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MINI-REVIEW

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Energy uncoupling in microbial growth under substrate-sufficient conditions

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Abstract It has been observed that the correlation between ATP and biomass formation is very poor, and the observed growth yield is lowered under substrate-sufficient conditions. This indicates that the excess substrate causes uncoupling between anabolism and catabolism, which leads to the dissipation of non-growth energy. However, a quantitative description of such uncoupling remains elusive. Based on a balanced substrate reaction, a growth-yield model in relation to residual substrate concentration for substrate-sufficient continuous cultures was developed. On the basis of this yield model, a coefficient governing the uncoupling of anabolism and catabolism was defined. A model describing the effect of the residual substrate concentration on this uncoupling coefficient was further proposed. These models agree with experimental data very well. It is clearly shown that, under substrate-sufficient conditions, the variation in growth efficiency is mainly due to energy uncoupling rather than to maintenance energy expenditure.

Introduction

The processes of energy-source degradation, ATP formation, monomer synthesis, macromolecular polymerization, DNA replication, and cell duplication are well understood. However, despite the abundance of information on the details of bacterial metabolism, there has been little quantitative information about bacterial energetic metabolism. Since Pirt (1965) postulated his well-known maintenance-energy equation, non-growth-associated energy requirements have been most usually attributed to maintenance. According to the Pirt theory, the observed growth rate has an effect on the observed growth yield, which can be described by Eq. 1 under substrate-limited conditions.

$$\frac{1}{Y_{obs}} = \frac{1}{Y_g} + \frac{m_s}{\mu_{obs}} \quad (1)$$

where Y_{obs} is the observed growth yield, Y_g is the true growth yield, μ_{obs} is the observed specific growth rate and m_s is the maintenance metabolism rate.

Many studies have been carried out to determine the maintenance coefficient and growth yield of microorganisms, using the Pirt equation (Pirt 1975; Stouthamer 1977; Jobses et al. 1985; Chang et al. 1993). Previous studies showed that, under substrate-sufficient conditions, the variation in respiration was far greater than the amount that could be ascribed to ATP production (Hempfling and Mainzer 1975; Westerhoff et al. 1982; Brooke et al. 1990). Substrate-sufficient cultures are known to have different metabolic behaviors from substrate-limited cultures with regard to the substrate removal rate, maintenance requirements and growth yield (Hueting and Tempest 1979; Tempest and Neijssel 1984; Brooke et al. 1990; Tsai and Lee 1990; Zeng and Deckwer 1995). In fact, much research shows that, under substrate-sufficient conditions, Y_{obs} decreases significantly with increasing residual substrate concentration for continuous and batch cultures (Rao and Gaudy 1966; Forrest 1969; Stouthamer 1977; Brooke et al. 1990; Chudoba et al. 1992; Yamane et al. 1992; Chang et al. 1993; Westerhoff et al. 1982; Ghigliazza et al. 1995).

Some of the variation in growth yield can be explained by maintenance-energy expenditures, but bacteria have other mechanisms of dissipating non-growth energy; that is, the cells waste ATP. In fact, under substrate-sufficient conditions, the correlation between ATP and biomass formation is very poor (Brooke et al. 1990; Stouthamer 1979). Lowered growth yields imply dissociation of catabolism from anabolism. Under substrate-sufficient conditions, energy generation

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from catabolism-associated substrate consumption is beyond that required for anabolism. This excess energy will be wasted (Stouthamer 1979; Westerhoff et al. 1982; Brooke et al. 1990; Tsai and Lee 1990). Thus, the interpretation of growth yield by the Pirt theory is questionable under substrate-sufficient conditions.

Tsai and Lee (1990) introduced the concept of overutilization of the substrate to explain the peculiar behavior of bacteria in substrate-sufficient cultures. However, few attempts have been made to establish a quantitative expression relating Y_{obs} to the residual substrate concentration, and to develop further a model describing the degree of energy uncoupling between anabolism and catabolism for substrate-sufficient continuous cultures. The specific objective of this work is to discuss energy uncoupling in a quantitative way.

Growth yield model

A culture of microorganisms can be classified as manifesting substrate-limited and substrate-sufficient growth according to the relative availability of the substrate. This work is only limited to substrate-sufficient continuous cultures. Under substrate-sufficient conditions, the substrate is consumed by microorganisms to form various intracellular metabolites and energy, which are then used for biomass formation, maintenance and product formation. Meanwhile the metabolites and energy may also be consumed in biomass turnover by energy spilling or through futile cycles. In reviewing the studies on bioenergetics, Harold (1986) pointed out that "there is something misleading about the fundamental assumption that the free energy of catabolism is fully conserved as ATP and expended necessarily either for biosynthesis or for useful work. Any departure from perfect coupling, either in the generation of ATP or in its utilization, will show up as a shortfall of the yield and exaggerate the apparent cost of cellular upkeep".

In an effort to account for the impact of energy uncoupling on growth yield, Liu (1996) postulated that the overall consumption of substrate (ΔS) should be taken to be the sums of the substrate consumed for growth (ΔS_g), the substrate used for maintenance (ΔS_m) and that part involved in energy spillage (ΔS_w), that is,

$$\Delta S = \Delta S_g + \Delta S_m + \Delta S_w \tag{2}$$

Equation 2 features the substrate consumption due to energy spillage with respect to the existing maintenance theory. If catabolism is tightly regulated to match the energy requirements of microorganisms, ΔS_w should be negligible. In fact, in cells, the regulation of substrate consumption may not effectively adapt to the exogenous substrate level (Forrest 1969; Stouthamer 1979; Tempest and Neijssel 1984; Fiechter and Seghezzi 1992; Russell and Cook 1995). In general, substrate-sufficient cultures have much higher substrate consumption rates and lower growth yields than substrate-limited cultures. This may be due to the overutilization of substrate by futile cycles and energy spilling, metabolic uncoupling, modification of the respiratory chain etc.

A semi-empirical model was proposed by Zeng and Deckwer (1995) for describing the excessive consumption of substrate in substrate-sufficient continuous culture (Δq_w):

$$\Delta q_w = (\Delta q_w)_{max} \frac{c_s - c_s^*}{c_s - c_s^* + K_s^*} \quad c_s \geq c_s^* \tag{3}$$

where Δq_w is the excessive consumption rate of substrate for a substrate-sufficient culture, $(\Delta q_w)_{max}$ is the maximum excessive consumption rate of substrate, c_s is the residual substrate concentration, c_s^* is the critical substrate concentration for substrate-limited growth, and K_s^* is the saturation constant. Dividing Eq. 2 by the increase in biomass (ΔX), we obtain Eq. 4.

$$\frac{\Delta S}{\Delta X} = \frac{\Delta S_g}{\Delta X} + \frac{\Delta S_m}{\Delta X} + \frac{\Delta S_w}{\Delta X} \tag{4}$$

The concept of growth yield leads to the following yield expressions (Liu 1996):
The true growth yield (Y_g):

$$Y_g = \frac{\Delta X}{\Delta S_g} = \frac{\mu_g}{q_g} \tag{5}$$

where μ_g and q_g are the true specific growth rate and the growth-related specific substrate consumption rate respectively.

The observed growth yield (Y_{obs}):

$$Y_{obs} = \frac{\Delta X}{\Delta S} = \frac{\mu_{obs}}{q_{obs}} \tag{6}$$

where μ_{obs} is the observed specific growth rate, and q_{obs} is the observed specific substrate consumption rate.
The pseudo-maintenance-related growth yield (Y_m):

$$Y_m = \frac{\Delta X}{\Delta S_m} = \frac{\mu_g}{m_s} \tag{7}$$

The pseudo-energy-spilling-related growth yield (Y_w):

$$Y_w = \frac{\Delta X}{\Delta S_w} = \frac{\mu_g}{\Delta q_w} \tag{8}$$

Substituting Eqs. 5–8 into Eq. 4 produces

$$\frac{1}{Y_{obs}} = \frac{q_g}{\mu_g} + \frac{m_s}{\mu_g} + \frac{\Delta q_w}{\mu_g} \tag{9}$$

Replacing Δq_w by Eq. 3 yields

$$\frac{1}{Y_{obs}} = \frac{q_g}{\mu_g} + \frac{m_s}{\mu_g} + \frac{(\Delta q_w)_{max}}{\mu_g} \frac{c_s - c_s^*}{c_s - c_s^* + K_s^*} \tag{10}$$

Under substrate-limited conditions, the Pirt theory shows that μ_{obs} equals μ_g, and Δq_w can be neglected, hence Eq. 10 simplifies to the well-known Pirt maintenance equation (Eq. 1). In experimental studies of substrate-sufficient continuous cultures, the input substrate concentration used is usually very high, given as 40 g glucose/l by O'Brien et al. (1980), and 50–150 mmol methanol/l by Brooke et al. (1990). Hueting and Tem-

pest (1979) reported that, for an ammonia-limited chemostat culture of *Klebsiella aerogenes* at a growth rate of 0.4 h^{-1}, for input glucose concentrations less than 7.5 g/l, the residual glucose concentrations were quite low. However, as the input glucose concentration increased from 7.5 g/l, excretion of partially oxidized metabolites was detected, which showed a glucose-sufficient condition, and the residual glucose concentrations increased sharply. A similar phenomenon was also reported by Brooke et al. (1990) in methanol-sufficient chemostat cultures of *Bacillus* strains. From these previous results, it appears that, under substrate-sufficient conditions, c_s would be much greater than c_s^*. Thus, Eq. 10 can be reduced to

$$\frac{1}{Y_{obs}} = \frac{q_g}{\mu_g} + \frac{m_s}{\mu_g} + \frac{(\Delta q_w)_{max}}{\mu_g}\frac{c_s}{c_s + K_s^*} \tag{11}$$

Obviously, the model can be simplified while sacrificing little in terms of accuracy. Eq. 11 can be rearranged to the following form:

$$\frac{1}{Y_{obs}} = \frac{1}{(Y_{obs})_{max}} + \frac{1}{(Y_w)_{min}}\frac{c_s}{c_s + K_s^*} \tag{12}$$

where $(Y_{obs})_{max}$ and $(Y_w)_{min}$ are the maximum observed growth yield under substrate-limited conditions, and the minimal energy-spilling-related growth yield, as described by Eqs. 13 and 14 respectively.

$$(Y_{obs})_{max} = \frac{\mu_g}{q_g + m_s} \tag{13}$$

$$(Y_w)_{min} = \frac{\mu_g}{(\Delta q_w)_{max}} \tag{14}$$

Within the limits of this study, $(Y_{obs})_{max}$ and $(Y_w)_{min}$ are considered to be independent of the residual substrate concentration. A simple graphical method is used to evaluate the model parameters. It is evident that $(Y_{obs})_{max}$ can be estimated from the curve of $1/Y_{obs}$ against c_s at $c_s = 0$. In order to determine $(Y_w)_{min}$ and K_s^*, Eq. 12 is rearranged to the following form:

$$\frac{1}{\frac{1}{Y_{obs}} - \frac{1}{(Y_{obs})_{max}}} = (Y_w)_{min}K_s^*\frac{1}{c_s} + (Y_w)_{min} \tag{15}$$

According to Eq. 15, plotting $1/[1/Y_{obs} - 1/(Y_{obs})_{max}]$ against $1/c_s$ should give a straight line. The intercept of this line is $(Y_w)_{min}$, and the slope equals $(Y_w)_{min}K_s^*$. Equation 12, for the first time, shows that, under substrate-sufficient conditions, the variation in growth efficiency is closely related to the degree of energy uncoupling. In previous studies, since the relationship between Y_{obs} and energy uncoupling was not thoroughly understood and quantified, the observed variation in growth yield even led Tempest and Neijssel (1984) to conclude that "yield values *per se* are not readily interpretable in precise bioenergetic and/or physiological terms and, unless treated with considerable circumspection, they may lead to the formation of concepts that are at best dubious".

Energy uncoupling model

Microbiologists have generally assumed that the yield of cells is directly proportional to the amount of ATP produced (Lehninger 1975; Brock and Madigan 1991). This assumption of a strict coupling between anabolism and catabolism is contradicted by the observation that bacteria can utilize an energy source even in the complete absence of growth. As pointed out earlier, under substrate-sufficient conditions, the variation in growth efficiency implies that there exists a discrepancy between the rate of ATP production by catabolism and the rate of ATP utilization by anabolism for growth purposes.

Consumption of ATP by bacteria is not directly related to growth and maintenance functions. Generally, such a phenomenon is referred to as energy uncoupling. In their review of the energetics of bacterial growth, Russell and Cook (1995) noted that "when bacteria are limited for energy sources, the free energy change of catabolic reactions is generally tightly coupled to the anabolic steps of cellular biosynthesis and total energy flux can be partitioned into growth and maintenance functions. If growth is limited by nutrients other than energy, however, bacteria can spill ATP in reactions that cannot be readily categorized as maintenance *per se*".

Within the scope of our review, there is still lack of a realistic assessment of the energy uncoupling because the P/O ratio and ATP production yield cannot be directly and simultaneously measured (Rao and Gaudy 1966; Stouthamer 1979). Under substrate-limited conditions, anabolism for growth tightly matches catabolism for energy generation; that is, the energy uncoupling is negligible (Forrest 1969; Stouthamer 1977, 1979). As Eq. 12 shows, under substrate-sufficient conditions, more substrate is required to obtain the same amount of energy production for microbial growth with than in substrate-limited cultures. This implies that ATP formed by catabolism is not used entirely in the formation of biomass.

Westerhoff et al. (1982) applied the principals of nonequilibrium thermodynamics to the study of bacterial growth. They found that microbial growth yields were 50% less than the theoretical values, and anabolism was incompletely coupled to catabolism. In this case, Liu and Chen (1997) considered that the difference between the observed growth yields under substrate-limited and substrate-sufficient conditions reflects the degree of uncoupling degree between catabolism and anabolism. They introduced a new parameter, the so-called energy-uncoupling coefficient, to describe the observed uncoupling between anabolism and catabolism under substrate-sufficient conditions. The energy-uncoupling coefficient was defined as:

$$E_u = \frac{(Y_{obs})_{max} - Y_{obs}}{(Y_{obs})_{max}} \tag{16}$$

where E_u is the energy-uncoupling coefficient. This parameter features reduction in the efficiency of converting

energy into cellular biosynthesis under substrate-sufficient conditions. For substrate-limited cultures, Y_{obs} would be close to $(Y_{obs})_{max}$ and the energy uncoupling minor.

After determination of $(Y_{obs})_{max}$ from Eq. 12, using a series of Y_{obs} and c_s data, the energy-uncoupling coefficient can be calculated from Eq. 16 for each corresponding residual substrate concentration. Substituting Eq. 12 into Eq. 16 produces the following substrate-dependent expression for the energy-uncoupling coefficient:

$$E_u = E_{u,max} \frac{c_s}{c_s + K_y^*} \tag{17}$$

where

$$E_{u,max} = \frac{(Y_{obs})_{max}}{(Y_{obs})_{max} + (Y_w)_{min}} \tag{18}$$

$$K_y^* = \frac{(Y_w)_{min}}{(Y_{obs})_{max} + (Y_w)_{min}} K_s^* \tag{19}$$

$E_{u,max}$ is the maximum energy-uncoupling coefficient, and K_y^* is the yield-related saturation constant. These two parameters can be determined from Eqs. 18 and 19 respectively. Equation 17 shows that the energy uncoupling is a function of the residual substrate concentration under substrate-sufficient conditions, and approaches a maximum as the residual substrate concentration is much higher than K_y^* value. It should be realized that Eqs. 12 and 17 provide quantitative information on the energetic metabolism of bacterial growth for the first time.

Model test

In order to test the proposed Y_{obs} and E_u models, the data published by Brooke et al. (1990) were used. These data were obtained during the growth of thermotolerant methylotrophic *Bacillus* strains in methanol-sufficient chemostat cultures. Figures 1 and 2 show a comparison between the observed growth yields with values computed using Eq. 12. The Y_{obs} model describes the experimental data very well. This, in turn, provides strong support for Eq. 12. With the values of $(Y_{obs})_{max}$, $(Y_w)_{min}$ and K_s^* obtained from Eq. 12, E_u, $E_{u,max}$ and K_y^* were calculated using Eqs. 16, 18 and 19 respectively. The effect of the residual methanol concentration on the energy-uncoupling coefficient is shown in Figs. 3 and 4 for nitrogen and potassium limitation respectively. As can be seen from these figures, Eq. 17 can provide a satisfactory description for the experimental data. As the residual methanol concentration exceeds 10 mmol/l, the energy-uncoupling coefficient reaches 0.7, meaning that about 70% of substrate consumption is dissociated from anabolism for growth.

Bauchop and Elsden (1960) indicated that *Enterobacter faecalis* diverted only 4% of the energy source to cell carbon. For substrate-sufficient batch cultures, Chang et al. (1993) also reported that more than 64% of

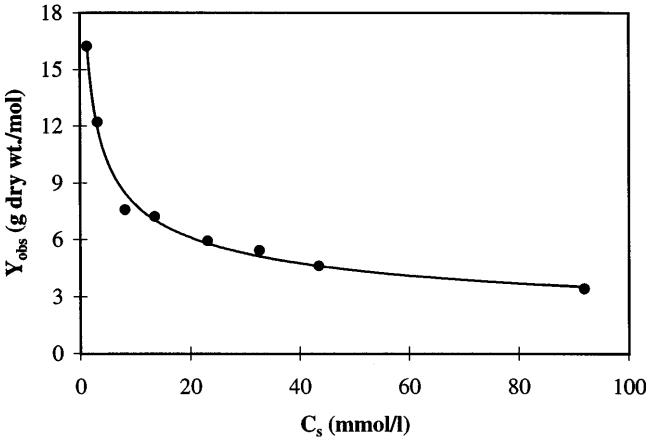

Fig. 1 Variation of Y_{obs} as a function of the residual methanol concentration in nitrogen-limited chemostat culture of *Bacillus* strain (dilution rate, $D = 0.2\,h^{-1}$) (data from Brooke et al. 1990). Equation 12 prediction is shown by *solid line*; $(Y_{obs})_{max} = 20.0$ g dry weight/mol, $(Y_w)_{min} = 4.18$ g dry weight/mol, $K_s^* = 23.6$ mmol/l

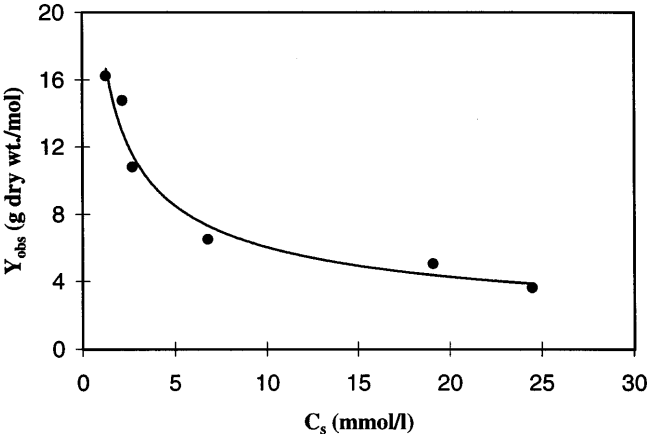

Fig. 2 Variation of Y_{obs} as a function of the residual methanol concentration in potassium-limited chemostat culture of *Bacillus* strain ($D = 0.2\,h^{-1}$) (data from Brooke et al. 1990). Equation 12 prediction is shown by *solid line*; $(Y_{obs})_{max} = 20.0$ g dry weight/mol, $(Y_w)_{min} = 2.5$ g dry weight/mol, $K_s^* = 22.4$ mmol/l

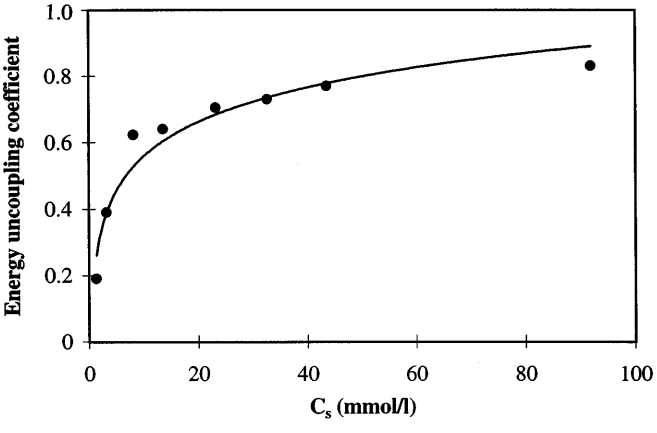

Fig. 3 Effect of residual methanol concentration on the energy-uncoupling coefficient in nitrogen-limited chemostat culture of *Bacillus* strain ($D = 0.2\,h^{-1}$). Equation 17 prediction is shown by *solid line*; $E_{u,max} = 0.83$, $K_y^* = 4.08$ mmol/l

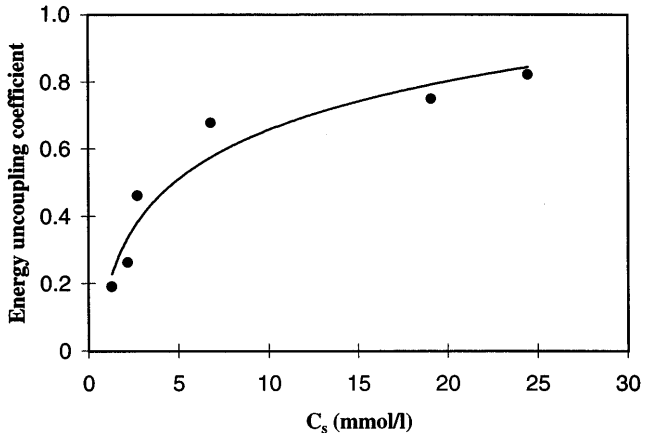

Fig. 4 Effect of residual methanol concentration on the energy-uncoupling coefficient in potassium-limited chemostat culture of *Bacillus* strain ($D = 0.2\,h^{-1}$). Equation 17 prediction is shown by *solid line*; $E_{u,max} = 0.89$, $K_y^* = 2.49$ mmol/l

the oxygen consumption did not contribute to microbial growth. A large energy dissipation was also observed in the carbon-sufficient cultures of *K. aerogenes* NCTC 418, which was not explained by the existing Pirt maintenance theory (Neijssel and Tempest 1976). We can therefore say that the proposed Y_{obs} and E_u models are capable of giving a theoretical basis for a correct and quantitative interpretation of energy uncoupling in bacterial growth under substrate-sufficient conditions.

Conclusion

A Y_{obs} model was developed for substrate-sufficient chemostat cultures. It was demonstrated that the observed growth yield is a net result of the interaction among growth, maintenance metabolism and energy losses due to energy uncoupling between anabolism and catabolism, futile cycling, modification of the respiratory chain and overflow metabolism etc. By analyzing the variation in patterns of growth yield, an energy-uncoupling coefficient was postulated for describing the dissociation of catabolism from anabolism under substrate-sufficient conditions. A substrate-dependent model of the energy uncoupling was then proposed for substrate-sufficient continuous cultures. It is clearly shown that catabolism seriously dissociates from anabolism at high residual substrate concentrations. As a result, the observed growth yield is greatly lowered. The proposed models are capable of giving a theoretical basis for a quantitative interpretation of the observed growth yield and energy uncoupling in relation to the residual substrate concentration for substrate-sufficient continuous cultures.

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