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Proposals for a Standardized Sample Handling Procedure for the Determination of Elemental Composition and Enthalpy of Combustion of Biological Material *

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SUMMARY

A procedure has been developed for the preparation of microbial biomass of standard, defined quality suitable for the determination of elemental composition and enthalpy of combustion. Furthermore methods for the determination of residual moisture and ash content of biomass samples have been established. The results indicate that samples should be prepared in a freeze-dried (lyophilized) state and that residual moisture content should be determined immediately prior to sample preparation for combustion calorimetry and elemental analysis. Results from such analysis should then be related to material which is first freeze- dried and subsequently oven dried (100 °C for 24 hours) as reference state.
The method outlined here for microbial biomass should prove suitable for biological samples from a wide variety of sources including both pure proteins, fats etc. as well as cells and tissues.

INTRODUCTION

Increasing interest has been shown over recent years into the development of material and energy balances describing microbial [ref. 1,2], mammalian cell [ref. 3] and tissue [ref. 4] growth and/or metabolism, particularly with respect to their use in developing control strategies for the optimization of laboratory and pilot scale processes [ref. 5,6].
In order to develop such balances it is essential to have precise and reproducible data for the elemental composition and enthalpy of combustion of the biological material. Whilst a considerable amount of data is available in the literature the methods by which the data has been obtained vary widely. Thus details concerning the growth conditions, physiological state of the cells and preparation of the material for subsequent analysis are frequently ill-defined or lacking [ref.1]. Furthermore,

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it is not always clear whether the residual water fraction of the material and ash fraction have been carefully measured and corrected for in the determination of elemental composition and enthalpy of combustion. Thus not all data has been corrected for, on an ash-free, water-free basis [ref. 1].
Recent work [ref. 4, 7,8] has shown that considerable errors may occur in the development of material and energy balances due to the absorption of atmospheric water by biological materials during sample preparation for analysis. Such errors would result in an overestimation of the hydrogen (H) and oxygen (O) content whilst affecting the actual weight of the samples.
Furthermore the nature of the reference state of the biological material, whether freeze-dried or oven dried, may also lead to errors due to degradation of organic material and loss of volatile components at elevated temperature.
It is the aim of the present paper to develop a method, primarily for microbial biomass but applicable to other biological materials, for the preparation of samples for elemental and enthalpy of combustion analysis. In addition methods and conditions for the determination of residual moisture and ash content of samples are presented.

MATERIALS

Cultures and Growth Conditions. *Debaromyces nepaliensis* (CBS 5921). *Zygosaccharomyces bailii* (NCYC 563), *Saccharomyces cerevisiae* whi 2^- (Dept. Genetics, University of Sheffield, UK) and *Lactobacillus helveticus* (G Milchforschung, Liebefeld, CH) were grown as batch cultures on a minimal salts medium with glucose (10 g l^{-1}) as limiting carbon and energy source. All cultures were grown in a 15 litre bioreactor (Model LI523, Bioengineering AG, CH) at 30 °C with carefully controlled pH (6.8) and aeration (1 litre air [litre medium]$^{-1}$ min^{-1}). *L Helveticus* was grown under anaerobic conditions.

Preparation of culture samples. All cells were harvested during the late exponential growth phase in order to avoid changes in cell composition associated with varying growth rate and the formation of secondary metabolic products. Harvesting was accomplished by centrifugation of the cells at 4000 g for 10 min (4 °C), washing the cell pellet with deionized water followed by centrifugation. The centrifugation/ washing procedure was repeated three times in order to remove all non-sedimenting solids and soluble extracellular materials which might interfere with subsequent

analytical procedures. Care must be taken that this washing procedure does not remove important soluble intracellular components.

The cell pellets after the final washing/centrifugation step were spread as a thin (2 mm) layer over the base of Petri dishes and stored at - 20 °C for 4 hours. The Petri dishes with frozen cell paste were placed in a freeze-drying cabinet with the refrigerated chamber pre-equilibrated to - 40 °C (LSL-Secfroid, Aclens, CH), and the vacuum pump initiated. Cells were freeze-dried (lyophilized) for 24 hours.

Following this initial freeze-drying step the dried cells were removed from the Petri dishes, pooled and ground manually to a fine homogeneous powder. The lyophilized cell powder was then distributed into freeze-drying flasks (20 ml) fitted with rubber septa and subjected to a second period of freeze-drying (12 h). At the end of this period the flasks were sealed under vacuum and stored at room temperature in the absence of light until required.

Dry weight determinations. The dry weight of the lyophilized cells was determined by placing a precise amount (0.5 g) into an oven (70 or 100 °C) for 12 to 72 h. The samples were removed periodically from the oven, placed in a desiccator containing phosphorus pentoxide (P_2O_5) and allowed to cool at room temperature under a water-free atmosphere, before re-weighing. The drying procedure was continued until the samples had reached constant weight.

Determination of the enthalpy of combustion of cells. Enthalpy of combustion of cells was determined by adiabatic combustion calorimetry (Model IKA C400, Janke & Kunkel KG, Staufen, FRG) at constant pressure and volume. Lyophilized cell powder (0.5 ot 1.0 g) was compressed into tablets incorporating a length of iron wire of known weight (to which a current is supplied in order to initiate the combustion) having a known enthalpy of combustion ($\Delta_c h_{wire}$ -7.087 kJ g^{-1}). Tablets were stored in a desiccator at room temperature until required.

One tablet at a time was removed from the desiccator and directly weighed or left to stand within the temperature controlled room housing the combustion calorimeter in order to equilibrate with the ambient humidity before determination of the moisture fraction. The tablet was then placed in the bomb of the combustion calorimeter together with 5 ml double distilled water and connected to the electrical contacts by means of the iron wire. The bomb was then filled to 36 atm (12 g) with pure O_2 and placed in the calorimeter containing 1874 g double distilled water and the temperature

allowed to stabilize at 25 °C. Combustion was initiated by application of a current to the ignition wires connected to the bomb and the final temperature recorded after 10 min. The bomb was then opened and the remaining iron wire collected and weighed.

The water remaining in the bomb was collected. A further small volume of double distilled water was used to wash the walls of the bomb and pooled with the former water for the determination of the HNO_3 and H_2SO_4 formed as oxidation products of the combustion process using standard methods [ref. 9].

The apparent heat capacity of the bomb (calibration constant, C) was determined using a standard compound (benzoic acid, Merk Prod. No. 134) having a known enthalpy of combustion [ref. 9].

The specific enthalpy of combustion, $\Delta_c h^o$ (kJ g^{-1} biomass in the reference state) is determined according to eqn. 1:

$$\Delta_c h^o \text{ (kJ g}^{-1}) = \frac{C \cdot \Delta T + (\Delta n_N \Delta_r H_N + \Delta n_S \Delta_r H_S + \Delta m_{wire} \Delta_c h_{wire})}{m^o} \quad \text{(eqn. 1)}$$

where : m^o is the standard (moisture-free, ash-free) mass of the biological sample used for combustion (g) : ΔT is the difference in temperature (°C) before and after combustion which yields a negative value for an exothermic reaction; Δn_S and Δn_N are the amounts of HNO_3 and H_2SO_4 formed during the combustion (g); and $\Delta_r H_N$ and $\Delta_r H_S$ are the enthalpies of reaction from HNO_3 (aq) and H_2SO_4 (aq) to N_2, H_2O, O_2 and S (kJ g^{-1}) ; Δm_{wire} and $\Delta_c h_{wire}$ are the mass (g) and specific enthalpy of combustion (kJ g^{-1}) of the iron wire eliminated in the combustion process.

A detailed explanation of the combustion calorimeter, its calibration together with the methods used for titration of the acid products of combustion and the numerical corrections for the acids formed are described in the literature [ref. 9].

Enthalpy of combustion measurements were repeated a minimum of 5 times for each biomass culture or standard sample.

Elemental analysis. The elemental composition of biomass samples was determined using a Perkin-Elmer, Model 2400 CHN analyzer (Perkin-Elmer Corp., USA). Acetanilide (Merk Prod. No. 11+) was used as a standard for calibration of the analyzer since, unlike benzoic acid, it contains nitrogen.

Lyophilized samples (1 to 3 mg) were carefully weighed in tin 'boats' and inserted into the oven of the analyzer (925 °C). The combustion products of

the analyzer were fed to a series of catalytic columns for the removal of sulphur, phosphorus and halogenic compounds. The resulting gas mixture was analysed by thermal conductivity detectors in order to determine the mass of C, H, and N which then were expressed as mass fractions, w (g g^{-1} of sample). The analyses with respect to H were then corrected for the contribution of the moisture and ash fraction, and expressed per unit standard (moisture-free, ash-free) biomass (eqn 2; compare eqn. 1 in ref. 4):

$$w_H{}^o = \frac{{}_{tot}w_H - 2/18\, w_{moisture} - {}_{ash}w_H\, w_{ash}}{1 - w_{moisture} - w_{ash}} \qquad \text{(eqn. 2)}$$

where $w_H{}^o$ is the standard hydrogen fraction (g g^{-1} m^o); $w_{moisture}$ and w_{ash} are the moisture fraction and ash fraction in the sample used for elemental analysis; ${}_{tot}w_H$ is the hydrogen fraction in biomass used for elemental analysis; 2/18 is the mass fraction in water; ${}_{ash}w_H$ is the mass fraction of hydrogen in ash.

The mass fraction of oxygen ($w_O{}^o$) was determined from eqn. 3:

$$w_O{}^o = 1 - (w_C{}^o + w_H{}^o + w_N{}^o) \qquad \text{(eqn. 3)}$$

where the standard C, H and N fractions (in parentheses) represent the mass fractions based on standard biomass and corrected for the contibution of ash (see eqn.2)

Ash determination. The ash content was determined by placing carefully weighed quantities (0.5 g) of lyophilized biomass samples in ceramic crucibles followed by incubation at 450, 600 or 750 °C for 6 hours. After incubation the samples were cooled to room temperature in a desiccator containing phosphorus pentoxide (P_2O_5) under a water-free atmosphere, and re-weighed.

In order to determine the accuracy of the determination the ash content for each sample was measured at least three times. Furthermore the elemental composition of the residue ('ash') resulting from incubation at each temperature was measured in order to determine the optimum temperature for the complete oxidation of the organic components of the samples.

Residual moisture determination. Lyophilized biomass samples contain varying amounts of water. Consequently results should be expressed on a water-free or dry weight basis.

Carefully weighed lyophilized biomass samples (0.5 g) were incubated at 100 °C for 24 - 48 hours, cooled to room temperature in a desiccator containing P_2O_5 under a water free atmosphere, and re-weighed. The actual period of incubation used was that required for achieving constant weight of the samples. Each determination was repeated a minimum of three times. The difference in mass was termed the "residual moisture content", distinct from the total residual water content which which may be higher than the determined moisture content due to crystalline water which does not evaporate at 100 °C [ref. 4] The residual moisture content, $m_{moisture}$ (g) divided by the mass of the sample is the residual moisture fraction, $w_{moisture}$ (g g^{-1}).

RESULTS AND DISCUSSION

The preparation of biological samples for elemental and combustion analysis probably represents one of the major sources of error for the development of material and energy balances. Thus it is important to develop a precise, reproducible method which can be applied to biological samples from a wide variety of sources, if data are to be compared, or control strategies developed.

The first step in the preparation of samples is the period in the growth phase or physiological state at which the cells or tissues are harvested and prepared for subsequent analysis. For microbial cultures growing in batch [ref. 10] or continuous [ref. 11] mode, it has been demonstrated that the elemental composition of the cells varies as a function of both growth rate and phase of the batch growth cycle. Furthermore, the composition of the growth medium, degree of reduction of the carbon and energy source [ref. 1,12] and the nature of the growth-limitation may all have an effect on the elemental composition and enthalpy of combustion of microbial cells [ref. 11].

Thus it is important to define the precise conditions of growth and/or physiological/metabolic state of the culture. In the work presented here all cultures were grown under batch conditions under glucose-limitation and harvested during the late exponential growth phase, where the growth rate (μ) was constant and close to the maximum specific growth rate (μ_{max}) for each organism under the conditions employed.

In the harvesting of microbial cells or other materials it is important that the cells are free from contamination by medium components which might interfere with subsequent analysis. This is particularly true where complex media containing yeast (or other) extracts, peptones, antifoam agents etc. are employed. It is usually sufficient, in cases where non-sedimenting solids are absent, to harvest cells by centrifugation followed by repeated washing of the cell paste with bi-distilled water. A washing step involving ethanol (20 - 40 %) may also be included in order to remove antifoam agents or extracellular lyophilic compounds, although the ethanol must then be completely washed away. Bi-distilled water for the washing procedure is preferred to isotonic solutions since the latter may influence ash determinations. However, care must be taken to ensure that the cells are not seriously affected by the osmotic stresses involved.

Once the cells are harvested it is important that they are conserved in a form which minimizes degradation and photo-oxidation. For this reason it is proposed that cells are lyophilized (freeze-dried) and stored in vials under vacuum. However, since it is difficult to control the amount of water remaining in lyophilized cell preparations, it is further proposed that moisture-free cell dry weight values are measured after a further drying period at 100°C which then is used as the reference state for dry biomass . In this case it is necessary to determine the residual moisture fraction of lyophilized biomass, in addition to determining the optimum temperature and duration for dry weight measurements.

Dry weight determinations are frequently measured after incubation of samples at 100 °C for 12 h . It has been suggested [ref. 8] that such an elevated temperature, whilst removing the water associated with the cells, may also result in the removal of volatile compounds and heat degradation of cellular components. In order to test this hypothesis, lyophilized cells (0.5 g) of *D. nepaliensis* NCYC 1441 and *Z. bailii* NCYC 563 were incubated at 70 °C and 100 °C until the final weight remained constant. The results (Figure 1) show that at 100 °C a constant weight was obtained after 24 hours incubation, whilst after 45 h a further weight loss was observed corresponding to degradation of cellular material as shown by elemental analysis. By contrast lyophilized samples incubated at 70 °C did not achieve constant weight even after 72 h incubation. Thus is was concluded that a temperature of 100 °C for a minimum of 24 h and maximum of 48 h, should be used for all further dry weight and residual moisture fraction determinations.

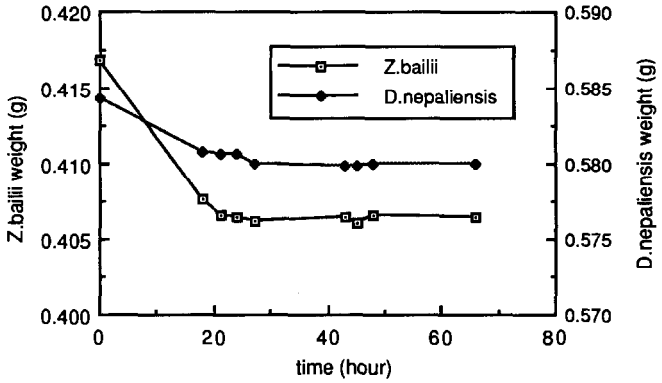

Figure 1 Measurement of the weight of *C.kefyr* and *D. nepaliensis* after incubation at 100°C, for the determination of dry weight conditions.

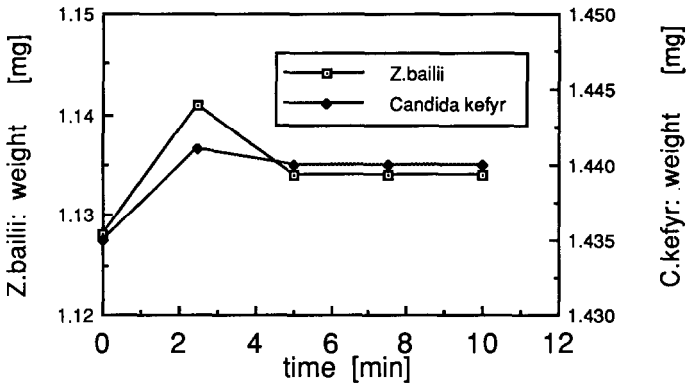

Figure 2 Absorption of atmosperic water by freeze- dried samples (dispersed powder form) of *Z. bailii* and *C. kefyr* . Temperature 27°C, Relative humidity 54%.

Lyophilized cell powder is very hygroscopic. Consequently, opening of vials for the preparation of samples for combustion and elemental analysis results in a rapid absorption of water. The amount of water absorbed is strain (and tissue [ref.4]) dependent, and varies with the temperature and humidity of the environment in which the samples are prepared. Consequently it is important to determine the residual water content under the operating conditions and to correct the combustion and elemental analysis data for this water.

In order to examine these effects lyophilized samples (1 - 3 mg) *C. kefyr* and *Z. bailii* were carefully weighed in tin boats used for elemental analysis, placed in Petri dishes and allowed to stand at the temperature and humidity of the laboratory housing the elemental analyzer. Samples were removed at intervals and re-weighed. The results (Figure 2) show that both culture samples rapidly absorbed atmospheric moisture. Within 2 min the weight of the samples had increased by 0.42 % (*C. kefyr*) and 1.15 % (*Z. bailii*). The weight then fell slightly as the humidity of the samples reached equilibrium with that of the atmosphere, resulting in an overall increase in weight of 0.35 % and 0.62 % for *C. kefyr* and *Z. bailii* respectively.When these samples were subsequently placed in a desiccator containing P_2O_5 and a water-free atmosphere, both culture samples showed a decrease in weight as water rapidly desorbed (Figure 3). Indeed after 3.5 h (*Z. bailii)* and 5.5 h (*C. kefyr*) the weight of the samples was less than the initial weight of the lyophilized samples (Figure 2). These results suggest that upon opening freeze-drying vials containing samples under vacuum, absorption of atmospheric water is so rapid that the initial weights determined in Figure 2 are already influenced by water absorption. Since the water absorbed from the atmosphere will influence the weight fraction of C, H, N and (by calculation) O determined by the elemental analyzer, it was decided that lyophilized samples used for elemental analysis should be allowed to stand at the laboratory temperature and humidity for 10 min before weighing and introduction to the analyzer. Samples treated in the same way should be used to determine the moisture content using the dry weight determination (incubation 100 °C for 24 - 48 h) as described earlier. The data from the elemental analyzer should subsequently be corrected for moisture and ash content.

Whilst samples used for elemental analysis are small (3 mg) and in dispersed powder form, the samples used for combustion analysis are larger (0.5 - 1,0 g) and compressed into a dense tablet form, consequently the

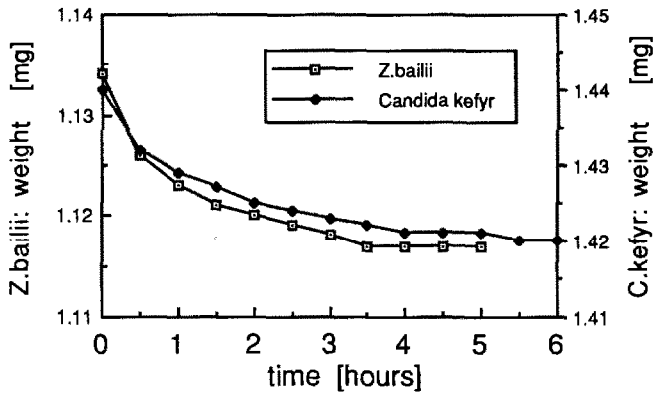

Figure 3 Desorption of water from hydrated, freeze- dried cells of *Z. bailii* and *C. kefyr* (from Figure 2) after incubation at atmospheric temperature (27°C) in a desiccator.

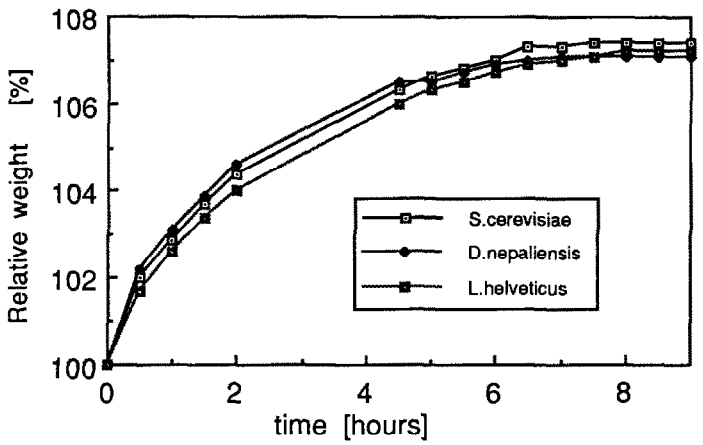

Figure 4 Absorption of atmospheric water by freeze- dried tablets (prepared for combustion calorimetry, 0.5g) of *S.cerevisiae*, *D. nepaliensis* and *L. helveticus*. Temperature, 27°C, relative humidity 55%.

uptake of atmospheric water would be expected to be different in the two cases. Consequently tablets (0.5 g lyophilized cells) were prepared and allowed to stand at the temperature and humidity of the laboratory housing the combustion calorimeter. The weight of the tablets was determined at intervals. The results (Figure 4) show that all strains tested absorbed water from the atmosphere, finally achieving a constant weight after approximately 7 h. Water uptake at equilibrium ranged from 7 % w/w for *L. helveticus* to 7.3 % for *S. Cerevisiae* whi 2⁻ and 7.1 % for *D. nepaliensis* .

When these hydrated tablets were returned to a desiccator containing P_2O_5 under a water-free atmosphere, ash tablets showed a decrease in weight over a period of 14 h followed by period (9 h) of relatively constant weight (Figure 5). This period of unchanging weight was shown to be due to the regular removal of the tablets from the desiccator for weight determinations, since a large decrease in weight was observed (Figure 5, 23 to 56 h) when tablets were not removed for a long (33 h) period. It can be concluded that during removal of the tablets from the desiccator water was absorbed from the atmosphere which approximated that lost to the desiccator during the period between weight determinations.

The final weight of the tablets stored in the desiccator did not always decrease to that of the original non-hydrated tablets even after incubation for more than 63 h (Figure 5). Thus it was concluded that tablets for combustion analysis should be prepared from lyophilized cell powder which had been open to the atmospheric conditions of the laboratory housing the combustion calorimeter for a minimum of 10 min before determination of the initial weight of the tablets and enthalpy of combustion. The combustion data should subsequently be corrected for the residual water content of the tablets as determined by dry weight measurements (incubation of the tablets at 100 °C for 24 - 48 h).

Microbial cells contain varying amounts of inorganic material termed ash which, whilst representing a fraction of the dry weight should not be considered as part of the elemental composition nor enthalpy of combustion. Thus is it important to determine the conditions for ash determination and to correct both enthalpy and elemental analysis data for the ash content.

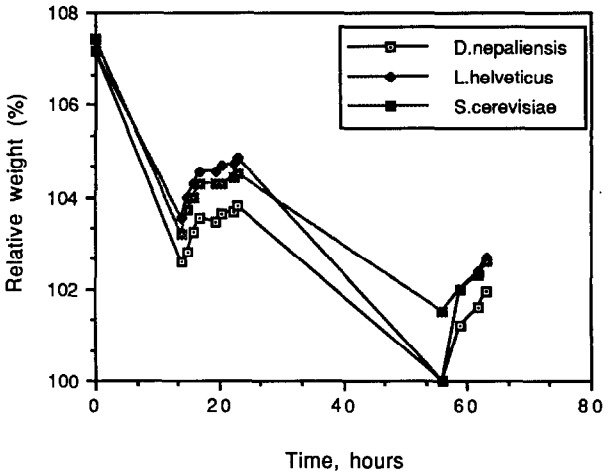

Figure 5 Desorption of water from freeze- dried tablets of cells from Figure 4 after placing in a desiccator at ambient temperature (27°C).

Samples (0.5 g) of *Z. bailii* and *D. nepaliensis* were allowed to stand in ceramic crucibles at room temperature for 10 min, weighed and incubated at 350 °C, 450 °C, 600 and 750 °C for 6 h. In the latter case the cells were initially incubated for 2 h at 450 °C followed by 4 h at 750 °C in order to avoid the aggressive combustion and sample loss experienced by direct incubation at 750 °C. At the end of the incubation period the samples were cooled in a desiccator and re-weighed.

The results (Table 1) show that for both culture samples a temperature of 350 °C for 6 h was insufficient for the complete removal of all organic material. This finding was corroborated by elemental analysis of the samples which showed (Table 2) that a considerable amount of carbon (mass fraction 18 - 33 %) remained in the samples.

Samples incubated at temperatures above 450 °C for 6 h gave similar ash values regardless of the temperature and contained less than 0.5 % mass fraction carbon. Consequently for all further work it was decided to incubate samples at 600 °C for 6 h. These conditions are similar to those reported elsewhere [ref. 13]. In biological samples containing significant amounts of

calcium carbonate, however, an ashing temperature of 500°C is preferable due to the loss of this component of the ash at higher temperatures [ref. 4].
Table 1 also shows that the ash content of different microbial cells may vary considerably. Thus *Z. bailii* contains approximately twice the amount of ash (9.78 ± 0.05 %) as *D. nepaliensis* (4.44 + 0.02 %), even though the growth medium and conditions were identical for both strains. It is thus essential that both elemental and enthalpy data for microbial biomass be corrected for ash content. This could be particularly important for halophilic or osmotolerant strains which may accumulate high levels of inorganic salts in order to maintain an osmotic balance with the environment.
Table 3 lists the enthalpy of combustion, elemental composition and ash fraction, as determined using the proposed sample preparation method, for the organisms used in the present study. These results show that significant differences exist between each of the yeast strains with respect to both $\Delta_c h^o$ and elemental compostion. Furthermore the value of $\Delta_c h^o$ for the bacterium *L. helveticus* (23.39 ± 0.06 kJ g^{-1}) is considerably higher than that obtained for yeasts (20.51- 21.22 kJ g^{-1}) which is in agreement with results reported elsewhere [1].

Table 3 Ash fraction, standard elemental composition and standard enthalpy of combustion, $\Delta_c h^o$ [kJ g^{-1}] data for a range of organisms determined according to the proposed method.

Strain	w_{ash} (%)	Standard elemental composition	Formula weight	$\Delta_c h^o$ (kJ g^{-1})
D.nepaliensis (CBS 5921)	4.44 ± 0.02	$CH_{1.8}O_{0.63}N_{0.09}$	25.07	20.88 ± 0.02
Z. bailii (NCYC 563)	9.78 ± 0.01	$CH_{1.64}O_{0.54}N_{0.13}$	24.14	20.51 ±0.09
S.cerevisiae whi2-	8.12 ± 0.01	$CH_{1.65}O_{0.49}N_{0.18}$	24.17	21.22 ± 0.05
L. helveticus	8.28 ± 0.06	$CH_{1.62}O_{0.38}N_{0.23}$	22.92	23.39 ± 0.06

Table 1: Ash fractions of yeasts at different temperatures

Sample	Ash fraction x 100 (%)			
	Temperature			
	450 °C	600 °C*	750 °C**	750 °C***
D. nepaliensis	4.89±0.38	4.44±0.02	4.72±0.08	4.64±0.02
Z. bailii	10.23±0.74	9.78±0.01	10.02±0.06	10.06±0.12

* Incubated for 2 h at 450 °C followed by 4 h at 600 °C
** Incubated for 2 h at 450 °Cm 4 h at 600 °C followed by 4 h at 750 °C
*** Incubated for 2 h at 450 °C followed by 4 h at 750 °C

Table 2 : Elemental composition of ash of cells burnt at 600 °C, 450 °C, 350 °C

Sample	Oven temperature	ash^{w}C	ash^{w}H	ash^{w}N
D. nepaliensis	600 °C	0.25 ± 0.06	- 0.09	- 0.03
	450 °C	0.3 ±0.15	0.17 ± 0.02	0.3 ± 0.13
	350 °C	31.56 ± 1.9	1.00 ± 0.11	11.90 ± 0.5
Z. bailii	600 °C	0.17 ± 0.04	- 0.04	- 0.03
	450 °C	0.18 ± 0.05	- 0.17 ± 0.02	0.36 ± 0.16
	350 °C	19.99 ± 0.4	0.51 ± 0.03	8.70 ± 0.009

ash^{w}C, ash^{w}H and ash^{w}N represent the mass fractions of carbon, hydrogen and nitrogen respectively, in the ash samples as determined by the elemental analyser and expressed relative to the mass of ash of sample.

CONCLUSIONS

The results show that the mass fractions of ash and residual moisture measured in a variety of microbial strains varies considerably. Since these effects could result in significant errors for the determination of elemental composition and enthalpies of combustion of cells, a method has been proposed for the determination of these parameters.

It should be emphasized however that the harvesting and preparation of lyophilized biomass should be carefully standardized for each microbial strain. Furthermore the method may require modification for cells with special characteristics, such as those which produce extracellular lipophilic products which may require additional washing steps in order to avoid contamination of the cellular material.

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REFERENCES

1 Cordier J.L., B.M. Butsch, B. Birou and U. von Stockar. 1987. 'The Relationship Between Elemental Composition and Heat of Combustion of Microbial Biomass'. App. Microbiol. Biotechnol. **25** : 305-312.

2 Prochazka G.J., W.J. Payne and W.R. Mayberry. 1970. 'Calorific Content of Certain Bacteria and Fungi'. J. of Bacteriology. **104** (2) : 646-649.

3 Randolph T.W., I.W. Marison, C. Berney, U. von Stockar. 1989. 'Bench-Scale Calorimetry of Hybridomas in Suspension Culture'. Biotechnology Techniques. **3** : 369-374.

4 Gnaiger E. and Bitterlich G. 1984 Proximate Biochemical Composition and Caloric Content calculated from Elemental CHN Analysis: a Stoichiometric Concept'. Oecologia. **62** : 289-298.

5 von Stockar U. and I.W. Marison. 1989. 'The Use of Calorimetry in Biotechnology'. Advances in Biochemical Engineering/ Biotechnology. **40** : 94-136.

6 Randolph T.W., I.W. Marison., D.E. Martens and U. von Stockar. 1990. 'Calorimetric Control of Fed-Batch Fermentation'. Biotech. Bioengineering (accepted for publication).

7 Butsch, B.M. 1987. 'Heat of Combustion and Elemental Analysis of Microbial Biomass' Internal Report, EPF- Lausanne, Switzerland.

8 Gnaiger E. and Shick J.M. 1985. 'A Methodological Note on CGN-Stoichiometric Analysis'. Cyclobios. Newsletter **2** : 2-4.

9 DIN 51900 Standard. 1977. 'Bestimmung des Brennwertes mit dem Bomben-Kalorimeter und Berechnung des Heizwertes.

10 Larsson C., A. Blomberg and L Gustafsson.1990 'The Use of Micro Calorimetric Monitoring in Establishing Continuous Energy Balances and in Continuous Determinations of Substrate and Product Concentrations of Batch-Grown *Saccharomyces cerevisiae*'. Biotechnol. Bioeng. (accepted for publication)

11 Ishikawa, Y. and M. Shoda. 1983. 'Calorimetric analysis of *Escherichia coli* in Continuous Culture'. Biotechnol. Bioeng. **25** : 1817-1827.

12 Birou B. 1986. 'Etude de la chaleur dégagée par des cultures microbiennes dans un fermenteur de laboratoire'. Ph.D. thesis, EPFL, Switzerland. 243 p.

13 Gerhard E. 1986. 'Determination de la composition élémentaire et de la chaleur de combustion d'une bactérie methanogène : *Methanobacterium thermoautotrophicum* sous différentes conditions de cultures'. Travail de diplôme - EPF-Lausanne.