Appl Microbiol Biotechnol (1987) 25:305–312

Applied and Microbiology Biotechnology

© Springer-Verlag 1987

Mini-review

The relationship between elemental composition and heat of combustion of microbial biomass

Jean-Louis Cordier*, Bertram M. Butsch, Bernard Birou, and Urs von Stockar

Institute of Chemical Engineering, Swiss Federal Institute of Technology, CH-1015 Lausanne, Switzerland

Summary. Four models taken from the literature, which permit calculation of heats of combustion from elemental analysis, are evaluated from a theoretical point of view. In order to obtain experimental values of heats of combustion with a higher degree of accuracy than those being available in the literature, an improved sample preparation technique based on lyophilisation of microbial biomass has been developed. Heats of combustion were determined by direct measurement in a calorimeter and compared to calculated values from each of the literature models. Giese's formula turned out to predict heats of combustion the most accurately. The enthalpy content of the bacteria investigated (23.13 ± 0.52 kJ/g) differs from that of yeasts (21.21 ± 0.47 kJ/g) in a significant manner.

Introduction

When setting up enthalpy balances around industrial fermentations for process calculations, data on the enthalpy content of all involved major substrates and products are needed. While this presents no problem for chemical compounds such as carbon and energy substrates or major fermentation products, it appears difficult to estimate the enthalpic content of dried microbial biomass from the literature due to an enormous scatter of the reported values which may differ by factors sometimes as much as 1.5 (Sedlaczek 1964; Prochazka et al. 1970; Prochazka et al. 1973; Belaich 1980; Ho and Payne 1979; Ishikawa et al. 1981; Ishikawa and Shoda 1983). Alternatively, the enthalpy content of dried biomass may be determined experimentally by burning samples in a calorimeter (Sunner and Mansson 1979), but the measurements are tedious and time consuming.

Because of these difficulties, an indirect determination of the heat of combustion based on elemental analysis, which is less time consuming to determine, has often been preferred (Luong and Volesky 1983). Several models are available from the literature that relate the heat of combustion of organic matter to its elemental composition.

In this paper four of these models are reviewed and compared with each other from a theoretical point of view. In order to submit these to an experimental test, the elemental composition of some microbial strains (*Escherichia coli, Methylotrophus methylophilus,* and *Kluyveromyces fragilis*) were determined and used to compute heats of combustion according to each model. The predictions of the four models are compared to the heats of combustion determined experimentally in a bomb calorimeter.

Since it is believed that the enormous scatter of microbial enthalpy contents given in the literature is due to unsuitable preparation of the dry biomass samples, development of a new, more reliable procedure for sample preparation was a prerequisite for the present work. Most importantly, the procedure involves lyophilisation of cells rather than drying at elevated temperature as suggested by Battley and DiBiase (1980).

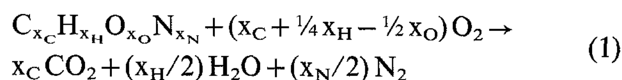
Review and comparison of models relating heats of combustion to elemental composition

The energy content of dry biomass ultimately depends on cellular composition which grossly can

* *Present address:* Nestec Ltd., Central Quality Assurance Laboratory, CH-1800 Vevey, Switzerland

Offprint requests to: U. von Stockar

be characterized by elemental analysis. Thornton (1917) was the first to show that the molar heat of combustion of many organic compounds is directly proportional to the number of oxygen atoms consumed during combustion. Somewhat later, Kharasch and Sher (1925) from the National Bureau of Standards proposed a similar concept; they correlated the enthalpy content of organic matter to the number of electrons transferred to oxygen, rather than to the number of oxygen atoms. In his extensive collection of experimental work, Kharasch (1929) demonstrated that the molar heats of combustion of a large number of organic compounds can be predicted within narrow limits by multiplying the number of equivalents of electrons transferred by 108.99 kJ. In reviewing both Thornton's (1917) work and the concept of "available" electrons that can be transferred to oxygen, Patel and Erickson (1981) used the "reductance degree" γ to calculate the number of available electron equivalents. Based on the following stoichiometry to describe the combustion of any organic compound



$$C_{x_C}H_{x_H}O_{x_O}N_{x_N} + (x_C + \tfrac{1}{4}x_H - \tfrac{1}{2}x_O)\,O_2 \rightarrow x_C\,CO_2 + (x_H/2)\,H_2O + (x_N/2)\,N_2 \tag{1}$$

the reductance degree γ is defined as the number of electrons transferred to oxygen per atom of carbon:

$$\gamma = \frac{4(x_C + \tfrac{1}{4}x_H - \tfrac{1}{2}x_O)}{x_C} \tag{2}$$

Thus

$$\gamma = 4 + x_H/x_C - 2\,x_O/x_C, \tag{3}$$

where x_C, x_H, x_O, and x_N are the stoichiometric indices of C, H, O, and N in the chemical formula, respectively. The molar heat of combustion thus becomes

$$\Delta H'_0 = Q\gamma x_C, \tag{4}$$

where Q is the heat evolved per number of available electron equivalents transferred to one gram atom of oxygen during combustion. Patel and Erickson (1981) assigned a value of 110.88 kJ/mol to the proportionality constant Q. They showed that the resulting expression predicts satisfactorily the enthalpy content of many organic compounds and thus could also be used to predict with a high degree of accuracy the heat of combustion of various types of ill-defined organic mixtures, including biomass. The authors named the corresponding estimation method after Thornton, the original inventor of this concept.

In his book "Energetics and Kinetics in Biotechnology", Roels (1983) undertakes another analysis of the proportionality of the heat of combustion of organic matter and the reductance degree γ. He confirms equation (4) implicitely, but based on a statistical analysis of only about 50 important chemical and biochemical compounds, he assigns a value of 115 kJ/mol to the coefficient Q.

Giese (1968) proposed a similar procedure for estimating the energy content of microbial biomass based on what he called the "reduction level" RL:

$$\Delta H'_0 = 4\,Q \cdot RL \cdot x_C, \tag{5}$$

where Q = 115.06 kJ/mol and where

$$RL = \frac{2\,x_C + 0.5\,x_H - x_O}{2\,x_C} \tag{6}$$

Giese's (1968) reduction level is thus a fourth of the reductance degree as defined by equation (3) and indicates the number of molecules of oxygen taken up per carbon atom during combustion according to equation (1).

The calculation procedure proposed by Ho and Payne (1979) is also based on the oxygen consumption during combustion as obtained from the stoichiometric equation (equation 1). Their model is modified by a correction factor which they justified at some length, but a formal analysis of their calculation procedure yields a very simple formula:

$$\Delta H_0 = 44.852 \cdot f_{CT}, \tag{7}$$

where f_{CT} is the mass fraction of carbon expressed on a basis free of ash and residual water. Indeed, the contents of the other elements do not need to be determined since they cancel each other.

Heats of combustion can also be estimated using a formula named after the French physicist Pierre Louis Dulong (Jarrier 1929), which originally was developed in order to estimate the energy content of fuels (Selving and Gibson 1945):

$$\Delta H_0 = 33.76\,f_{CT} + 144.05\,(f_{HT} - f_{OT}/8), \tag{8}$$

where f_{CT}, f_{HT}, and f_{OT} denote the mass-fractions of C, H, and O, respectively, in the ash-free sam-

ple. The coefficients in equation (8) are derived from the enthalpy contents of carbon and hydrogen in their elemental states (Luong and Volesky 1983).

All of these models may be used to predict heats of combustion of dried biomass from an elemental analysis. While equations (7) and (8) directly indicate the enthalpy content in kJ/g of ash free dry matter, use of equations (4) and (5) requires knowledge of the "chemical formula" of dry biomass for evaluation of the reductance degree. The chemical formula is usually computed from elemental analysis as follows:

$$x_C = f_{CT}/12 \quad (9.1)$$

$$x_H = f_{HT} \quad (9.2)$$

$$x_N = f_{NT}/14 \quad (9.3)$$

$$x_O = f_{OT}/16 \quad (9.4)$$

This procedure implicitly defines the formula mass to be 1 g. When, therefore, equations (4) and (5) are used in conjunction with equations (9) in order to estimate the enthalpy content of biomass, the results will be expressed in kJ per gram of dry, ash-free matter. Another common practice consists of reducing the chemical formula of biomass to represent a "C-mol" by setting $x_C = 1$. Equations (4) and (5) then simplify to

$$\Delta H'_0 = Q\gamma \quad (10)$$

or

$$\Delta H_0 = \frac{Q}{M'_x} \cdot \gamma, \quad (11)$$

where M'_x means the molar mass of 1 C-mol, which is given by the expression $12/f_{CT}$.

It is evident that all models may be written in a form similar to that of equation (4) by substituting the mass fractions in equations (7) and (8) by means of equations (9). In this case, another reductance degree γ^* has to be introduced, which must be defined separately for each model (see Table 1):

$$\Delta H'_0 = Q \cdot \gamma^* \cdot x_C \quad (12)$$

For pure organic compounds with a defined chemical formula, equation (12) yields the molar heat of combustion. For ill-defined organic mixtures such as dry biomass, the heat of combustion is obtained in kJ per gram, provided x_C is determined using equation (9.1).

Table 1. Comparison between the constants Q and γ^* needed to calculate the heat of combustion after Thornton, Giese, Roels, Ho and Payne, and Dulong, where $\Delta H'_0 = Q \cdot \gamma^* \cdot x_C$

Model	Q [kJ/mol]	γ^* [−]
Thornton (1917)	110.88	$4 + x_H/x_C - 2x_O/x_C$
Giese (1968)	115.06	$4 + x_H/x_C - 2x_O/x_C$
Roels (1983)	115.00	$4 + x_H/x_C - 2x_O/x_C$
Ho and Payne (1979)	112.13	4.80
Dulong (Luong and Volesky 1983)	101.28	$4 + 1.4223 \cdot (x_H/x_C - 2x_O/x_C)$

As can seen from Table 1, the models of Thornton (Patel and Erickson 1981), Roels (1983), and Giese (1968) are formally identical, differing only by the value assumed for the proportionality constant Q. Moreover, the models of Giese and Roels yield virtually the same numerical results. The model of Ho and Payne (1979) assumes a constant reductance degree of 4.80, if the value proposed by the authors is used for Q. Finally, the model of Dulong (Luong and Volesky 1983) uses a definition of γ^* giving a slightly different weight to x_H and x_O than would be derived from stoichiometry.

Materials and methods

Chemicals. Reagents were bought from Fluka (Buchs SG, Switzerland) or from Merck (Darmstadt, FRG), yeast extract from Difco Laboratories (Detroit, Mich., USA), O_2 (99.998%) from Carbagas (Lausanne, Switzerland), benzoic acid standard from the National Bureau of Standards (Washington, D.C., USA), and standard acetanilide from Perkin Elmer Corp. (Norwalk, Conn., USA).

Strains. Bacillus thuringiensis var. *kurstaki* LBGB 4432 was received from Dr. Lüthi, Swiss Federal Institute of Technology, Department of Microbiology, Zürich, Switzerland. *Escherichia coli* W (ATCC 9637) was obtained from the department of Microbiology of the University of Sheffield, Sheffield, England. *Enterobacter cloacae* was isolated in our laboratory. *Methylophilus methylotrophus* AS 1 was obtained from ICI, Billingham, England. The following yeasts were received from the Northern Regional Research Laboratory (NRRL), ARS Culture Collection, U.S. Dept. of Agriculture, Ill. (USA): *Candida boidinii* NRRL 2332, *Candida lipolytica* NRRL 1094, and *Kluyveromyces fragilis* NRRL 1109.

Growth of microorganisms. The organisms were grown in fermenters of 2 or 5 l (Bioengineering, Wald ZH, Switzerland), mostly as batch cultures in synthetic or semisynthetic media. Indications are given in gram per liter. *E. coli* and *Eb. cloacae* were cultivated in the medium as described by Davis and Mingioli (1950); instead of glucose 15, glycerol 10 was employed in some cases. *B. thuringiensis* was cultivated as described by Yousten and Rogoff (1969). *M. methylotrophus* was grown in a medium containing CH_3OH 4, NaH_2PO_4 1.56, $(NH_4)_2SO_4$ 1.8,

$MgSO_4 \cdot 7H_2O$ 0.2, $FeCl_3 \cdot 6H_2O$ 0.001, H_2O to 1000 ml, and 1 ml of trace element solution containing $FeSO_4 \cdot 7H_2O$ 0.5, $CuSO_4 \cdot 5H_2O$ 0.1, $MgSO_4 \cdot 7H_2O$ 0.05, H_3BO_3 0.007, H_2SO_4 conc. 0.2 ml, $CaCl_2 \cdot 2H_2O$ 1.32, $CaCl_2$ 0.01, Na_2MoO_4 0.01, and $ZnSO_4 \cdot 7H_2O$ 0.05, and H_2O to 1000 ml. After autoclaving the pH was adjusted to pH 7 and maintained during growth by means of 4 N NaOH. The following medium was used to grow yeasts as batch cultures: $(NH_4)_2SO_4$ 2, $(NH_4)_2HPO_4$ 0.64, KCl 0.29, $MgSO_4 \cdot 7H_2O$ 0.25, $CaCl_2 \cdot 2H_2O$ 0.1, yeast extract 2, deionized water to 1000 ml, and 2 ml of trace element and vitamin solution containing $CuSO_4 \cdot 5H_2O$ 0.068, $FeCl_3 \cdot 6H_2O$ 0.48, $ZnSO_4 \cdot 7H_2O$ 0.3, $MnSO_4 \cdot 2H_2O$ 0.35, biotin 0.001, mesoinosit 2, Ca-pantothenate 1, pyridoxine · HCl 0.05, thiamine · HCl 0.2, HCl conc. 3—4 drops, and H_2O to 100 ml. After autoclaving, the pH was adjusted to 5.5 by means of NaOH. The medium was supplemented with glucose · H_2O 11, lactose 15, galactose 15, ethanol 10, or methanol 4. For the cultivation of *K. fragilis* in the chemostat the following medium was used: $(NH_4)_2HPO$ 2.5, $(NH_4)_2SO_4$ 2.5, $MgSO_4 \cdot 7H_2O$ 2.5, $CaCl_2 \cdot H_2O$ 2.5, yeast extract 4.0, glucose · H_2O 44, and 2.5 ml of trace and vitamin solution containing H_3BO_3 1.0, $ZnSO_4$ 1.0, $MnCl_2$ 1.0, $FeCl_3$ 0.5, $CuSO_4$ 0.1, KI 0.1, biotin 0.007, meso-inositol 25, 3-aminopropionic acid 2.5, folic acid 0.002, niacinic acid 0.4, 4-aminobenzoic acid 0.2, pyridoxine · HCl 0.4, riboflavin 0.2, thiamin · HCl 0.4, and H_2O to 1000 ml. The pH was adjusted to 4. Solutions containing trace elements or vitamins had been sterilized by filtration.

Preparation of cells. To prevent lysis, cells were harvested while still in the exponential phase in a vessel cooled by ice water under vigorous stirring. Nevertheless, in some cases a thin layer of brown debris could be seen at the top of the sediment after centrifugation (15 min at 4620 *g*) and was, if present, removed carefully. Washing was performed three times with ice-cold water. The cell paste obtained was resuspended in some water, poured into Petri dishes as thin layers, frozen over night at −20° C, and lyophilised for 24 h in a Minilyo II apparatus (Dr. Morand SA, Aclens-Lausanne, Switzerland). The cells were then pestled and the powder was filled into penicillium flasks, again lyophilised for 6 hours and then sealed under vacuum. Tablets of samples (see below) took up considerable amounts of moisture when taken out of the desiccator and allowed to equilibrate with the surrounding for 15 to 30 min. To correct for this residual water, samples of cells ranging from 0.1 to 0.7 g were weighed before and after drying at 105° C for 24 hours. To determine the ash content, these cells were burnt to constant weight in a Bunsen flame in quantities as indicated above.

Determination of heat of combustion of the cells. An adiabatic calorimeter of type IKA C400 (Janke & Kunkel KG, Staufen i. Br., FRG) placed in a temperature controlled room was used for the determinations of heats of combustion. A sample of about 1 g was first compressed together with the ignition wire (made of pure iron; 0.12 mm in diameter; 0.0063 kJ/cm; received from the manufacturer of the calorimeter) and precisely weighed afterwards. After connecting its two ends to the two electrical contacts inside the bomb, it was closed and filled with 12 g of O_2 (36 atm) and placed inside the calorimeter. After equilibration and recording of the initial temperature combustion was initiated. The heat capacity of the calorimeter (C) was determined according to the following formula (DIN 51900) using benzoic acid (Merck no. 134) as a standard

$$C = \frac{\Delta H_{0B} \cdot m_B + Q_w}{\Delta t}, \tag{13}$$

where ΔH_{0B}, m_B, Q_w, and Δt are the heat of combustion of benzoic acid (26.473 kJ/g at 20° C according to DIN 51900), its mass, the heat evolved due to burning of the wire, and the difference of temperature observed before and after combustion, respectively. C values obtained by means of Merck's standard were equal when compared to the standard of the National Bureau. Under the operating conditions employed, C was found to be 9.739 ± 0.010 kJ/° K (standard deviation; $n = 20$). The heats of combustion ΔH_0 of biomass samples (m representing biomass free of ash and residual water) could then be calculated as

$$\Delta H_0 = \frac{(C \cdot \Delta t) - (Q_{NO} + Q_S + Q_w)}{m}, \tag{14}$$

where Q_{NO} and Q_S mean heats of formation of, respectively, the HNO_3 and the H_2SO_4 evolved during combustion. The amounts of these acids were determined by titration as described elsewhere (DIN 51900).

Elemental analysis. The elemental analyser (Perkin-Elmer, model 240B, Perkin-Elmer Corp., Norwalk, Conn., USA) was calibrated by means of acetanilide. 1—2 mg of sample (crystals of standard or cell powder) were inserted into the oven and burnt. Then a stream of O_2 carried the products of combustion through a bed of catalysts designed to oxidize them completely and to remove sulphur, phosphorus, and halogens (Culmo 1969). In the reduction column, nitrogenous oxides were transformed into N_2, and oxygen was thereby completely removed. The products thus formed (CO_2, H_2O, and N_2) were flushed into a cell by helium at ambient temperature and pressure. The gas mixture was analysed by thermal conductivity detectors to determine the amounts of C, H, and N in g per g of sample. The analyses with respect to H were then corrected for the contribution of residual water as follows:

$$f_H = f_{H\,app} - \frac{2}{18} \cdot f_{H_2O}, \tag{15}$$

where f_H, $f_{H\,app}$, and f_{H_2O} are the true content of H, the content of H as indicated by the elemental analyser, and the content of residual water, respectively. The fraction of O (f_O) was computed as follows:

$$f_O = 1 - (f_C + f_H + f_N + f_{ash} + f_{H_2O}), \tag{16}$$

where f_C, f_H, f_N, f_{ash}, and f_{H_2O} are the contents of C, H, N, ash, and residual water, respectively.

Results and discussion

The heats of combustion determined for various microorganisms on various substrates are listed in Table 2. Since the standard deviation never exceeded 1.3%, the results may be regarded as highly reproducible. It is noteworthy that the enthalpies of formation of HNO_3 and H_2SO_4 contributed only little to the heats of combustion: 0.055 ± 0.013 and 0.049 ± 0.038 kJ/g, respectively, and in sum they never exceeded 0.290 kJ/g; the ferric wire gave rise to 0.127 ± 0.090 kJ/g ($n = 97$).

Table 2. Heats of combustion of some bacteria and yeasts on various substrates

Strain	Substrate	n	ΔH_0 [kJ/g]
B. thuringiensis	glucose	4	22.08 ± 0.03
Eb. cloacae	glucose	6	23.22 ± 0.14
	glycerol	6	23.39 ± 0.12
E. coli	glucose	4	23.04 ± 0.06
	glycerol	6	22.83 ± 0.07
M. methylotrophus	methanol	6	23.82 ± 0.06
C. boidinii	glucose	6	20.41 ± 0.18
	ethanol	4	20.40 ± 0.14
	methanol	5	21.52 ± 0.09
C. lipolytica	glucose	7	21.34 ± 0.16
K. fragilis	lactose	6	21.54 ± 0.07
	galactose	6	21.78 ± 0.10
	glucose	6	21.66 ± 0.19
	glucose[a]	6	21.07 ± 0.07
		6	21.30 ± 0.10
		6	20.66 ± 0.26
		6	21.22 ± 0.14

[a] Chemostat instead of batch culture; dilution rates were 0.036, 0.061, 0.158, and 0.227 h^{-1}, respectively

The data suggest that there are specific values of the heat of combustion for the bacteria and yeasts investigated being 23.13 ± 0.52 ($n = 32$) and 21.21 ± 0.47 ($n = 64$) kJ/g, the difference being significant (t-test, $p = 0.01$). Also, if the ΔH_0 value of any bacterium is compared to that of any yeast, they always differ significantly. With one exception (*Eb. cloacae* compared to *E. coli*, both grown on glucose) the ΔH_0 values are also significantly different from one species to another within the bacteria investigated, but to a much smaller extent within the yeasts (Table 3).

Investigations of a similar kind were described by Prochazka et al. (1973), although they used species different from ours in most cases. However, as opposed to our results, they found mean values of 22.6 and 22.3 kJ/g for bacteria and yeasts, respectively, and their values varied greatly within any one group. On the other hand, they determined ΔH_0 of *Anacystis nidulans* as being 18.4 kJ/g, and of two strains of *Chlorella pyrenoidosa* being 18.2 and 19.8 kJ/g, respectively. For *Pseudomonas fluorescens* values between 20.3 and 20.7 kJ/g were reported by Mennett and Nakayama (1971). It seems that algae have lower heats of combustion than bacteria and yeasts. On the basis of these results, it appears that yeasts, bacteria, and algae are all characterized by a specific value of the heat of combustion. However, significant differences between bacteria and yeasts only emerge if the measurements are done precisely enough. Indeed, recent values for *E. coli* published by Dermoun et al. (1985) are 22.9, 22.6, and 22.6 kJ/g for succinate, acetate, and glucose as substrates, respectively; these values are close to ours, suggesting lyophilization being the method of choice.

In some cases significant differences caused by different substrates were observed (Tables 2 and 3). This agrees with the observation of Prochazka et al. (1970) who measured different mean values of ΔH_0 on complex and defined medium, respectively (22.8 and 22.1 for an unidentified bacterium, 22.7 and 19.4 for *Clostridium pasteurianum,* and 22.5 and 23.7 kJ/g for a *Pseudomonas* sp.). Besides, they reported a value of 26.7 (complex medium) for *Aerobacter aerogenes* (= *Enterobacter aerogenes*, Cowan 1974), whereas Ho and Payne (1979) found an average value of 23.2 kJ/g for the same organism (defined medium containing glucose). These differences might be due to the use of complex and defined media.

The variation of the dilution rates had no influence on the heat of combustion of *K. fragilis* (Table 2). Similar findings were reported for *Enterobacter aerogenes* by Ho and Payne (1979).

Table 3. Statistical comparisons between different yeasts (data taken from Table 2). + and − mean significant and non-significant differences, respectively (t-test, $p = 0.01$)

		C. boidinii Glucose	ethanol	methanol	*C. lipolytica* Glucose
C. lipolytica	glucose	+	+	−	
K. fragilis	lactose	+	+	−	−
	galactose	+	+	+	+
	glucose	+	+	−	+
	glucose[a]	+	+	+	+
		+	+	+	−
		−	−	+	+
		+	+	+	−

[a] see Table 2

Elemental contents of three species have been analysed (Table 4). In agreement with many authors, the nitrogen contents we found in the present study were significantly higher in the bacterial strains tested than in the fungus *K. fragilis*, whereas the oxygen contents showed a significant difference in the reverse sense. This confirms the general observation that bacteria have a higher nitrogen contents in comparison to fungi (Stokes and Gunness 1946; Reusser et al. 1958). In general N-contents of bacteria range from 11 to 14% (ash content included; Bauer and Ziv 1976; Dermoun and Belaich 1980; Mennett and Nakayama 1971; Shimizu et al. 1978; Stouthamer 1977) whereas *Saccharomyces cerevisiae* contains 6.3 to 9.0% of nitrogen (Barford and Hall 1979a, b; Dekkers et al. 1981; Harrison 1967; De Kok and Roels 1980). These differences in elemental composition clearly account for the observed variation of enthalpy contents between the two groups of organisms.

Some authors investigated the influence of the dilution rate on the elemental composition of biomass. For *Saccharomyces cerevisiae* grown in the chemostat under glucose limitation Dekkers et al. (1981) found some indications that the N-contents increase if the dilution rate is raised. In this context Herbert (1975) presents very convincing results for *Klebsiella aerogenes* and *Candida utilis*. Under carbon limitation, nitrogen contents were nearly invariable whereas under nitrogen limiting conditions, N-contents increased in parallel with the dilutions rate to reach the same value as under C limiting conditions. Similar observations were made by Esener et al. (1982) in a glycerol limited chemostat culture of *Klebsiella pneumoniae*. Although the influence of dilutions rate on elemental composition was not investigated in the present study, the insensitivity of the heat of combustion of *Kluyveromyces fragilis* to variations of dilution rate would tend to confirm the observations of Herbert (1975) under non-limiting conditions with respect to nitrogen. The influence of dilution rate under nitrogen limiting conditions on both heat of combustion and elemental composition will be elucidated by further research.

The best fit between predicted and observed values of heats of combustion (Table 5) was obtained by way of Giese's (1968) formula (Eq. 5 or 12), followed by those of Thornton (1917; equation 4 or 12) and of Ho and Payne (1979; equation 1 or 12). The model of Roels (1983) yields virtually identical numerical values as compared with Giese (1968) and has not been reported separately on Table 5.

The worst fit was obtained by Dulong's (Luong and Volesky 1983) formula (Eq. 8). Considering the fact that for the calculation according to Ho and Payne (1979) only the C-content is relevant, the agreement with the observed values is surprisingly good. Nevertheless, for obtaining precise measurements the combustion of cells remains an indispensible task, since most predicted values in Table 5 are significantly different from the observed ones.

Conclusions

Several models are available from the literature that permit to predict the heat of combustion of dry microbial biomass based on its elemental composition. A formal comparison shows that they all are very similar and can be reduced to equation (12), differing only with respect to the

Table 4. Elemental analyses (in mass %) of *E. coli, M. methylotrophus,* and *K. fragilis*

Strain	Substrate	*n*	Residual water	Ash	*n*	Elemental analysis			
						f_C	f_H	f_N	f_O
E. coli	glucose	6	0.84 ±0.05	7.53 ±0.22	2	45.84 ±0.15	6.50 ±0.01	13.39 ±0.04	25.90 ±0.19
M. methylotrophus	methanol	6	1.06 ±0.06	6.17 ±0.25	2	47.22 ±0.02	6.75 ±0.06	13.62 ±0.04	25.18 ±0.05
K. fragilis	lactose	6	1.13 ±0.21	7.22 ±0.20	5	43.66 ±0.26	6.46 ±0.06	8.08 ±0.08	33.45 ±0.37
	galactose	6	0.84 ±0.14	7.77 ±0.21	3	44.65 ±0.14	6.52 ±0.07	8.64 ±0.11	31.58 ±0.28
	glucose	6	1.08 ±0.15	6.54 ±0.14	7	45.92 ±0.36	6.69 ±0.04	8.11 ±0.08	31.66 ±0.44

Table 5. Indices of molecular formulas of species investigated and comparison between predicted heats of combustion and the observed values (taken from Table 2). In the last five columns values of ΔH_0 are given according to the models of Ho and Payne (1979), Dulong (Luong and Volesky 1983), Giese (1968), and Thornton (1917), and as determined by combustion (obs). Predicted values which do not differ significantly from the observed ones ($p=0.01$, t-test) are printed in italics

Strain	Substrate	Indices of molecular formula ($\times 100$)						ΔH_0 [kJ/g]				
		x_C	x_H	x_N	x_O	γ	M'_x	Ho	Dulong	Giese (Roels)	Thornton	obs
E. coli	glucose	4.169	7.09	1.044	1.767	4.853	23.988	22.44	22.01	*23.28*	22.43	23.04
		±0.013	±0.01	±0.003	±0.018	±0.005	±0.076	±0.07	±0.10	±0.10	±0.10	±0.06
M. methylotrophus	methanol	4.242	7.28	1.049	1.696	4.917	23.575	22.83	22.79	*24.00*	23.13	23.82
		±0.002	±0.06	±0.003	±0.018	±0.018	±0.010	±0.01	±0.12	±0.10	±0.09	±0.06
K. fragilis	lactose	3.970	7.05	0.630	2.281	4.627	25.190	*21.37*	19.66	21.13	20.37	21.54
		±0.023	±0.07	±0.006	±0.028	±0.046	±0.149	±0.13	±0.26	±0.24	±0.23	±0.07
	galactose	4.072	7.13	0.675	2.160	4.690	24.559	*21.92*	20.54	*21.97*	21.17	21.78
		±0.013	±0.08	±0.009	±0.022	±0.024	±0.079	±0.07	±0.20	±0.18	±0.17	±0.10
	glucose	4.143	7.24	0.634	2.142	4.713	24.162	22.28	21.02	22.44	*21.63*	21.66
		±0.033	±0.04	±0.006	±0.028	±0.017	±0.189	±0.17	±0.25	±0.24	±0.23	±0.19

numerical values assigned to the various coefficients in this equation (see Table 1).

Measured heats of combustion show significantly different values for most strains. More important, the heats of combustions of different groups of organisms (yeasts, bacteria, algae, etc.) seem to cluster around means that are typical for each group and significantly different from that of the other groups. More measurements are needed to establish these means, but significant differences between them only emerge if the measurements are carried out very carefully, and especially employing a rigorous sample preparation technique, e.g. based on lyophilisation. The features observed in heats of combustion are mirrored by the elemental compositions of the strains, which are somewhat less tedious to determine.

For the limited number of strains tested in this study, the formulae of Giese (1968) and Roels (1983) (see equations (9.1) and (12) and Table 1) seem to predict the measured heats of combustion most accurately, followed by those of Thornton (1917) and Ho and Payne (1979). Again this conclusion needs further experimental testing. Although the prediction of all four models may seem to lie quite close together, most of them still differ significantly from the experimental values so that the true heats of combustion can so far only be found by experimental determination in a bomb calorimeter.

Acknowledgements. We thank Prof. Dr. E. Kovats and Mrs. K. Grogg-Szabo of the Institute of Technical Chemistry, Swiss Federal Institute of Technology, Lausanne, for giving us access to the elemental analyser. Thanks are due to Prof. Dr. C. Robinson of the University of Waterloo, Canada, for reviewing the manuscript. This work was supported by the Swiss National Foundation for Scientific Research.

Nomenclature

C,	heat capacity of the calorimeter (kJ/°K)
f_{ash},	content of ash (g/g)
f_C,	content of carbon as measured by the elemental analyser (g/g)
f_{CT},	content of carbon corrected for ash and residual water (g/g)
f_H,	content of hydrogen corrected for residual water (g/g)
$f_{H\,app}$,	content of hydrogen as measured by the elemental analyser (g/g)
f_{H_2O},	content of residual water (g/g)
f_{HT},	content of hydrogen corrected for ash and residual water (g/g)
f_N,	content of nitrogen as measured by the elemental analyser (g/g)
f_{NT},	content of nitrogen corrected for ash and residual water (g/g)
f_O,	calculated content of oxygen (g/g)
f_{OT},	content of oxygen corrected for ash and residual water (g/g)
m,	mass of cells corrected for ash and residual water (g)
m_B,	mass of benzoic acid (g)
n,	number of separate samples tested (–)
M'_x,	molar mass (g/C-mol)
Q,	heat evolved per number of available electron equivalents transferred to one gram atom of oxygen during combustion (kJ/mol)
Q_{NO},	heat of formation of HNO_3 (kJ)
Q_S,	heat of formation of H_2SO_4 (kJ)
Q_w,	heat of combustion of the iron wire (kJ)
x_i,	i = C, H, O, N, stoichiometric index (–)

γ, degree of reductance (–)
γ^*, general degree of reductance (–)
ΔH_0, heat of combustion of cells (kJ/g)
$\Delta H'_0$, molar heat of combustion (kJ/mol, kJ/C-mol)
ΔH_{0B}, heat of combustion of benzoic acid (kJ/g)
Δt, difference of temperature before and after combustion (°C)

Subscripts: C, carbon; H, hydrogen; N, nitrogen; O, oxygen

References

Barford JP, Hall RJ (1979a) An examination of the crabtree effect in *Saccharomyces cerevisiae:* the role of respiratory adaptation. J Gen Microbiol 114:267–275

Barford JP, Hall RJ (1979b) Investigation of the significance of a carbon and redox balance to the measurement of gaseous metabolism of *Saccharomyces cerevisiae.* Biotechnol Bioeng 21:609–626

Battley EH, DiBiase G (1980) Convenient method of preparing microbial cells for bomb calorimetry. Biotechnol Bioeng 22:2417–2418

Bauer S, Ziv E (1976) Dense growth of aerobic bacteria in a bench-scale fermentor. Biotechnol Bioeng 18:81–94

Belaich JP (1980) Growth and metabolism in bacteria. In: Beezer AE (ed) Biological Microcalorimetry. Academic Press, London, pp 1–42

Cowan ST (1974) Gram-negative facultatively anaerobic rods. In: Buchanan RE, Gibbons NE (eds) Bergey's Manual of Determinative Bacteriology, 8th ed. The Williams and Wilkins Co, Baltimore, pp 290–383

Culmo R (1969) Automatic microdetermination of carbon, hydrogen, and nitrogen: improved combustion train and handling techniques. Mikrochimica Acta 1:175–180

Davis BD, Mingioli ES (1950) Mutants of *Escherichia coli* requiring methionine or vitamin B 12. J Bacteriol 60:17–28

De Kok HE, Roels JA (1980) Method for the statistical treatment of elemental and energy balances with application to steady state continuous culture growth of *Saccharomyces cerevisiae* CBS 426 in the respiratory region. Biotechnol Bioeng 22:1097–1104

Dekkers JGJ, De Kok HE, Roels JA (1981) Energetics of *Saccharomyces cerevisiae* CBS 426: Comparison of anaerobic and aerobic glucose limitation. Biotechnol Bioeng 23:1023–1035

Dermoun Z, Belaich JP (1980) Microcalorimetric study of *Escherichia coli* aerobic growth: theoretical aspects of growth on succinic acid. J Bacteriol 143:742–746

Dermoun Z, Belaich JP (1980) Microcalorimetric study of *Escherichia coli* aerobic growth: theoretical aspects of growth on succinic acid. J Bacteriol 143:742–746

DIN 51900 (1977) Bestimmung des Brennwertes mit dem Bomben-Kalorimeter und Berechnung des Heizwertes. Deutsches Institut für Normung, Beuth Verlag, Berlin

Esener AA, Roels JA, Kossen NWF (1982) Dependence of the elemental composition of *Klebsiella pneumoniae* on the steady-state specific growth rate. Biotechnol Bioeng 24:1445–1449

Giese AC (1968) Cell Physiology, 3rd ed. WB Saunders Co, Philadelphia, p 412

Harrison JS (1967) Aspects of commercial yeast production. Process Biochemistry 2:41–45

Herbert D (1975) Stoichiometric aspects of microbial growth. In: Dean ACR (ed) Continuous culture 6: application and new fields. Ellis Horwood, Chichester, pp 1–30

Ho KP, Payne WJ (1979) Assimilation efficiency and energy contents of prototrophic bacteria. Biotechnol Bioeng 21:787–802

Ishikawa Y, Nonoyama Y, Shoda M (1981) Microcalorimetric study of *Escherichia coli* in batch culture. Biotechnol Bioeng 23:2825–2836

Ishikawa Y, Shoda M (1983) Calorimetric analysis of *Escherichia coli* in continuous culture. Biotechnol Bioeng 25:1817–1827

Jarrier P (1929) The various methods of calculating the calorific values of solid fuels. Fuel 8:486–491

Kharasch MS (1929) Heats of combustion of organic compounds. Bur Stand J Res 2:359–430

Kharasch MS, Sher B (1925) The electronic conception of valence and heats of combustion of organic compounds. J Phys Chem 29:625–658

Luong JHT, Volesky B (1983) Heat evolution during the microbial process — estimation, measurement, and applications. Adv Biochem Eng 28:1–40

Mennett RH, Nakayama TOM (1971) Influence of temperature on substrate and energy conversion in *Pseudomonas fluorescens.* Appl Microbiol 22:772–776

Patel SA, Erickson LE (1981) Estimation of heats of combustion of biomass from elemental analysis using available electron concepts. Biotechnol Bioeng 23:2051–2067

Prochazka GJ, Payne WJ, Mayberry WR (1970) Calorific content of certain bacteria and fungi. J Bacteriol 104:646–649

Prochazka GJ, Payne WJ, Mayberry WR (1973) Calorific contents of microorganisms. Biotechnol Bioeng 15:1007–1010

Reusser F, Spencer JFT, Sallans HR (1958) *Tricholoma nudum* as a source of microbiological protein. Appl Environ Microbiol 6:5–8

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier Biomedical Press, Amsterdam, 330 pp

Sedlaczek L (1964) Heat balance of growth of some bacterial cultures. Acta Microbiologica Polonica 13:101–111

Selvig WA, Gibson FH (1945) Calorific Value of Coal. In: Lowry HH (ed) Chemistry of Coal Utilization. John Wiley & Sons, Inc, New York, vol 1, pp 132–144

Shimizu T, Furuki T, Waki T, Ichikawa K (1978) Metabolic characteristic of denitrification by *Paracoccus denitrificans.* J Ferment Technol 56:207–213

Stokes JL, Gunness M (1946) The amino acid composition of microorganisms. J Bacteriol 52:195–207

Stouthamer AH (1977) Theoretical calculations on the influence of the inorganic nitrogen source on parameters for aerobic growth of microorganisms. Antonie van Leeuwenhoek 43:351–367

Sunner S, Mansson M (1979) Combustion Calorimetry, Pergamon Press, Oxford

Thornton WM (1917) The relation of oxygen to the heat of combustion of organic compounds. Philos Mag 33:196–203

Yousten AA, Rogoff MH (1969) Metabolism of *Bacillus thuringiensis* in relation to spore and crystal formation. J Bacteriol 100:1229–1236

Received May 9, 1986/Revised August 25, 1986