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# An Apparent Anomaly in the Calculation of Ash-Free Dry Weights for the Determination of Cellular Yields

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**The use of ash-free dry weights results in a determination of CHON-containing cell mass that is 5 to 6% less than that determined on the basis of total cellular composition. This discrepancy derives from the fact that phosphorus and sulfur contribute greater weight to ash as oxides and salts of P and S than the contribution they make as part of the cellular fabric. In addition, in the absence of P and S, the unit carbon formula for this mass becomes changed, on the basis of an equivalent number of available electrons per unit of carbon formula weight.**

A knowledge of the quantity of biomass produced from a substrate is important in biotechnology and is essential to an understanding of the thermodynamics of microbial growth. The quantity of such material is usually expressed in terms of ash-free dry weights. The conventional method for arriving at ash-free dry weights is to subtract from the dry weight the percentage remaining as ash after combustion. The relative percentages of C, H, and N in the ash-free material that have been determined analytically for whole cells are then are assumed to be the same for the ash-free biomass, the percentages of O being calculated by difference. In a recent and rigorous consideration of the elemental analysis of microbial cells Gurakan et al. (2) reiterated the conventional opinion that ash represents a fraction of the dry weight that should not be considered as part of the elemental composition nor as material that contributes to the enthalpy of combustion. Thus, the unit carbon formulae (UCF) representing the composition of the four microorganisms they studied consist only of C, H, O, and N in the proportions in which these are found in cells. On the other hand, the cellular fabric also consists of P and S, and the question can be raised as to whether representations of cellular fabric should better contain these elements. Quantitative analyses of the elemental compositions of various microorganisms are largely missing from the literature. Of those that exist, one of the most complete is that given by Battley (1) for *Escherichia coli* K-12 cells. By summing up the quantities of C, H, O, N, P, and S in the various macromolecular components in *E. coli* K-12 cells as compiled by Neidhardt (3) and adding to these the quantities of cellular ions, Battley was able to account for most of what composes the cells. In this manner, a UCF of $CH_{1.595}O_{0.374}N_{0.263}P_{0.023}S_{0.006}$ was constructed that had a UCF weight of 24.190 g, to which must be added the weight of 0.846 g of ions within the cells to give a total dry weight of 25.036 g comprising all of the dry cellular material associated with 1 UCF weight of cells. The nature of all of the substances resulting from the combustion of cellular material is seldom considered, the products conventionally taken to be $CO_2$(g), $H_2O$(1), $N_2$(g), and ash. The purpose of this paper is to investigate whether the weight of the oxides and salts of P and S in the ash is greater than the weight contributed by phosphate and sulfide as part of the cellular fabric.

Battley's (1) data can be used as a model set of data for studying the use of ash-free dry weights for calculating cellular yields when these yields are expressed in terms of C, H, O, and N only. The percentage composition of the elements composing *E. coli* K-12 cells that contribute to the ash formed on combustion is given in Table 1, together with the calculated weights of what can be considered reasonably to be the products of the oxidation. Any other substances in the cells, such as trace elements, are not considered in that they are present in extremely small quantities. It is apparent that the weight of salts plus oxides in the ash is considerably greater than that of the cellular components from which they arise. Because the ions other than biphosphate do not change their weight on combustion, the difference is due largely to the presence of phosphorus and sulfur in the cells.

Table 2, column *A*, shows a list of the quantities of elements making up 100 g (dry weight) of *E. coli* K-12 cells as determined from the actual composition (1), from which the quantities of C, H, O, and N make up 93.04 percent of the dry weight. Table 2, column *B*, shows the values for C, H, O, and N that would be found by the conventional method of determination, whereby the ash is subtracted from the total dry weight to give an ash-free dry weight of cellular material. It is this latter value that is conventionally reported as the ash-free yield of cells, and it is apparent that by this method, the yield is calculated to be about 5.5% less than the true value with respect to the C, H, O, and N composing most of the fabric of the cells. The reason for the excess weight of ash can be seen from the following equation that has been taken to represent the combustion of the cellular material:

$$\begin{aligned} &CH_{1.595}O_{0.374}N_{0.263}P_{0.023}S_{0.006}\ (\text{cells}) + 1.25\ O_2(g) \\ &\quad + 0.006\ K_2O(c) \rightarrow 1.000\ CO_2(g) + 0.1315\ N_2(g) \\ &\quad + 0.00575\ P_4O_{10}(c) + 0.006\ K_2SO_4(l) \\ &\quad + 0.006\ K_2SO_4(l) + 0.797\ H_2O(l) \end{aligned} \quad (1)$$

where $K_2O$(c) originates as intracellular potassium ion prior to combustion. On the left side of equation 1, 0.374 g-atoms of O from the cells + 2 × 1.250 g-atoms from $O_2$(g) consumed during combustion + 0.006 g-atoms from $K_2O$(c) = 2.880 total g-atoms of O that are available for the combustion. On the right side of the equation, 2.000 g-atoms of O used in the formation of $CO_2$(g) + 0.797 g-atoms used in the formation of water = 2.797 g-atoms of O that are used in the formation of products of combustion other than ash. The difference, 2.880 g-atoms − 2.797 g-atoms = 0.083 g-atoms of O, is thus utilized

TABLE 1. Quantities of elements forming ash in 100 g of dried *E. coli* K-12 cells[a]

| Element | Wt in cells | Wt as ash |
|---|---|---|
| Phosphorus | 2.80 g from cellular fabric | 6.41 g as $P_4O_{10}$(c) |
| | 0.62 g as $HPO_4^{2-}$ in cells | 0.46 g as $P_4O_{10}$(c) |
| Potassium | 1.85 g as $K^+$ in cells | Combined with 2.28 g $SO_4^{2-}$ (from oxidation of S in fabric) |
| | 0.10 g as $K^+$ in cells | Combined with 0.12 g of $SO_4^{2-}$ in cells |
| | 0.05 g as $K^+$ in cells | Combined with 0.05 g of $Cl^-$ in cells |
| Sulfur | 0.76 g from cellular fabric (oxidized to 2.28 g of $SO_4^{2-}$) | 4.13 g as $K_2SO_4$(c) |
| | 0.19 g as $SO_4^{2-}$ in cells | 0.24 g as $MgSO_4$(c) |
| | 0.12 g as $SO_4^{2-}$ in cells | 0.17 g as $CaSO_4$(c) |
| | 0.12 g as $SO_4^{2-}$ in cells | 0.22 g as $K_2SO_4$(c) |
| Iron | 0.20 g as $Fe^{3+}$ in cells | 0.29 g as $Fe_2O_3$(c) |
| Magnesium | 0.05 g as $Mg^{2+}$ in cells | Combined with $SO_4^{2-}$ |
| Calcium | 0.05 g as $Ca^{2+}$ in cells | Combined with $SO_4^{2-}$ |
| Chlorine | 0.05 g as $Cl^-$ in cells | 0.11 g as KCl(c) |
| Total | 6.96 g containing 3.40 g of ions. | 12.03 g of ash solids |

[a] Data were calculated by the method of Battley (1), with the correction that $Fe_2O_3$ is formed from $Fe^{3+}$ and not $FePO_4$, which would be unstable at the appropriate ashing temperature of 600°C determined by Gurakan et al. (2).

TABLE 2. Comparison of the true percentages of the elements composing 100 g (dry weight) of *E. coli* K-12 cells with those obtained by the conventional and derived methods of ash-free dry weight determinations

| Element | *A*: true (g%)[a] | *B*: conventional (g%) | $[(A - B)/A] \times 100$ (%) | *C*: derived (g%) | $[(A - C)/A] \times 100$ (%) |
|---|---|---|---|---|---|
| Cellular fabric | | | | | |
| Carbon | 47.99[b] | 45.38[c] | | 46.50[d] | |
| Hydrogen | 6.42[b] | 6.07[c] | | 6.33[d] | |
| Oxygen | 23.93[b] | 22.62[c] | | 20.87[d] | |
| Nitrogen | 14.70[b] | 13.90[c] | | 14.26[d] | |
| Subtotal 1 | 93.04 | 87.97 | 5.45 | 87.97 | 5.45 |
| Phosphorus | 2.80[b] | | | | |
| Sulfur | 0.76[b] | | | | |
| Subtotal 2 | 96.60 | | | | |
| Cellular ions | | | | | |
| Potassium | 2.00 | | | | |
| Biphosphate | 0.62 | 12.03 ash solids[e] | | 12.03 ash solids[e] | |
| Sulfate | 0.43 | | | | |
| Iron | 0.20 | | | | |
| Magnesium | 0.05 | | | | |
| Calcium | 0.05 | | | | |
| Chlorine | 0.05 | | | | |
| Subtotal 3 | 3.40 | | | | |
| Total | 100.00 | 100.00 | | 100.00 | |

[a] Data calculated from Tables 2 and 3 of reference 1.
[b] These values were obtained by multiplying the percentages of elements in the UCF from Table 2 of reference 1 by 0.966 to convert them to percentages of the total weight of 25.036 g comprising cells plus ions.
[c] These values were obtained by subtracting from 100 the total ash value from column 2 in Table 1 and calculating the weights of CHON by using the relative percentages of these elements from column *A* above.
[d] These values were obtained by subtracting from 100 the total ash value from column 2 in Table 1 and calculating the weights of CHON from the derived UCF in equation 2 in the text.
[e] This value was taken from Table 1.

during combustion in the formation of ash from the biomass. This quantity of O weighs 1.328 g. The UCF weight of the cells is 24.190 g. The percentage increase in weight due to added oxygen in the formation of the ash is then (1.328/24.190) × 100 = 5.49%, which is almost identical to that given in Table 2.

An equally important question concerns the composition of the UCF that is obtained on the basis of the conventional assumptions. Are the relative proportions of the C, H, O, and N atoms in the ash-free UCF actually the same as they would be with respect to the whole cells? Conventionally, the proportions of C, H, and N are determined with whole cells, with the proportion of oxygen being calculated by the difference after the weight of ash has been subtracted from the weight of whole cells. However, it is possible theoretically to convert the UCF containing CHONPS into one containing only CHON while still retaining an equivalent number of available electrons, thus maintaining the energy level of the "organic" part of the biomass, as follows:

$$CH_{1.595}O_{0.374}N_{0.263}P_{0.023}S_{0.006}\ \text{(cells)}$$
$$+\ 0.055\ H_2O(l) \rightarrow CH_{1.624}O_{0.337}N_{0.263}\ \text{(derived cells)}$$
$$+\ 0.023\ H_3PO_4(aq) + 0.006\ H_2S(aq) \qquad (2)$$

In equation 2, the biomasses represented as cells and derived cells both have 4.161 available electrons, thus retaining the same organic energy content in the latter as in the former. However, it is evident that the proportions of C, H, O, and N differ from the true proportions, as shown in column *C* of Table 2.

In conclusion, an accurate determination of what goes on materially and energetically with respect to the growth of cells requires an acknowledgement that phosphate and sulfide are not just ash but are integral parts of the biomass. As Battley (1) has suggested, the ion-free dry weight is a better criterion of biomass yield and involves the inclusion of P and S in UCF. If the quantity of ions in exponentially grown *E. coli* cells is taken as about 3.5%, an analytical determination of the percentages of C, H, N, P, and S plus the percentage of ions enables an accurate estimate of the percentage of O to be determined by difference. This would enable the calculation of a UCF and of a biomass yield that are much more representative of the true nature of the growth process.

## REFERENCES

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