Mass and energy balance constraints on the biological production of chemicals from coal

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Several organic chemicals, including methane and ethanol, may be produced by the bioprocessing of coal. This may be done either by direct microbial attack on the coal, or indirectly by the bioprocessing of solubilized coal. As in chemical liquefaction and gasification, the relative amounts of the various products that can be produced are severely constrained by mass and energy balance considerations. The main differences in biological processing are that water is a ubiquitous reactant, carbon dioxide a common product, and that some of the carbon and nitrogen in the coal may go to the synthesis of new biomass rather than products. The conventional biotechnological yield analysis applied to coal processing has several interesting consequences. The mass balance reduces to a balance of available electrons, and coal has a similar oxidation/reduction state to both carbohydrates and biomass. This makes high product yields feasible particularly under anaerobic conditions, although leaving open the question of whether the relevant hydrolase enzymes exist. Recommendations are made on products, and combinations of two products, that may be made with high yields and economic return. The energy balance provides little extra information. A general intracellular energy balance can be written in terms of the production and consumption of ATP, but much of the necessary information on the metabolic pathways is currently not available for coal processing micro-organisms.

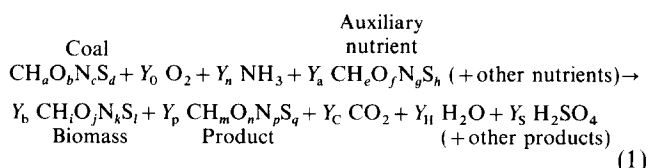(Keywords: coal; bioprocessing; chemicals)

Several proposals have been made for the bioprocessing of coal to produce useful liquid or gaseous chemicals[1]. They have included direct microbial production of methane, microbial production of chemicals from solubilized coal[2], and a chemical gasification followed by a microbial product synthesis[3]. New micro-organisms, biochemical pathways and potential products are reported at regular intervals. It is timely to ask where attention should be focused and which products and processes, if any, are commercially promising. This is a complex economic balance, involving questions of market size, process rates, product separation costs, etc. An important factor in this overall equation is the yield of the process, that is the amount of product that can be obtained from unit mass of coal. The objective of this paper is to apply the standard theory of bioprocess yields to the particular problem of predicting the possible yields from coal bioprocesses.

The standard theory[4,5] is essentially a set of mass and energy balances applied to the particular conditions of a bioprocess. It provides insight into the main factors governing the yield of a product, fixes definite upper limits on the yield, and thus allows a preliminary economic analysis that will eliminate some products from consideration and allow a more rational choice between those that remain. The theory contains little information about metabolic pathways or the internal processes of micro-organisms, and it can even be applied to hypothetical bioprocesses for which no micro-organisms have yet been isolated. Experience seems to indicate that if a reaction involving naturally occurring substances is stoichiometrically possible and thermodynamically advantageous in dilute aqueous solution then there will exist a micro-organism that will catalyse it.

THE MASS BALANCE

The simplest way to monitor the substrates and products in a fuel bioprocessing operation is to write a pseudo chemical reaction. This reaction is best written in terms of carbon equivalents, that is the amount of organic matter that contains 1 mol of carbon. Nitrogen and sulphur will be included in the formulae because they are often important in fuel bioprocessing, and are significant microbial nutrients.

$$\underset{\text{Coal}}{CH_aO_bN_cS_d} + Y_0\,O_2 + Y_n\,NH_3 + Y_a\,\underset{\text{nutrient}}{CH_eO_fN_gS_h}\;(+\text{other nutrients})\rightarrow$$

$$\underset{\text{Biomass}}{Y_b\,CH_iO_jN_kS_l} + \underset{\text{Product}}{Y_p\,CH_mO_nN_pS_q} + Y_c\,CO_2 + Y_{II}\,H_2O + Y_s\,H_2SO_4\;\underset{(+\text{other products})}{} \tag{1}$$

The Y values are yields expressed as carbon equivalents or moles of a compound produced or consumed per carbon equivalent of fuel. Converting these to a dry/mass basis requires correction for the inorganic (ash) constituents of the compound. For example

$$\text{Biomass yield from fuel (wt/wt)} = \frac{Y_b M_b(1 - r_f)}{M_f(1 - r_b)} \tag{2}$$

where M is the mass of a carbon equivalent and r is the

mass fraction of mineral matter ('ash') in the dry compound ($r \sim 0.08$ for biomass).

Note that reaction (1) shows ammonia as a nutrient and water as a product. This is not necessarily so; the metabolism of coal and the auxiliary nutrients may involve more hydrolysis than dehydration steps and more deamination than amination. Water would then be a nutrient, ammonia a product and both Y_n and Y_H, as calculated here, would be negative.

If the possible other nutrients and products are ignored, Equation (1) contains eight unknown yields, and five element balances (C, H, N, O, S) can be written for it. Using these element balance equations to eliminate Y_N, Y_C, Y_H and Y_S gives the result

$$\gamma_p Y_p + \gamma_b Y_b + 4 Y_0 = \gamma_f + \gamma_a Y_a \qquad (3)$$

This equation is essentially an oxidation/reduction balance over reaction (1). The γ coefficients represent the oxidation/reduction state of a compound, specifically the number of available electrons per carbon equivalent of each compound. Thus the definition for the fuel is

$$\gamma_f = 4 + a - 2b - 3c + 6d \qquad (4)$$

Typical values of γ for different ranks of coal, different types of (dried) biomass and several chemicals that could be produced by bioprocessing are shown in *Table 1*. This type of analysis is useful only because the γ values for biomass are surprisingly constant between species. This is confirmed by larger compilations of data on the elemental compositions of living material[6]. The data for the yeast *Candida utilis* shows that the carbon source used to grow a micro-organism also makes little difference to its elemental composition. A rapidly growing microbe has a lower value of γ (mainly due to increased RNA production) but the difference is not of major significance.

Table 1 also shows that the γ values for coal are not only fairly consistent between ranks, but also very close to the values for biomass. This may seem surprising in view of the very different elemental composition of the coal, yet it reflects the fact that coal is made from living matter. The coalification process consists mainly of natural reactions (dehydration, deamination) that do not

alter the oxidation/reduction state of the starting material.

Most of the chemicals that we want to produce by liquefaction or gasification of coal are fuels which are, by definition, reduced compounds such as methane ($\gamma = 8$), methanol ($\gamma = 6$) and others shown in *Table 1*. The oxidation/reduction state of the biomass (and coal) is seen to be more comparable to that of carbohydrate ($\gamma = 4$). Among bulk chemicals that could be produced from coal only acetic acid has a comparable γ value.

THE GENERAL PRODUCT YIELD

Equation (3) can easily be generalized for the common situation where several products are made. Each product adds an additional term to the left-hand side of the equation. The auxiliary nutrients may be specific product precursors (e.g. phenyl acetic acid in penicillin production) or less well defined compounds like yeast extract that provide a mixture of precursors for biomass growth. It is assumed that each additional nutrient is associated with the formation of a specific product, and that the ratio of nutrient consumed to product (or biomass) produced is a constant (e.g. for biomass $Y_{ab} = Y_a/Y_b$). Equation (3) becomes

$$\sum_{\text{cells and products}} Y_p(\gamma_p - \gamma_a Y_{ap}) = \gamma_f - 4Y_0 \qquad (5)$$

Note that, for the purposes of this yield analysis, biomass can be treated as just another product. In coal bioprocessing it is unlikely (on economic grounds) that chemical precursors would be added to direct the formation of specific products, so the biomass term will be the only one in the summation which involves an auxiliary nutrient. The effect of this nutrient is to reduce the amount of fuel required to make biomass, and thus to decrease the 'cells' term in the summation. In the limit where the auxiliary nutrient (yeast extract?) has the same composition as the biomass ($\gamma_a = \gamma_b$) and provides all the precursors for cell growth ($Y_{ab} = 1$) the 'cells' term is zero. This situation produces the highest possible, or 'theoretical', product yields but, since yeast extract costs $5 \, \$ \, lb^{-1}$ and coal $0.01 \, \$ \, lb^{-1}$ it is unlikely to be a commercial strategy.

The most obvious consequence of Equation (5) is that the more oxygen is consumed by a process (higher Y_0) the lower the total yield of products. The same applies to most other, externally supplied, terminal electron acceptors, (NO_3^-, SO_4^{2-}) but not to CO_2, which can be reduced to CH_4, a useful product, by methanogenic bacteria metabolizing hydrogen. A consortium of fermentative and methanogenic organisms similar to that used in anaerobic digestion could not only produce the highest yields, but also avoid the costs and problems associated with aerating a coal slurry[7].

THE SINGLE PRODUCT

The above discussion outlines an optimum commercial process. It would be based on anaerobic metabolism and make a single product, since the cost of separating multiple products can be prohibitive. The bioreactor would be continuous and contain a high concentration of biomass to offset the low specific rates of anaerobic metabolism. The biomass must be immobilized and

Table 1 Values of the γ parameter

Substance	Composition of organic fraction	γ
Coals (typical)		
Anthracite	$CH_{0.5} O_{0.03} N_{0.02} S_{0.01}$	4.44
Bituminous	$CH_{0.7} O_{0.06} N_{0.02} S_{0.01}$	4.58
Sub-bituminous	$CH_{0.8} O_{0.15} N_{0.02} S_{0.01}$	4.50
Lignite	$CH_{0.8} O_{0.22} N_{0.02} S_{0.01}$	4.36
Biomass (dried)		
Yeast (*C. utilis*)		
Glucose $\mu = 0.08 \, h^{-1}$	$CH_{1.82} O_{0.47} N_{0.19}$	4.32
$\mu = 0.45 \, h^{-1}$	$CH_{1.84} O_{0.56} N_{0.20}$	4.12
Ethanol $\mu = 0.06 \, h^{-1}$	$CH_{1.82} O_{0.46} N_{0.19}$	4.33
$\mu = 0.43 \, h^{-1}$	$CH_{1.84} O_{0.55} N_{0.20}$	4.13
Bacteria (*A. aerogenes*)	$CH_{1.78} O_{0.33} N_{0.24}$	4.40
Activated sludge	$CH_{1.4} O_{0.4} N_{0.2}$	3.99
Possible products		
Carbohydrate	CH_2O	4.0
Acetic acid	CH_2O	4.0
Ethanol	$CH_3O_{0.5}$	6.0
Octane	$CH_{2.25}$	6.25
Methane	CH_4	8.0

Table 2 Possible products from sub-bituminous coal

Product	γ_p (electrons equiv.$^{-1}$)	Y_p' (equiv. equiv.$^{-1}$)	Price (c lb^{-1})	Possible return (c lb^{-1} coal)
Propionic acid	4.67	0.96	34	43
Butanediol	5.5	0.82	16	16
Ethanol	6.0	0.75	30	28
Methane	8.0	0.56	7.3	3.5

slow-growing to maximize product yields and minimize the cost of providing auxiliary growth nutrients. The question is what product should be produced?

The first constraint is that an anaerobic process cannot produce a single product that is more oxidized than the substrate. However the values in *Table 1* show that most products of interest are fuels or chemicals with $\gamma_p > \gamma_f$, so this is not a serious restriction. The maximum possible, or 'theoretical', yield of the product is $Y_p' = \gamma_f/\gamma_p$ (Equation (5) with $Y_b = Y_0 = 0$) so it is sensible to look for a product with γ_p only slightly larger than γ_f. Some potential candidates are listed in *Table 2*. They are all known end-products of fermentative metabolism, although micro-organisms capable of producing them from coal have not necessarily been isolated. Also shown in *Table 2* are their approximate current prices. Multiplying these prices by the theoretical yields gives the maximum possible financial return per pound of coal processed. This must be considerably larger than the price of coal (approximately 1 c lb^{-1}) to make a feasible process. On this basis ethanol and propionic acid appear very promising and methane less so. However it must not be forgotten that the manufacture of methane gas does not involve the considerable costs involved in separating the other two products from the fermentation media.

TWO PRODUCTS

Most fermentative metabolic pathways produce not a single product but a mixture of two or more[5]. For two products, the 'theoretical yield' situation (Equation (5) with $Y_0 = Y_p = 0$) must be represented not by a single value but by a graph such as *Figure 1* (for CH_4 and H_2 over the entire range of γ_f values for coal) or *Figure 2* (for ethanol and acetic acid from a coal with $\gamma_f = 4.5$). These graphs are useful in several ways. They show clearly what combinations of yields are feasible (anything below the constant γ_f line) and what are ruled out by stoichiometry (anything above the line). They therefore provide a useful check on experimental data. For example the yields of methane and hydrogen from a Texas lignite ($\gamma_f = 4.52$) reported by Barik *et al.*[2] are shown in *Figure 1*. They fall comfortably within the feasible region, and it is immediately apparent from the graph how much the yields could be improved either by improving the microbial culture or by using a more reduced lignite. Note however that the yield of 511 cm^3 CH_4 g^{-1} coal ($Y_{p2} = 0.73$) claimed in the same report would be outside the feasible region, suggesting perhaps that some methane was generated from the auxiliary nutrient.

The 'feasible' region on these graphs may be further constrained by biochemical hypotheses. For example there is no known mechanism by which non-photosynthetic anaerobic bacteria can produce molecular hydrogen from water. The broken line in *Figure 1* shows

the maximum yield of H_2 that could be produced from the hydrogen in the lignite ($CH_{0.89}\ N_{0.016}\ S_{0.12}\ O_{0.20}$) and the data fall within this more constrained area.

When there are two products one of them can be more oxidized than the coal without violating the requirements of stoichiometry. This situation is illustrated for acetic acid ($\gamma_{p1} = 4$) and ethanol ($\gamma_{p2} = 6$) from a typical coal ($\gamma_f = 4.5$) in *Figure 2*. It creates the possibility of a truly optimal situation in which all the coal carbon is converted into products, with no net production of CO_2. This happens along the section of the $(Y_{p1} + Y_{p2}) = 1$ line that is in the feasible region. Compare this with the methane/hydrogen situation (*Figure 1*) where $0.55 \geqslant Y_{p2} \geqslant 0$, implying that anywhere from 45 to 100% of the coal carbon must be converted to CO_2.
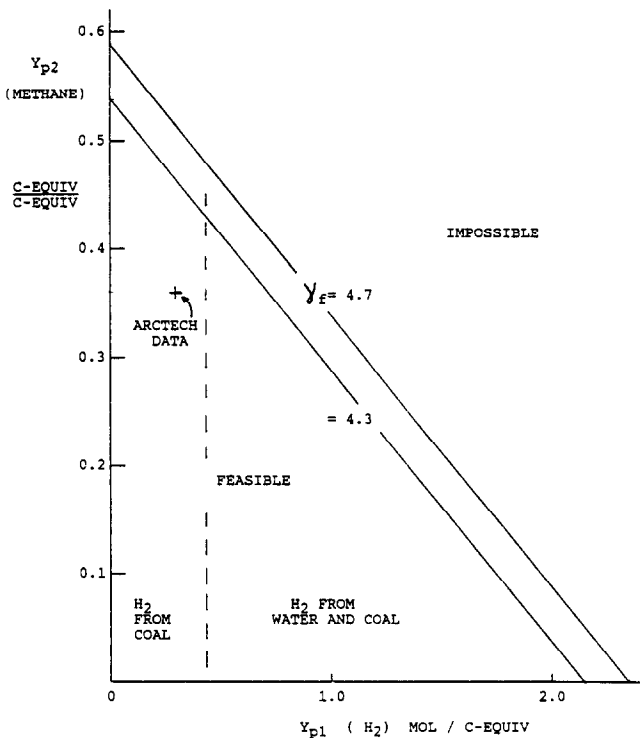

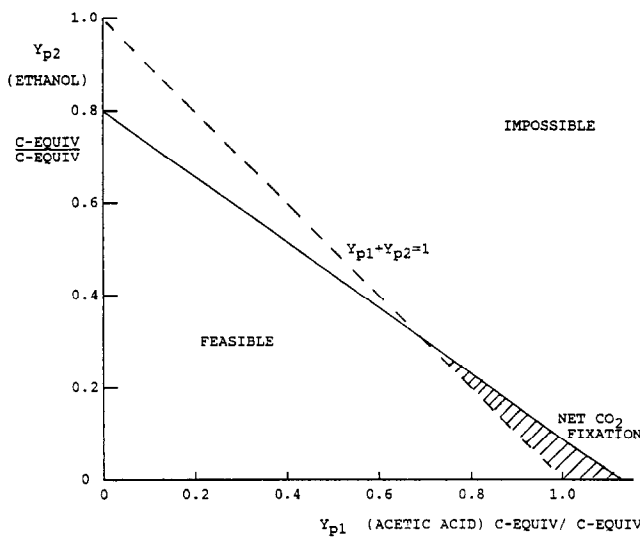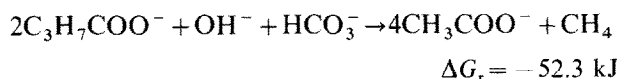**Figure 1** Theoretical yields of methane and hydrogen

Figure 2 Theoretical yields of ethanol and acetic acid from a typical coal ($\gamma_f = 4.5$)

In the area above the $(Y_{p1} + Y_{p2}) = 1$ line inside the feasible region in *Figure 2*, CO_2 is not a product but a reactant. This may seem unlikely, but is not excluded either by stoichiometry or by the energy balance which will be shown in the next section, to a first approximation, to be identical to the mass balance (greater precision requires knowledge of the free energy of formation of the coal's organic matter). Furthermore, if the reduced product is methane, there is no biochemical barrier to a net fixation of CO_2. Besides the methanogenic bacteria that produce methane from CO_2 and hydrogen, there are several examples of exergonic carboxylation reactions catalysed by the acetogenic bacteria. For example, one step in the digestion of glucose to methane is the carboxylation of butyrate[8]

$$2C_3H_7COO^- + OH^- + HCO_3^- \rightarrow 4CH_3COO^- + CH_4$$

$$\Delta G_r = -52.3 \text{ kJ}$$

Long chain organc acids are known to be a major component of solubilized coal, and it is conceivable that a carefully controlled coal bioprocess continuously provided with CO_2 could result in a series of such reactions with a net fixation of carbon and production of acetate and methane with a total carbon yield greater than unity.

THE ENERGY BALANCE

The other main constraint on the formation of products is that they do not violate the conservation of energy. The energy balance for a bioprocess can be written in several different ways. For example, an energy balance over the entire process can be written in terms of the heats of combustion (ΔH = heat of combustion per C-equivalent of the substrates and products).

$$\sum_{\text{cells and products}} Y_p(\Delta H_p + Y_{ap}\Delta H_a) = \Delta H_f - Q \qquad (7)$$

Here Q is the heat generated per C- equivalent of fuel consumed. It is a common observation that the heat of combustion of a compound is approximately proportional to its degree of reduction; i.e. $\Delta H = K\gamma$. Substituting this into Equation (7) and subtracting Equation (5) gives a standard result for metabolic heat production.

$$Q = 4KY_O \qquad (8)$$

Note that for a fermentative process ($Y_O = 0$) the approximation $\Delta H = K\gamma$ makes Equations (5) and (7) identical, and thus the energy balance would produce no extra information. The implication that no metabolic heat would be generated ($Q = 0$) in this case is not correct but results from inexactitudes in the assumption that $\Delta H = K\gamma$ (K is in the range 109–130 kJ mol^{-1} electrons depending on the compound). Heat is generated during fermentative processes, although far less than in aerobic processes.

An alternative way of writing the conservation of energy for a bioprocess is to balance the production and consumption of ATP, the cell's main energy carrier, inside the cell. The general equation is[4]

$$\sum_{\text{cells and products}} \alpha_p Y_p = \alpha_f - m \qquad (9)$$

$\alpha_f = N + \gamma_f(P/O)/2$ is the amount of ATP that would be

generated by substrate-level (first term) and oxidative (second term) phosphorylation during the complete catabolism of one C-equivalent of fuel.

$\alpha_p = (1/Y_{ATP}) + (N - N_p)/Y_c + (\gamma_p - \gamma_a Y_{ap})(P/O)/2$ is the total ATP cost to the cell of making one C-equivalent of product. The first term gives the actual consumption of ATP in the anabolic pathways. This extension of the Y_{ATP} concept from biomass to any product has been discussed by Andrews[4]. For a catabolic product Y_{ATP} is infinite by definition. The second and third terms account for the ATP and reducing power (in the form of NADH etc.) that the cell cannot produce due to the diversion of intermediates from the catabolic pathway to the anabolic reactions that form the product. The contribution of substrate-level phosphorylation (second term) is usually small enough to be ignored in respiratory processes. Adding an auxiliary nutrient that provides better precursors reduces the oxidative phosphorylation contribution (third term) by reducing the amount of catabolic intermediates that must be diverted to product formation. When the auxiliary nutrient provides all the precursors and electrons needed for product formation (or growth in the case of biomass) then this term is zero. Note that the auxiliary nutrient may also increase the value of Y_{ATP}. In the case of biomass growth for example, it is obviously easier (less energy consuming) for the cell to make new biomass from pre-formed nucleotides and amino acids than if it must synthesize these compounds from intermediates in the catabolic pathway[9].

Equation (9) is general and it can usually be greatly simplified, for example in aerobic processes (substrate-level phosphorylation negligible), fermentative processes ($P/O = 0$) or in cases where no metabolic products are produced. It can be very useful for processes involving well-studied metabolic pathways giving, for example, quite accurate predictions for aerobic cell yield on carbohydrates ($\gamma_f = 4$) using the Embden–Meyerhof pathway ($N = 1/3$), the common cytochrome chain ($P/O \sim 2.5$) and the usual estimate of $Y_{ATP} \sim 10$ g mol^{-1}. Unfortunately, in coal bioprocessing neither the substrate nor the metabolic pathways leading to the products of interest are well characterized. Values of the energy parameters N, (P/O), etc., are not known, so application of Equation (9) would be premature. Research in this area should be encouraged.

CONCLUSIONS

The amounts and types of products that can be produced by any type of coal bioprocessing are constrained by the requirements of stoichiometry and energy conservation. The critical parameter is the number of available electrons per carbon equivalent in the organic fraction of the coal. This value shows no systematic variation with coal rank, but is always close to the value for carbohydrate and biomass ($\gamma \sim 4$). This reflects its origin as living matter and implies that, in this respect at least, coal is a reasonable substrate for biological activity.

Anaerobic (fermentative) processes will give higher product yields than processes based on respiratory metabolic pathways where an external electron acceptor is provided. The only possible exception is methanogenic metabolism in which CO_2 acts as the electron acceptor, being reduced to CH_4. With certain combinations of

products from coal, it may be possble to use these organisms to 'fix' externally supplied CO_2. For fermentative metabolism, the maximum 'theoretical' yields of various combinations of products can be calculated directly from the mass balances. These provide an excellent yardstick with which to judge experimental data.

In order to obtain useful extra information from the energy balance equation several metabolic parameters, including the production of ATP by substrate-level and oxidative phosphorylation, must be known. This requires further study of coal bioprocessing organisms.

ACKNOWLEDGEMENTS

REFERENCES

1 Srivastava, R. D., Campbell, I. M. and Blaustein, B. D. *Chem. Eng. Prog.* 1989, **85**, 45
2 Barik, S., Wyza, R. and Isbister, J. D. in 'Proceedings of the Symposium on the Biological Processing of Coal and Coal-Derived Substances', EPRI no. ER6572, 1989
3 Vega, J. L., Elmore, B. B., Ackerson, M. D., Clausen, E. C. and Gaddy, J. L. in 'Proceedings of the Symposium on the Biological Processing of Coal and Coal-Derived Substances', EPRI no. ER6572, 1989, pp. 3–69
4 Andrews, G. F. *Biotechnol. Bioeng.* 1988, **33**, 256
5 Papoutsakis, E. T. and Meyer, C. L. *Biotechnol. Bioeng.* 1985, **27**, 67
6 Atkinson, B. and Mavituna, F. 'Biotechnology and Biochemical Engineering Handbook', Nature Press, 1983
7 Andrews, G. F. and Quintana, J. 'Mixing and Mass Transfer in the Aerated Trough Bioreactor', 1st International Symposium on the Biological Processing of Coal, Orlando, FL, May 1990
8 McCarty, P. L. in 'Water Pollution Microbiology' (Ed. R. Mitchell), Wiley, 1972, p. 91
9 Forrest, W. W. and Walker, D. J. *Adv. Microbial. Physiol.* 1971, **5**, 213

NOMENCLATURE

ΔH	Heat of combustion per C-equivalent
M	Weight of a C-equivalent
m	Maintenance requirement for ATP
N	Moles ATP produced by substrate level phosphorylation per C-equivalent of compound
(P/O)	Oxidative phosphorylation ratio
Q	Metabolic heat release per C-equivalent of fuel
r	Mass fraction of mineral matter
Y	Yield; moles or C-equivalent of compound per C-equivalent of fuel
α	Total moles ATP involved in breakdown or production of a C-equivalent of compound
γ	Available electrons per C-equivalent of compound

Subscripts

a	Auxiliary nutrient
b	Biomass
f	Fuel
o	Oxygen
p	Product