}}$	3.1	3.5[c]	3.6	1.9[d]	2.05	1.1	1.7
$Q_{S_{CRIT}}$	0.51	0.62	0.9	0.33	0.2[e]	0.2[e]	0.25
Y_{S_R}	0.49	0.48	0.5	0.52	0.33[f]	0.33[f]	0.44
Y_{S_F}	0.078	0.052	0.11 (0.05)[a]	0.052[g]	0.11	0.20	0.12
$Q_{O_{20}}$ [h]	0.6	1.7	0.9	0.9	—	—	0.4
$Q_{O_{2CRIT}}$ [h]	—[i]	8.0	13.5 (6.7)[a]	5.0[d]	3.7	3.7	3.8
$^{M}Y_{O_2}$	34.5	47.6	43.5[j]	29.4[d]	17.5[k]	17.5[k]	28.6
β ($<\mu_{CRIT}$)	1.33	1.85	1.7	1.5	0.6	0.6	1.2
β (at μ_{MAX})	2.1	0.77	0.85	0.5	0.6	1.5	1.4

[a] Values in parentheses are for unadapted *S. cerevisiae* UNSW 703100
[b] Obtained by extrapolation to $Q_E = 0$ (see *Figure 7*)
[c] Value used by Sonnleitner and Käppeli[39]
[d] Value given by Käppeli and Sonnleitner[40]
[e] Obtained by extrapolation of respirofermentative Q_S values to μ_{CRIT} (see *Figure 7*)
[f] Equals $\mu_{CRIT}/Q_{S_{CRIT}}$
[g] Given by $(\mu_{MAX} - \mu_{CRIT})/(Q_{S_{MAX}} - Q_{S_{CRIT}})$
[h] Values in mmol g^{-1} h^{-1}
[i] Q_{O_2} declined for $D > D_{CRIT}$ (see *Figure 12*)
[j] Linear relation obtained only for $D < 0.175$ h^{-1} (see *Figure 8*)
[k] $Q_{O_{20}}$ was assumed to be zero, thus $m_{O_2} = Q_{O_{2CRIT}}/\mu_{CRIT}$

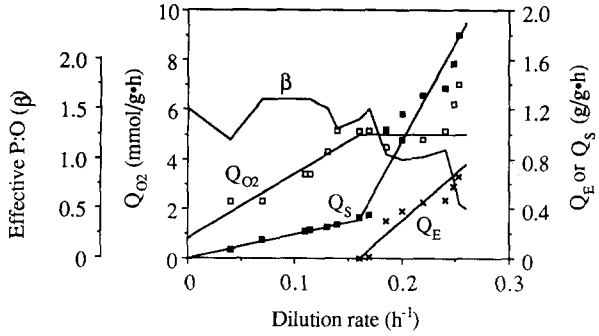

Figure 6 Glucose metabolism by *S. uvarum*. Symbols same as *Figure 4*. Data from Petrik *et al.* (ref. 35)

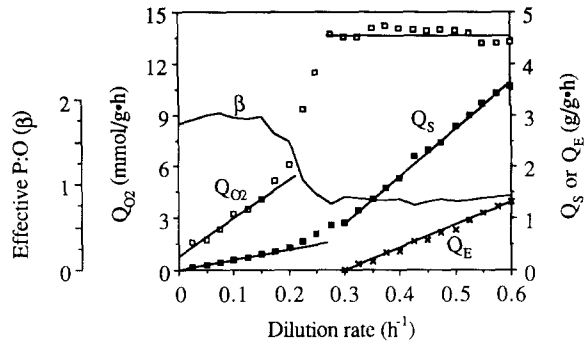

Figure 8 Glucose metabolism by *S. cerevisiae* UNSW 703100. Symbols same as *Figure 4*. Data from Barford and Hall (ref. 33)

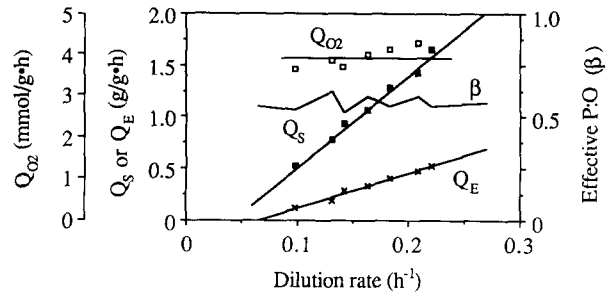

Figure 7 Glucose metabolism by *S. pombe* ATCC 26189. Symbols same as *Figure 4*. Data from Forrest (ref. 81)

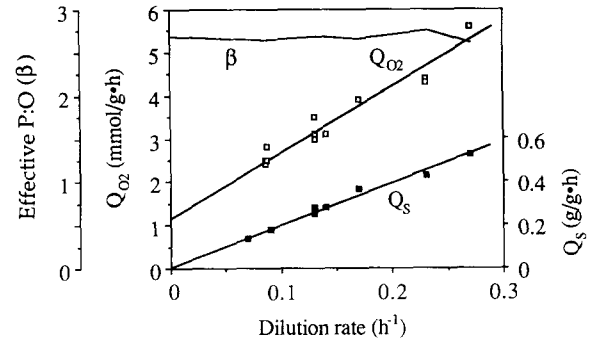

Figure 9 Xylose metabolism by *C. shehatae* under fully aerobic conditions. Symbols same as *Figure 4*. Data from Alexander *et al.* (ref. 25)

mmol g^{-1} h^{-1}, which was used for $Q_{O_{2CRIT}}$ in *Table 1*. Metabolism of *S. cerevisiae* UNSW 703100 on glucose (*Figure 8*) was not everywhere consistent with *Figure 2*. For $\mu > \mu_{CRIT}$ and $\mu \leq 0.15$ h^{-1} the data were consistent and could be analyzed to give the parameters in *Table 1*.

Figure 9 shows data for *C. shehatae* on xylose under fully aerobic conditions.[25] Both relations were clearly linear and were analyzed as shown in *Figure 2* for $\mu < \mu_{CRIT}$. The parameters obtained appear in *Table 2*. Data for *Candida utilis*[17] and *Candida parapsilosis*[30] under fully aerobic conditions were analyzed in the same way with the results shown in *Table 2*.

Figure 10 shows data for continuous culture of *C. shehatae* on xylose under oxygen limitation.[25] The data were consistent with *Figure 3* and the parameters

Table 2 Parameters for respiration-unlimited yeasts

Yeast	*Candida shehatae*		*C. utilis*	*C. parapsilosis*
Aeration condition	Fully aerobic	Semiaerobic	Fully aerobic	Fully aerobic
Reference	25	25	17	30
Sugar	Xylose	Xylose	Glucose	Glucose
μ_{MAX}	0.28	0.28	0.53	0.75
$Q_{S_{MAX}}$	0.55	0.55	1.0	1.3
Y_{S_R}	0.51	—	0.53	0.9 ($\mu < 0.5$)[a]
$Q_{O_{20}}$	0.0012	—	—	0.0004
$^{M}Y_{O_2}$	66.7	—	—	62.5
β (fully aerobic)	2.6	2.6	—	2.7
$^{OL}Y_{O_2}$	—	35[b]	—	—
β_{OL}	—	1.75	—	—

[a] Large amounts of yeast extract were used, which apparently was assimilated into biomass resulting in an unusually high yield from glucose. For dilution rates less than 0.5 h^{-1}, the yield remained constant at a high value close to 0.9 g g^{-1}. As the dilution rate approached μ_{MAX}, the yield dropped somewhat (see ref. 30)

[b] A $^{OL}Y_{O_2}$ of 35 g mol^{-1} has also been reported for oxygen-limited growth of *Candida* sp. XF217 on xylose,[80] giving this yeast an estimated β_{OL} of 1.75

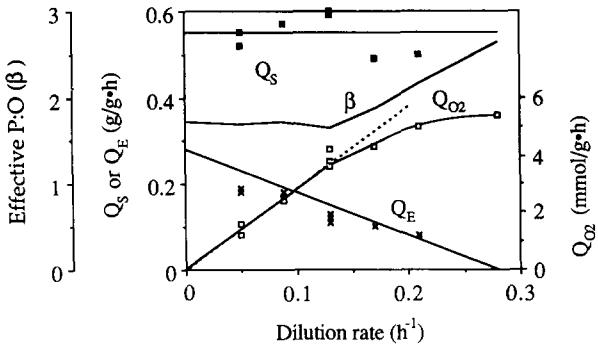

Figure 10 Xylose metabolism by *C. shehatae* under semiaerobic conditions. Symbols same as *Figure 4*. Data from Alexander *et al.* (ref. 25)

obtained are shown in *Table 2*. A ${}^{OL}Y_{O_2}$ of 35 g mol^{-1} for oxygen-limited batch culture of *Candida* sp XF217 has been reported[77] from which β_{OL} of 1.75 was obtained (see footnote 2, *Table 2*).

Interpretation of metabolism in terms of β

Figures 4 through *10* also show β calculated as a function of μ using equation (9). Examination of the variation of β with μ for respiration-limited yeasts reveals two general trends. In the *Saccharomyces* yeasts (*Figures 4, 6,* and *8*), β declines by about one unit as μ increases from zero to μ_{MAX}, whereas β remains approximately constant for *S. pombe* (*Figures 5* and *7*).

For *S. cerevisiae* UNSW 703100 (*Figure 8*), the drop in β occurs for $\mu < \mu_{CRIT}$. This drop in β occurs because of the increase in the slope of the relation between Q_{O_2} and μ for $\mu > 0.15$ h^{-1}. The change in β was calculated assuming a constant Y_{ATP} of 10 g mol^{-1}. Alternatively, a change in Y_{ATP} at constant β could also account for the increase in slope. These two ways of interpreting *Figure 8* are not equivalent. This can be seen by comparing the overall and marginal values of β and Y_{ATP} as shown in *Table 3*. The marginal value of β refers to the (small) increase in ATP production resulting from a (small) increase in oxygen uptake. Similarly, the marginal Y_{ATP} refers to an increase in μ resulting from an increase in Q_{ATP}. The marginal values of β suggest that the decline in β overall with increasing μ is consistent with two parallel respiratory systems, one having a β of about 2 and the other having a much lower value. Similarly, the marginal Y_{ATP} suggests that variable overall Y_{ATP} results from the operation of three parallel growth processes, two having Y_{ATP} values of about 10 and one having a much lower value.

The metabolic patterns displayed by *S. cerevisiae* UNSW 703100 in *Figure 8* are consistent with either multiple parallel growth processes having different Y_{ATP} values or multiple respiratory systems having different β (i.e. P:O) values. The latter hypothesis is the more plausible, because multiple respiratory pathways having the necessary characteristics are known to exist. In contrast, there are no known mechanisms consistent with multiple growth processes with different Y_{ATP} values. In order for Y_{ATP} to vary, the amount of ATP required for protein, nucleic acid, lipid, or carbohydrate synthesis would have to change. All biochemical evidence suggests that the ATP requirements are fixed characteristics of the pathways. Thus, we will assume that the variable respiratory system hypothesis is correct.

For *S. cerevisiae* H1022 and *S. uvarum* (*Figures 4* and *6*), a drop in β occurs for μ greater than μ_{CRIT}. Recall from the model development that when the product of β and Q_{O_2} is not constant for $\mu > \mu_{CRIT}$, Y_{S_F} is not equal to the anaerobic cell yield. For example, the cell yield observed for anaerobic glucose culture of *S. cerevisiae* H1022 is 0.10 g g^{-1}.[80] The glycerol-corrected yield (Y_{S_F}) is 0.11 g g^{-1}. This correction is required because glycerol-forming metabolism, which is necessary for maintenance of the redox balance,[17,81] does not contribute to ATP production. Thus, the observed anaerobic cell yield is consistent with the predicted value of $Y_{ATP}/90$ for a Y_{ATP} of 10 g mol^{-1}. Y_{S_F} for this same yeast in aerobic culture is 0.052 (*Table 1*). This low value is a consequence of the loss of respiratory efficiency manifest by the decrease in β shown in *Figure 4*. Because of the declining β, fermen-

Table 3 Interpretation of glucose metabolism by *S. cerevisiae* UNSW 703100 in terms of marginal and overall values for Y_{ATP} and β[a]

Specific growth rate (μ)	Y_{ATP}		β	
	Overall	Marginal	Overall	Marginal
0.050	9.8	9.1	1.8	1.8
0.075	8.9	8.9	1.8	1.7
0.100	8.2	8.7	1.8	1.7
0.125	8.9	8.2	1.8	1.6
0.150	8.9	6.8	1.8	1.2
0.175	8.0	4.2	1.6	0.7
0.200	7.6	2.9	1.5	0.4
0.225	5.6	2.3	1.1	0.2
0.250	4.9	2.6	0.9	0.2
0.275	4.5	4.3	0.8	0.4
0.300	4.6	13.8	0.8	—
0.325	4.8	11.4	0.8	—
0.350	5.0	10.5	0.8	—
0.375	5.2	9.3	0.8	—
0.400	5.4	8.6	0.8	—
0.425	5.4	9.4	0.8	—
0.450	5.6	10.7	0.8	—
0.475	5.8	10.8	0.8	—
0.500	5.8	9.4	0.8	—
0.525	6.0	9.6	0.8	—
0.550	6.1	11.1	0.8	—

[a] Marginal Y_{ATP} is defined as $\Delta\mu/\Delta Q_{ATP}$. Marginal β is defined as $\frac{1}{2}\Delta Q_{ATP}/\Delta Q_{O_2}$. ΔQ_{O_2} was calculated as $\Delta Q_{O_2} = (Q_{O_{2i-1.5}} - Q_{O_{2i-1.5}})/3$, where $Q_{O_{2i+1.5}}$ refers to the average of $Q_{O_{2i+1}}$ and $Q_{O_{2i+2}}$ and $Q_{O_{2i-1.5}}$ refers to the average of $Q_{O_{2i-1}}$ and $Q_{O_{2i-2}}$. ΔQ_{ATP} was calculated in an analogous fashion with Q_{ATP} determined by equation (6). An increment size of $\Delta\mu = 0.025$ h^{-1} was employed throughout. Overall values for β and Y_{ATP} were determined from equations (8) and (9)

Table 4 Y_{S_F} values for various respiration-limited yeasts

Yeast	μ_{CRIT}	μ_{MAX}	Y_{S_F}[a]	Ref.
S. cerevisiae H1022	0.30	0.45	0.054 ± 0.006	34
S. cerevisiae H1022	0.25	0.45	0.078 ± 0.005	29
S. cerevisiae strain 211	0.30	0.65	0.07[b]	89
S. cerevisiae	0.25	0.45	0.08 ± 0.02	30
S. cerevisiae UNSW 703100	0.30	0.60	0.11 ± 0.01	33
S. uvarum H2055	0.16	0.26	0.06 ± 0.03	35
S. pombe UNSW 707100	0.11	0.29	0.13 ± 0.03	36
S. pombe	0.14	0.32	0.09 ± 0.01	90
S. pombe	0.08	0.29	0.13 ± 0.03	82
S. pombe ATCC 26189				
Glucose	0.065	0.27	0.12 ± 0.02	78
Glucose + xylulose (adapted)	0.105	—	0.12 ± 0.01	78
B. intermedius	0.14	0.185	0.09 ± 0.04	17
B. lambicus DSM 70001	—[c]	0.14	0.22[d]	82

[a] The data presented are the average and standard deviation of the set of values calculated from $Y_{S_F} = \Delta\mu/\Delta Q_S$ using all possible combinations of μ and Q_S

[b] Biomass was expressed as cell counts, giving $Y_{S_F} = 8.9 \times 10^{11}$ cell mol^{-1}. A value of 1.2×10^{12} was obtained for the cell yield in anaerobic continuous culture. Thus, Y_{S_F} was about 70% of the anaerobic yield. If a value of 0.10 g g^{-1} is assumed for the anaerobic yield,[80] we obtain an approximate value of 0.07 g g^{-1} for Y_{S_F}

[c] *Brettanomyces* yeasts show both acetate and ethanol production. For *B. intermedius*, two values of μ_{CRIT} occur,[17] one at which acetate production begins (0.14 h^{-1}) and one at which ethanol production begins (0.17 h^{-1})

[d] Respirofermentative metabolism occurred at all dilution rates with an invariant cell yield of 0.22 g g^{-1}

tation-derived ATP must be used to make up for the loss of respiration-derived ATP. As a result, less ATP is available for the formation of new biomass, and the apparent yield of biomass from fermentative metabolism (Y_{S_F}) is lower. It must be stressed that the true fermentative yield is unaffected by declining respiratory efficiency. It remains equal to its anaerobic value of $Y_{ATP}/90$. In general, the model parameter Y_{S_F} will be equal to the actual fermentative cell yield only when respiratory deficiency is constant. When it is not, Y_{S_F} is only an apparent yield and has no simple energetic interpretation.

A declining value of $\beta Q_{O_{2CRIT}}$ will necessarily give a value of Y_{S_F} lower than $Y_{ATP}/90$, which for a Y_{ATP} of 10, is equal to 0.11. Changes in β reflect changes in the relative rates of normal versus alternate respiration. For constant $Q_{O_{2CRIT}}$, a decrease in β implies a decrease in the rate (i.e. repression) of normal respiration ($^{N}Q_{O_2}$). A decrease in $Q_{O_{2CRIT}}$ at constant β likewise implies a decrease in $^{N}Q_{O_2}$. Thus, Y_{S_F} values lower than 0.11 may be used as an indicator of repression of normal respiration. This idea is exploited in *Table 4*, in which Y_{S_F} values for a number of yeasts appear. The Y_{S_F} values for *S. cerevisiae* (except UNSW 703100) and *S. uvarum* were significantly smaller than 0.11, indicating that repression occurs in these yeasts. The values for *S. pombe* were reasonably close to 0.11, indicating that repression does not occur in these yeasts. The assignment of *S. pombe* to the Crabtree-negative category is in accordance with its characterization as *petite*-negative[27] and incapable of anaerobic growth.[26]

Also shown are values for two *Brettanomyces* species. For *B. lambicus*, no definite D_{CRIT} was observed and the cell yield was approximately constant for all dilution rates.[83] For *B. intermedius*, a definite D_{CRIT} was observed, allowing the calculation of a Y_{S_F} of 0.12 ± 0.04. Based on this value, this yeast is tentatively assigned to the Crabtree-negative category.

Instead of inferring repression of normal respiration from changes in β, $^{N}Q_{O_2}$ can be calculated as a function of Q_S, μ, and a reference β value (β_N) using equation (8):

$$^{N}Q_{O_2} = [\mu/(2_{ATP}) - Q_S/180 + \beta_0 Q_{O_{2_0}}]/\beta_N \qquad (31)$$

β_N is calculated using equation (9) for a reference condition in which Q_{O_2} is assumed to be composed of 100% normal respiration. $^{N}Q_{O_2}$ calculated by equation (31) can be compared with enzymatic and other data to provide evidence for the reality of repression of normal respiration. Examples of the application of equation (31) follow.

Figure (11) shows Q_{O_2} and titers of several respiratory enzymes for *S. uvarum* before and after a shift in dilution rate from 0.14 h^{-1} ($<\mu_{CRIT}$) to 0.21 h^{-1} ($>\mu_{CRIT}$).[37] Also shown are biomass and ethanol concentrations. Prior to the shift, Q_{O_2} was assumed to be 100% normal respiration, allowing a value for β_N of 1.37 to be obtained from equation (9) (values for β_0 and $Q_{O_{2_0}}$ of 1.5 and 0.9 were obtained for *S. uvarum* from *Table 1*). This value of β_N was then used in equation (31) to calculate the effects of the shift on $^{N}Q_{O_2}$. Both the cytochrome titers and $^{N}Q_{O_2}$ declined to about half their values before the shift. Malate dehydrogenase fell even further. This correspondence between declining enzymatic titers and $^{N}Q_{O_2}$ strongly suggests that (normal) respiratory repression occurred as a result of this shift, implying that a Crabtree effect operates in *S. uvarum*.

Figure 12 shows Q_{O_2} data for continuous culture of

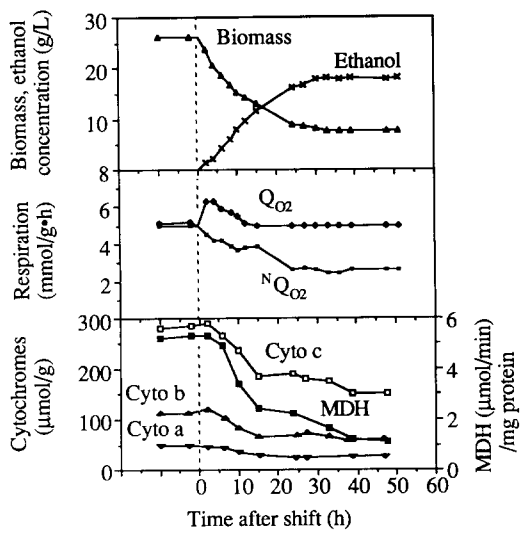

Figure 11 Effect of shift in dilution rate on glucose metabolism by *S. uvarum*. At the time indicated by the broken vertical line, the dilution rate was shifted from 14 h^{-1} ($<\mu_{CRIT}$) to 0.21 h^{-1} ($>\mu_{CRIT}$). Shown are cell density (▲), ethanol concentration (×), Q_{O2}, (◆), $^{N}Q_{O2}$ (■), and titers of cytochromes *a* (▼, *b* (△), *c* (□), and malate dehydrogenase (■). Data from Käppeli *et al.* (ref. 38)

S. cerevisiae H1022 from three researchers, von Meyenberg[29] and the groups of Barford[32] and Rieger.[34] The data of each are consistent with different intercepts ($Q_{O_{2_0}}$) and so the intercept-corrected rate ($Q_{O_2} - Q_{O_{2_0}}$) was plotted. The respirative data for the Barford and Rieger groups fall on the same line; thus the slope ($1/^{M}Y_{O_2}$) and, hence, β (by equation 16) is the same for both. Here we see the generality of β among the data of different workers, which is indicative of its validity as a fundamental concept.

The data of von Meyenberg are clearly different from the other two. Energetics analysis shows that the respiratory efficiency for $D < D_{CRIT}$ was low ($\beta = 1.3$, *Table 1*) compared with the others ($\beta = 1.85$). The suspected nutritional limitation in the medium used by von Meyenberg appears to have produced a less efficient respirative metabolism. β increased with dilution rate for respirofermentative metabolism ($D > D_{CRIT}$), however, rising to 2.1 at μ_{MAX} (*Table 1*). Apparently, the Q_{O_2} measured by von Meyenberg for large dilution rates was composed solely of normal respiration. $^{N}Q_{O_2}$ was calculated with equation (31) ($\beta_N = 1.85$) using the data of Rieger *et al.* and was compared with the Q_{O_2} of von Meyenberg (*Figure 12*). The normal respiratory component ($^{N}Q_{O_2}$) of the overall respiration observed by Rieger and co-workers decreased with dilution rate in a remarkably similar way as the Q_{O_2} (apparently all normal respiration) measured by von Meyenberg. Thus, the use of a nutritionally sufficient medium by Rieger *et al.* did not eliminate repression, but simply served to mask it.

Since the Q_{O_2} data of Barford *et al.* are essentially identical to those of Rieger *et al.*, we conclude that repression of normal respiration (i.e. the Crabtree effect) occurs with *S. cerevisiae* H1022, regardless of adaptation, medium, or other "handling" effects.

Thus far, we have inferred that (normal) respiratory repression appears to be common in yeasts of the genus *Saccharomyces*, but not in other respiration-limited genera such as *Schizosaccharomyces* or *Brettanomyces*. This repression is consistent with the observations of von Meyenberg[28] and the enzymatic findings of Käppeli *et al.*[37] The major exception to this generalization was *S. cerevisiae* UNSW 703100, whose value of Y_{S_F} puts it into the Crabtree-negative category (see *Table 4*), in sharp opposition with the assignment of all the other *S. cerevisiae* strains as Crabtree-positive. Recall that this yeast was adapted to respirofermentative growth, which results in an increase in μ_{MAX} and $Q_{O_{2CRIT}}$. The unadapted strain is very similar to *S. cerevisiae* H1022 in that it has similar $Q_{S_{MAX}}$, $Q_{O_{2CRIT}}$ and μ_{MAX} values (*Table 1*). Assuming that μ_{CRIT} for the unadapted strain is the same as that for the adapted strain (and *S. cerevisiae* H1022), the Y_{S_F} value for the unadapted strain of *S. cerevisiae* UNSW 703100 is much smaller than 0.11, indicating that a Crabtree effect does occur with the unadapted yeast but not with the adapted one. The hypothesis of Barford and Hall that respiratory repression can be removed by adaptation to a fully respiratory condition appears to be partially correct. It is true for *S. cerevisiae* UNSW 703100, but not for H1022. Adaptation appears to involve selection for a variant strain with a depressed alternate respiration. The sudden drop in β and increase in Q_{O_2} shown in *Figure 8* and analyzed in *Table 3* can be interpreted as an overflow of respiration into the alternate pathway as the normal pathway approaches saturation.

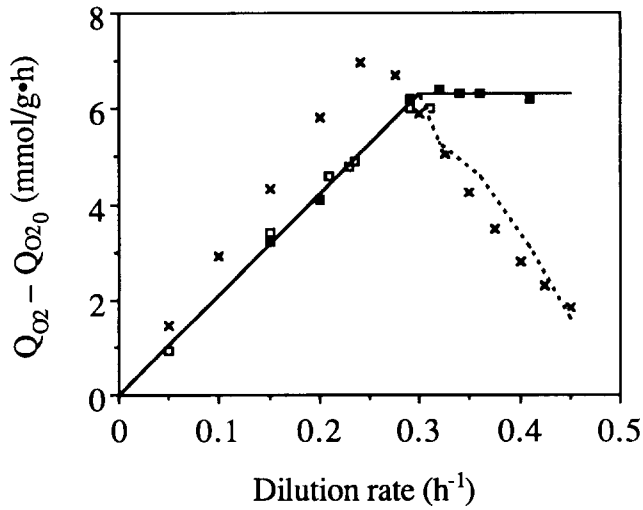

Figure 12 Comparison of normalized Q_{O_2} data for *S. cerevisiae* H1022 from three groups of workers. $Q_{O_{2_0}}$ was subtracted from Q_{O_2} to put all the data on the same basis. Data from von Meyenberg[29] (×), Rieger and co-workers[34] (■), and Barford and co-workers[33] (□). The broken line shows $^{N}Q_{O_2}$ calculated from the data of Rieger *et al.*[34]

Discussion

Comparison of the present model with the model of Sonnleitner and Käppeli

The primary difference between this model and that of Sonnleitner and Käppeli[39,40] is that energetics considerations have a prominent position in the former, whereas stoichiometric considerations dominate the latter. In the stoichiometric approach, overall metabolism is considered as the sum of two independent, parallel reactions corresponding to respirative and fermentative metabolism. These reactions have fixed stoichiometric coefficients that are determined from mass balances on carbon, oxygen, and hydrogen using cell elemental composition data and the corresponding experimentally determined cell yields (i.e. Y_{S_R} and Y_{S_F}). Y_{S_R} is obtained directly from aerobic growth data. Similarly, Y_{S_F} could be obtained from anaerobic growth data. However, the value so obtained did not give a good fit of aerobic fermentation data when used in the model. Thus, Y_{S_F} was determined from the aerobic fermentation data using a goodness-of-fit criterion. The Y_{S_F} value obtained in this way was only half the value consistent with anaerobic growth, leading Sonnleitner and Käppeli to hypothesize that different nutritional requirements or ethanol inhibition may be responsible for the difference. As described earlier, the energetics model interprets this difference as a side effect of respiratory repression. In reality, the fermentative cell yield is not different; it only appears to be so.

The energetics model has the advantage of explanatory power. For example, the metabolic patterns shown in *Figure 8* are inconsistent with a fixed stoichiometry. Analysis of these patterns using the energetics model shows that they are very likely the result of the action of multiple respiratory systems. On the other hand, the stoichiometric model can be readily extended to substrates other than sugars, for which biosynthetic oxygen demand must be considered, whereas the energetics model cannot.

Implications of differences in relation between β and μ among yeasts

Comparison of the parameters for respiration-unlimited (*Table 2*) and respiration-limited (*Table 1*) shows that β values for respirative (fully aerobic) metabolism of the former are at least 50% higher than those for respirative metabolism ($<\mu_{MAX}$) of the latter, suggesting that at least one proton translocation site is inactive in the mitochondria of respiration-limited yeasts. Under normal growing conditions, all three sites are active in mitochondria from the respiration-unlimited yeasts *C. utilis*[45,46,68,69,83] and *E. magnusii*.[83,84] The existence of site I in *Saccharomyces* yeasts has been a matter of controversy for a long time.[83] It is often reported to be present in stationary phase[83,85] or starved cells.[86] However, cells harvested from exponential[83] or early stationary phase[85,87] are generally reported to lack site I. Site I can be induced by aerobic incubation under starved conditions.[87] The finding here of a lower apparent P:O for *Saccharomyces* yeasts is consistent with the frequently observed absence of site I activity in actively growing cells.

Beta for *C. shehatae* under oxygen limited conditions (i.e. β_{OL}) is smaller than β under fully aerobic conditions (*Table 2*). Also, β_{OL} rises with increasing dilution rate (*Figure 10*). The data shown are for culture on xylose, whose utilization gives rise to a redox imbalance that results in NADH overproduction in the cytoplasm.[21] Up to 6.7 mmol of cytoplasmic NADH per gram xylose can be produced, depending on the activity of NADH-linked xylose reductases, which recycle NADH back to NAD.[88] Since xylose utilization for *C. shehatae* is constant at 0.55 g g^{-1} h^{-1} under semiaerobic conditions (*Table 2*), a constant flux of up to 3.6 mmol g^{-1} h^{-1} of cytoplasmic NADH is generated, which requires an oxygen utilization rate of up to 1.8 mmol g^{-1} h^{-1} for its oxidation. In actuality, not all of this NADH is re-oxidized; some is used for production of xylitol.

Recall that oxidation of cytoplasmic NADH proceeds with a P:O of 2 rather than 3 (*Figure 1*). Thus, at low dilution rates, when Q_{O_2} is small, a large fraction of Q_{O_2} will be used to oxidize cytoplasmic NADH and β will be approximately 2. As dilution rate and Q_{O_2} increase, a decreasing fraction of the total respiration results from oxidation of cytoplasmic NADH, and β will gradually increase. Under fully aerobic conditions, rates of xylose and oxygen utilization rise together with dilution rate (*Figure 9*). Thus, oxidation of cytoplasmic NADH is never a major fraction of the total respiration and P:O values are high.

Concluding remarks

It has been shown that the Crabtree effect can be identified by apparent fermentative cell yield (Y_{S_F}) values in aerobic continuous culture that are less than 0.11 g g^{-1} as shown in *Table 4*. If Q_{O_2} data are available, one can identify a Crabtree effect directly by a drop in the (calculated) normal respiratory rate ($^{N}Q_{O_2}$) associated with the onset of respirofermentative metabolism at D_{CRIT}.

Yeasts identified as Crabtree-positive using one of the above methods fall into the first of three distinct regulatory categories identifiable using the model. Crabtree-positive yeasts are necessarily respiration-limited. In addition, they are *petite*-positive and able to grow anoxically in suitable media, indicating that respiration is totally optional for these yeasts. The prototype Crabtree-positive yeast is *S. cerevisiae*.

Crabtree-negative, respiration-limited (CNRL) yeasts fall into the second category. They show aerobic fermentation but no detectable repression and are *petite*-negative. Respiration is more important in these yeasts. Although they are capable of utilizing fermentative metabolism for growth, they will not grow in the complete absence of oxygen. Examples of CNRL yeasts are *S. pombe* and, possibly, *B. intermedius*.

The third category are the respiration-unlimited yeasts, which are necessarily Crabtree-negative, so this term need not be applied to them. They are incapable of anaerobic growth and do not form stable *petite* mutants. The growth of respiration-unlimited yeasts has the greatest requirement for oxygen. In semiaerobic culture showing low to moderate growth rates, the growth of these yeasts is proportional to oxygen utilization.[25,79] That is, the oxygen-limited growth of respiration-limited yeasts is consistent with a constant $^{OL}Y_{O_2}$, regardless of the extent of fermentation, implying that fermentative metabolism is irrelevant to growth (i.e. Y_{S_F} is always zero).

The subtle difference in the ability of respiration-limited and respiration-unlimited yeasts to make use of fermentative metabolism for growth is well illustrated by a comparison of the growth characteristics of *S. pombe* ATCC 26189 on xylulose with those of *C. shehatae* on xylose. Both of these yeasts are Crabtree-negative and incapable of anaerobic growth. The primary difference between them is their respiratory capacity.

Under excessive aeration, *C. shehatae* develops the faster growth rate (0.28 vs 0.23 h^{-1}) and utilizes sugar far more efficiently (cell yield on sugar of 0.5 vs 0.2 g g^{-1}). When oxygen is in short supply, the relevant growth yield is $^{OL}Y_{O_2}$, the cell yield from oxygen for oxygen-limited growth. Consider the situation in which oxygen availability limits the extent of respirative growth to half its maximal value. Under these conditions, *C. shehatae* shows a μ of 0.14 h^{-1} and a $^{OL}Y_{O_2}$ of 35 g mol^{-1} (*Figure 10*). In contrast, *S. pombe* would show a μ of about 0.19 h^{-1} ($= \mu_{MAX} - \mu_{CRIT}/2$) and a $^{OL}Y_{O_2}$ of 103 g mol^{-1} ($= 2\mu/Q_{O_{2CRIT}}$). Thus, under 50% oxygen limitation, *S. pombe* enjoys 35% faster growth at three times the cell yield.

When oxygen availability falls to zero, the advantage of *S. pombe* over *C. shehatae* vanishes, as neither can grow, even with glucose as substrate. Crabtree-positive yeasts like *S. cerevisiae* can grow, however, and so possess a clear advantage over all others under conditions of extreme oxygen limitation or anaerobic conditions.

The ecological significance of respiratory limitation is apparent. A respiration-limited yeast, by virtue of its restricted respiration capacity and ability to grow fermentatively, obtains an advantage over respiration-unlimited yeasts under conditions of carbon sufficiency and oxygen limitation at the cost of a disadvantage under conditions of oxygen sufficiency and carbon limitation. Crabtree-positive yeasts gain the extra advantage of anaerobic growth at the expense of respiratory repression.

Finally, we note that the model findings used to detect the Crabtree effect are directly testable using respiratory inhibitors. For example, the great increase in Q_{O_2} in respirative metabolism of adapted *S. cerevisiae* UNSW 703100 (*Figure 8*) is associated with a drop in β, which implies that an alternate respiration develops. If this is true, as much as half of the Q_{O_2} observed for $D > 0.25$ should be cyanide resistant. The CN-insensitive respiration detected (if any) could be tested for its sensitivity to salicyl hydroxamate (SHAM) in order to classify it as HAS or AZS. If it is the former, SHAM could be added to cultures to suppress HAS respiration and allow direct observation of normal respiration for comparison to $^{N}Q_{O_2}$. Rotenone sensitivity of semiaerobic xylose cultures versus fully aerobic xylose cultures of respiration-unlimited yeasts can be used to verify whether site I is active (*Figure 1*). Since mostly cytoplasmic NADH is oxidized by the former, we should expect no effect of rotenone on these cultures, whereas some effect of rotenone should be observable for the latter.

Alternative evidence for decreased efficiency of respiration at high dilution rates has recently been provided by Postma and co-workers.[91] They observed an inflection in the Q_{O_2} versus μ relation and a decrease in the cell yield similar to that shown in *Figure 8*. They attributed this change to a decrease in respiratory efficiency. The change in inflection was accompanied by a marked decrease in specific activity of acetyl-CoA synthetase and the appearance of acetate. Incorporating propionate into the medium caused a similar decrease in cell yield. They hypothesized from these observations that organic acids uncouple the proton driving force in the mitochondria, thereby decreasing P : O.

Further research into the mechanisms determining respiratory efficiency and metabolite partitioning in both Crabtree-positive and Crabtree-negative yeasts is still required.

Nomenclature

AF	yeasts that ferment under strictly aerobic conditions
AR	yeasts that do not ferment when oxygen is present in excess
Q_S	specific sugar uptake rate (g g^{-1} h^{-1})
Q_E	specific ethanol production rate (g g^{-1} h^{-1})
Q_{O_2}	specific oxygen uptake rate (mol or mmol g^{-1} h^{-1})
$^{N}Q_{O_2}$	specific rate of normal respiration (mol or mmol g^{-1} h^{-1})
μ	specific growth rate (h^{-1})
Q_R	specific rate of sugar utilization by respirative metabolism (g g^{-1} h^{-1})
Q_F	specific rate of sugar utilization by fermentative metabolism (g g^{-1} h^{-1})
Q_{ATP}	specific rate of ATP production/consumption (mol g^{-1} h^{-1})
$Q_{O_{2CRIT}}$	maximum specific oxygen uptake rate (mol or mmol g^{-1} h^{-1})
$Q_{O_{2_0}}$	intercept of relation between Q_{O_2} and μ for respirative metabolism
μ_{MAX}	maximum specific growth rate (h^{-1})
μ_{CRIT}	critical specific growth (dilution) rate (h^{-1})
$Q_{S_{MAX}}$	maximum specific sugar uptake rate (g g^{-1} h^{-1})
$Q_{S_{CRIT}}$	Q_S value at μ_{CRIT} (g g^{-1} h^{-1})
Y_{S_R}	yield of biomass from sugar in respirative metabolism (g g^{-1})

Y_{S_F} apparent yield of biomass from sugar in fermentative metabolism (g g^{-1})
Y_E yield of ethanol from sugar (g g^{-1})
Y_{O_2} yield of biomass from oxygen (g mol^{-1})
$^{M}Y_{O_2}$ marginal yield of biomass from oxygen (g mol^{-1}), also the reciprocal of the slope of the linear relation between Q_{O_2} and μ for respirative metabolism
$^{OL}Y_{O_2}$ yield of biomass from oxygen for oxygen-limited growth (g mol^{-1})
Y_{ATP} yield of biomass from ATP (g mol^{-1})
Ψ_E theoretical ethanol yield, equal to 0.51 g g^{-1}
β apparent P : O for respiration as a whole
β_0 β at $\mu = 0$
β_{CRIT} β at $\mu = \mu_{CRIT}$
β_{MAX} β at $\mu = \mu_{MAX}$
D dilution rate (h^{-1})
K_S substrate affinity constant (g l^{-1})
S sugar concentration (g l^{-1})

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