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Determination of the Carbon-Bound Electron Composition of Microbial Cells and Metabolites by Dichromate Oxidation

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The applicability of the silver sulfate–acid dichromate oxidation (chemical oxygen demand) method for determining the carbon-bound electron compositions of microbial cells, substrates, and metabolic by-products was evaluated. An approach for approximating the carbon-bound electron composition of microbial cells from CHN data is also presented. Ten aliphatic and aromatic carboxylic acids, 17 amino acids, and 8 sugars generally gave 96 to 101% (mainly ≥98%) recovery with 0.0625 N dichromate (digestion mixture of 10 ml of sample–10 ml of 0.25 N dichromate–20 ml of Ag_2SO_4-amended concentrated H_2SO_4). Recoveries of nicotinic acid (5%) and methionine (65%) were incomplete; arginine (125%) and two purine and three pyrimidine bases (105 to 120%) were overestimated. The validity of 0.0625 N dichromate for determining the carbon-bound electron composition of bacterial cells was supported by theoretical analysis of the carbon-bound electron composition of hypothetical bacterial cell material (defined monomer composition) and by the compatibility of elemental and dichromate oxidation-derived carbon-bound electron compositions of typical bacterial cells.

The efficiency of heterotrophic microbial growth is a function of the relative amount of carbon/energy substrate assimilated for cell biosynthesis versus the amount dissimilated for energy. Recognition of the importance of standardizing efficiency in terms of the relative partitioning of substrate carbon-bound electrons for assimilatory and dissimilatory purposes is widespread, although terminology has yet to be standardized. Thus, electron equivalents (e^-eq) (8), available electrons (4, 16), oxygen equivalents (7, 8, 13), and reduction equivalents (3, 14, 22) are integral components of recently developed or commonly used growth efficiency concepts and equations. As illustrated in Tables 1 and 2, calculation of the C-bound electron composition of microbial substrates, metabolic by-products, or cell materials is readily accomplished from a knowledge of elemental composition, using the half-reaction approach (8). Alternatively (equation 3), C-bound electron composition may be expressed indirectly, as the amount of O_2 required for combustion of organic materials (7); e.g., for typical bacterial cell material (Table 1, line 11):

$$C_4H_7O_{1.5}N + 6.5H_2O \rightarrow 4CO_2 + NH_3 + 17e^- + 17H^+ \quad (1)$$

$$17e^- + 17H^+ + 4.25O_2 \rightarrow 8.5H_2O \quad (2)$$

$$C_4H_7O_{1.5}N + 4.25O_2 \rightarrow 4CO_2 + NH_3 + 2H_2O \quad (3)$$

Dichromate oxidation has long been used for microbial growth characterization (2, 9, 20) and for determining soil and plant organic matter (1, 6) and the chemical oxygen demand of organic wastes (11, 17). Based on a relatively restricted range of biochemicals, organic C compounds and organic N compounds are, with few known exceptions (e.g., benzene, toluene, and pyridine), quantitatively oxidized to CO_2 and CO_2 plus NH_4^+, respectively; but efficient oxidation of certain organics, particularly acetate-related compounds, requires an Ag_2SO_4 catalyst (11, 17). In other words, Ag_2SO_4-catalyzed acid dichromate oxidation potentially provides a technique for direct experimental determination of the carbon-bound electron compositions of biological materials (e.g., $C_4H_7O_{1.5}N$ typical bacterial cell):

$$C_4H_7O_{1.5}N + 6.5H_2O \rightarrow 4CO_2 + NH_4^+ + 17e^- + 16H^+ \quad (1)$$

$$17e^- + 39.67H^+ + 2.83Cr_2O_7^{2-} \rightarrow 5.67Cr^{3+} + 19.83H_2O \quad (4)$$

$$C_4H_7O_{1.5}N + 23.67H^+ + 2.83Cr_2O_7^{2-} \rightarrow 4CO_2 + NH_4^+ + 5.67Cr^{3+} + 13.33H_2O \quad (5)$$

For interconversions between e^-eq and other mass parameters: 1 e^-eq of organic material (e.g., 1,000/24 = 41.7 mmol, or 180/24 = 7.5 g of glucose) ≡ 1 e^-eq of ferrous ammonium sulfate

TABLE 1. *Carbon-bound electron composition of bacterial cells*[a]

No.	Elemental composition (%)					Empirical formula and half reaction[b]	Formula wt (g/mol)	C-bound electron composition[b]	
	Organic				Inorganic			Equivalent wt (g/e⁻eq)	e⁻/C (e⁻eq/mol)
	C	H	O	N					
						Experimentally based cell formula			
1	48.85	6.43	20.44	14.91	9.37	$C_{3.82}H_{5.99}O_{1.20}N \rightarrow 3.82CO_2 + NH_4^+ + 15.87e^-$	93.92	5.92	4.15
2	47.48	7.10	22.42	13.00	10.00	$C_{4.26}H_{7.59}O_{1.51}N \rightarrow 4.26CO_2 + NH_4^+ + 18.61e^-$	107.77	5.79	4.37
3	45.60	7.59	24.71	12.09	10.00	$C_{4.40}H_{8.73}O_{1.79}N \rightarrow 4.40CO_2 + NH_4^+ + 19.75e^-$	115.89	5.87	4.49
4	46.14	6.31	24.00	13.55	10.00	$C_{3.97}H_{6.47}O_{1.55}N \rightarrow 3.97CO_2 + NH_4^+ + 16.25e^-$	103.34	6.36	4.09
5	46.52	7.23	21.86	14.39	10.00	$C_{3.77}H_{6.98}O_{1.33}N \rightarrow 3.77CO_2 + NH_4^+ + 16.40e^-$	97.33	5.93	4.35
6	49.25	6.78	19.57	14.40	10.00	$C_{3.99}H_{6.54}O_{1.19}N \rightarrow 3.99CO_2 + NH_4^+ + 17.12e^-$	97.29	5.68	4.29
7	47.48	7.20	22.41	12.91	10.00	$C_{4.29}H_{7.75}O_{1.52}N \rightarrow 4.29CO_2 + NH_4^+ + 18.87e^-$	108.52	5.75	4.40
						Hypothetical monomer composition-based cell formula			
8	47.72	6.44	23.72	12.07	10.05	$C_{4.61}H_{7.41}O_{1.72}N \rightarrow 4.61CO_2 + NH_4^+ + 19.41e^-$	116.03	5.98	4.21
						Hypothetical rounded-off cell formula			
9	42.34	7.11	28.20	12.34	10.00	$C_4H_9O_2N \rightarrow 4CO_2 + NH_4^+ + 17e^-$	113.47	6.67	4.25
10	47.78	5.61	25.46	11.14	10.00	$C_5H_7O_2N \rightarrow 5CO_2 + NH_4^+ + 20e^-$	125.69	6.28	4.00
11	46.44	6.82	23.20	13.54	10.00	$C_4H_7O_{1.5}N \rightarrow 4CO_2 + NH_4^+ + 17e^-$	103.46	6.09	4.25

[a] Calculated from data of: (1) Roberts et al. (18) for *E. coli*; (2) Hill (5) for *K. pneumoniae*; (3) Mayberry et al. (10) for aquatic bacteria; (4-6) A. Unluturk, J. M. Wasileski, M. A. Vargas, and R. F. Harris (unpublished) for *A. crystallopoietes*, *E. coli* and *S. lipoferum*, respectively; (7) Stouthamer (22) for *Parococcus dentrificans*; (8) Morowitz (12) and Stouthamer (21); (9) Payne (16); (10) McCarty (8); (11) R. F. Harris (unpublished). Except for lines 1 and 8, a 10% inorganic composition was assumed. Percent organic oxygen for lines 2, 3, and 7 was calculated as 100 − (percent C + percent H + percent N + 10). Data expression to the second decimal is for bookkeeping rather than precision identification purposes.

[b] The cell C-bound electron/cell N ratio is identified in the half reaction.

(1,000/1 = 1,000 mmol) reduces one e⁻eq dichromate (1,000/6 = 166.7 mmol) ≡ 1 e⁻eq of oxygen (1,000/4 = 250 mmol of O_2, or 32 × 1,000/4 = 8,000 mg of chemical oxygen demand).

The major objective of this paper is to evaluate the applicability of the Ag_2SO_4-acid dichromate method (17) for determining the carbon-bound electron compositions of microbial substrates, cells, and metabolic by-products. An approach for approximating the C-bound electron compositions of microbial cells from CHN data is also presented.

MATERIALS AND METHODS

Materials. Reagent grade chemicals were obtained from standard commercial sources. No purity validation or repurification was attempted, but appropriate corrections for water content based on oven-drying of subsamples for 24 h at 105°C were made for heat-stable compounds. Cells of *Escherichia coli, Cellulomonas cartalyticum,* and *Azotobacter vinelandii* were obtained from glucose-limited batch cultures grown on a trace element-amended mineral salts medium E (15) modified to contain (per liter), 200 millielectron equivalents (me⁻eq) of glucose (1.5 g), 15 mmol of NH_4^+-N, and 60 mmol of potassium phosphate buffer, pH 6.8.

Pure compounds were dissolved in organic-free distilled water (distilled water passed through a Milli-Q-Water Purification System [Millipore Corp., Bedford, Mass.] contains <1 me⁻eq of dichromate-oxidizable material per liter) to provide 100-ml 0.1 N (100 me⁻eq/liter) stock solutions and analyzed immediately; dissolution of such compounds as the purine and pyrimidine bases required addition of a few drops of 10 N NaOH. Bacterial cell suspensions were washed twice with phosphate buffer (0.06 M, pH 6.8) between centrifugations, diluted to about 100 me⁻eq/liter in distilled water, and then acidified (1 drop of concentrated H_2SO_4 per 10-ml suspension) for refrigerated storage before analysis.

Methods. The method used for determining the C-bound electron compositions of microbial biochemicals was essentially the same as the standard silver sulfate-acid dichromate reflux method for determination of the chemical oxygen demand of organic wastes (17). All glassware was thoroughly cleaned with dichromate cleaning solution and rinsed with organic-free distilled water before use. Unless specified otherwise, a 10-ml sample of an aqueous solution containing 10 me⁻eq of test material (10 ml of distilled water for the blank) was placed in a 300-ml, flat-bottomed reflux flask containing about 10 glass beads followed by addition of 10 ml of 0.25 N standard potassium dichromate solution. Twenty milliliters of silver sulfate-amended concentrated sulfuric acid (22 g of Ag_2SO_4 per 9-pound (ca. 4.08-kg) bottle of concentrated acid, about 3 days allowed for dissolution) was then added slowly with continuous swirling and mixing; the neck of the flask was rinsed with <1 ml of distilled water (to prevent the flask from freezing to the condenser) followed by immediate connection of the flask to a water-cooled condenser positioned over a precalibrated hot plate. The flask was refluxed with gentle boiling (140 to 145°C) for 2 h. Digestion at less than boiling resulted in incomplete oxidation for some biochemicals; vigorous boiling caused poor reproducibility. The 2-h digestion was preceded by a 10- to 15-min

warm-up and followed by a 30-min cooling period. Following cooling, the condenser was rinsed down with about 10 ml of distilled water. The flask was removed, diluted with 50 ml of distilled water, and cooled to room temperature in a cold water bath (about 10 min). The excess dichromate in the diluted digestion mixture was then titrated with standard 0.10 N (approximately) ferrous ammonium sulfate, using ferroin indicator (2 drops) and a magnetic stirrer. A consistent titration technique is critical for high precision: before titrating, the sides and neck of the flask should be rinsed with organic-free distilled water; the stirrer should not be turned on until titration is to begin; refluxed samples should not be permitted to stand for an extended time period. The end point is the first sharp color change from blue-green to orange or reddish brown (even though the blue-green color may reappear within minutes). The carbon-bound e^-eq content, s(me$^-$eq/liter), of the sample was calculated as follows:

$$s = \frac{(a - b)N \times 1{,}000}{V} \quad (6)$$

where a is the volume (milliliters) of $Fe(NH_4)_2(SO_4)_2$ used for the digested blank, b is the volume (milliliters) of $Fe(NH_4)_2(SO_4)_2$ used for the sample, N is the normality (e^-eq/liter) of the $Fe(NH_4)_2(SO_4)_2$, and V is the volume (milliliters) of sample added to the digestion flask (usually 10 ml).

RESULTS AND DISCUSSION

Applicability of dichromate oxidation for determining carbon-bound electron composition. In general (Table 2), the diverse aliphatic and aromatic carboxylic and amino acids and sugars evaluated showed ≥96% (mainly ≥98%) recovery with 0.0625 N dichromate (10 ml of 0.25 N dichromate in 40 ml of digestion mixture). Increasing the dichromate concentration twofold, to 0.125 N, caused only a slight increase in recovery (e.g., sugar recovery increased from 96 to 97%), but, consistent with the findings of Moore and Walker (11), decreasing the concentration through 0.03125 to 0.00625 N frequently, although not always (e.g., succinate), caused a marked decline in recovery (Table 3). Proline was particularly sensitive to dichromate concentration, with recoveries ranging from 97% with 0.125 N to only 50% with 0.00625 N dichromate. The low recovery of nicotinic acid (Table 2) is consistent with the refractory nature of pyridine (17). Methionine was the only other compound of those evaluated that showed markedly incomplete recovery with 0.0625 N dichromate (Table 2). Whether the low recovery of methionine was due to resistance to oxidation or loss of volatile intermediate oxidation products (e.g., organic and/or inorganic sulfides or elemental S) is not known, but preliminary digestion in an ice bath to retard possible loss of volatile intermediates did not improve recovery (unpublished data). Elemental S was incompletely recovered by 0.0625 N dichromate oxidation (Table 2). Cystine, the other S-containing amino acid analyzed, gave high recoveries with ≥0.0625 N dichromate (Tables 2 and 3), consistent with complete oxidation of the organic S to sulfate (Table 2). Overestimations of C-bound electron composition occurred for arginine (120 to 125%) and heterocyclic N purine and pyrimidine bases (105 to 120%) (Tables 2 and 3). The most likely explanation of this phenomenon is partial oxidation of the organic N to N_2 or more oxidized N species rather than quantitative release of the N as ammonia. In line with the trend shown by most pure compounds (Table 3), C-bound electron recoveries for bacterial cell systems decreased progressively (down to about 90% recovery) as dichromate normality was lowered from 0.0625 to 0.00625 (Table 4). Precision on an absolute concentration basis was high for each dichromate concentration, usually less than ±2 and frequently less than ±1 me$^-$eq/liter, but deviations expressed on a percent basis understandably increased with decreasing sample/dichromate concentration (Table 3).

The applicability of 0.0625 N dichromate oxidation for determining the C-bound electron composition of microbial cells was evaluated (Table 5) by comparing the theoretical versus dichromate oxidation-derived C-bound electron compositions of hypothetical, typical bacterial cell material (12, 21). Oxidation with 0.0625 N dichromate should recover 98, 96, 101, and 97% of the C-bound electrons of the bacterial protein, polysaccharide, nucleic acid, and lipid components, respectively, giving a total recovery for the intact cell material of about 98% (Table 5). Further evidence of the validity of the 0.0625 N dichromate oxidation method for determining the C-bound electron composition of bacterial cells is given by the compatibility of the dichromate oxidation-derived C-bound electron compositions of cells of *E. coli, Spirillum lipoferum,* and *Arthrobacter crystallopoietes* with C, H, O, N, and dry-weight composition data typical of bacterial cells (Table 1).

In practice, the choice of dichromate system (Table 6) will be dictated by sample C-bound electron concentration and, within certain limits, will be a compromise between accuracy (recoveries for dichromate concentration, 0.125 N ≃ 0.0625 N ≫ 0.00625 N), sample size (e.g., sample volume), and precision (25 to 60% dichromate consumption optimum). The accuracy of 0.126 N dichromate is somewhat higher than that of 0.0625 N dichromate (Table 3), but the latter has been validated experimentally over a broader range of materials (Tables 2 and 3). Dichromate concentrations of <0.0625 N suffer

TABLE 2. *Applicability of 0.0625 N dichromate oxidation for determining the carbon-bound electron composition of microbial substrates and cell components*

Compound	Assumed half reaction[a]	e^- eq (me^-eq/g)		
		Theoretical	Observed[b]	
			Mean	% of theoretical[c]
Acetate	$NaC_2H_3O_2 \cdot 3H_2O \rightarrow Na^+ + 2CO_2 + 8e^-$	58.79	57.7[d]	97.9 ± 0.4
	$NaC_2H_3O_2 \rightarrow Na^+ + 2CO_2 + 8e^-$	97.53	96.5	98.9 ± 0.0
Adenine	$C_5H_5N_5 \rightarrow 5CO_2 + 5NH_3 + 10e^-$	74.00	78.6[d]	106.2 ± 4.3
Alanine	$C_3H_7O_2N \rightarrow 3CO_2 + NH_3 + 12e^-$	134.68	133.7[e]	99.2 ± 0.5
Arabinose	$C_5H_{10}O_5 \rightarrow 5CO_2 + 20e^-$	133.22	127.9	96.0 ± 0.2
Arginine	$C_6H_{15}O_2N_4Cl \rightarrow 6CO_2 + 4NH_3 + \frac{1}{2}Cl_2 + 23e^-$	109.18	131.6[d]	120.5 ± 1.9
	$\rightarrow 6CO_2 + 4NH_3 + Cl^- + 22e^-$	104.43	131.6[d]	126.0 ± 1.9
Asparagine	$C_4H_8O_3N_2 \cdot H_2O \rightarrow 4CO_2 + 2NH_3 + 12e^-$	79.93	79.8	99.9 ± 0.2
Butyrate	$NaC_4H_7O_2 \rightarrow Na^+ + 4CO_2 + 20e^-$	181.67	176.6[d]	97.2 ± 0.3
Cellobiose	$C_{12}H_{22}O_{11} \rightarrow 12CO_2 + 48e^-$	140.23	134.2	95.7 ± 0.2
Citrate	$Na_3C_3H_5O_7 \cdot 2H_2O \rightarrow 3Na^+ + 6CO_2 + 18e^-$	61.20	60.2[e]	98.4 ± 0.2
Cystine	$C_6H_{12}O_4S_2N_2 \rightarrow 6CO_2 + 2NH_3 + 2H_2SO_4 + 34e^-$	141.49	135.5[d]	95.8 ± 2.9
Cytosine	$C_4H_5ON_3 \cdot H_2O \rightarrow 4CO_2 + 3NH_3 + 10e^-$	77.45	91.9[e]	118.7 ± 0.8
Formate	$NaCHO_2 \rightarrow Na^+ + CO_2 + 2e^-$	29.41	29.7[e]	100.9 ± 0.0
Fructose	$C_6H_{12}O_6 \rightarrow 6CO_2 + 24e^-$	133.21	127.9	96.0 ± 0.2
Gluconate	$NaC_6H_{11}O_7 \rightarrow Na^+ + 6CO_2 + 22e^-$	100.85	96.8[d]	96.0 ± ± 0.3
Glucose	$C_6H_{12}O_6 \rightarrow 6CO_2 + 24e^-$	133.21	127.9[d]	96.0 ± 0.8
Glutamic acid	$C_5H_9O_4N \rightarrow 5CO_2 + NH_3 + 18e^-$	122.34	122.2[e]	99.9 ± 0.0
Glutamine	$C_5H_{10}O_3N_2 \rightarrow 5CO_2 + 2NH_3 + 18e^-$	123.16	123.4[e]	100.0 ± 1.2
Glyceraldehyde	$C_3H_6O_3 \rightarrow 3CO_2 + 12e^-$	133.2	125.0[d]	93.9 ± 0.4
Glycerol	$C_3H_8O_3 \rightarrow 3CO_2 + 14e^-$	151.99	145.2[e]	95.5 ± 0.0
Glycine	$C_2H_5O_2N \rightarrow 2CO_2 + NH_3 + 6e^-$	79.93	78.1	97.7 ± 0.4
Glycolic acid	$C_2H_4O_3 \rightarrow 2CO_2 + 6e^-$	78.90	79.3[e]	100.5 ± 0.0
Guanine	$C_5H_5ON_5 \rightarrow 5CO_2 + 5NH_3 + 8e^-$	52.93	61.3[e]	115.9 ± 2.8
Histidine	$C_6H_9O_2N_3 \rightarrow 6CO_2 + 3NH_3 + 20e^-$	128.90	136.0[d]	105.5 ± 3.3
Isoleucine	$C_6H_{13}O_2N \rightarrow 6CO_2 + NH_3 + 30e^-$	228.69	225.7	98.7 ± 0.2
Leucine	$C_6H_{13}O_2N \rightarrow 6CO_2 + NH_3 + 30e^-$	228.69	223.0[e]	97.5 ± 0.2
Lysine	$C_6H_{15}O_2N_2Cl \rightarrow 6CO_2 + 2NH_3 + \frac{1}{2}Cl_2 + 29e^-$	158.77	156.5[d]	98.6 ± 2.3
	$\rightarrow 6CO_2 + 2NH_3 + Cl^- + 28e^-$	153.30	156.5[d]	102.1 ± 2.4
Malic acid	$C_4H_6O_5 \rightarrow 4CO_2 + 12e^-$	89.49	88.2[e]	98.6 ± 0.5
Mannitol	$C_6H_{14}O_6 \rightarrow 6CO_2 + 26e^-$	142.72	137.7[d]	96.5 ± 0.8
Methionine	$C_5H_{11}O_2NS \rightarrow 5CO_2 + NH_3 + H_2SO_4 + 30e^-$	201.06	131.9[d]	65.6 ± 2.3
	$\rightarrow 5CO_2 + NH_3 + S + 24e^-$	160.85	131.9[d]	82.0 ± 2.8
Nicotinic acid	$C_6H_5O_2N \rightarrow 6CO_2 + NH_3 + 22e^-$	178.70	7.9[e]	4.4 ± 2.2
Phenylalanine	$C_9H_{11}O_2N \rightarrow 9CO_2 + NH_3 + 40e^-$	242.15	238.0	98.3 ± 0.2
Phthalate	$KHC_8H_4O_4 \rightarrow K^+ + 8CO_2 + 30e^-$	146.89	146.0[d]	99.4 ± 0.4
Proline	$C_5H_9O_2N \rightarrow 5CO_2 + NH_3 + 22e^-$	191.09	180.0[d]	94.2 ± 1.0
Propionate	$NaC_3H_5O_2 \rightarrow Na^+ + 3CO_2 + 14e^-$	145.74	141.4[d]	97.0 ± 0.2
Pyruvate	$NaC_3H_3O_3 \rightarrow Na^+ + 3CO_2 + 10e^-$	90.88	89.3[d]	98.3 ± 0.8
Ribose	$C_5H_{10}O_5 \rightarrow 5CO_2 + 20e^-$	133.22	130.2[d]	95.7 ± 0.6
Serine	$C_3H_7O_3N \rightarrow 3CO_2 + NH_3 + 10e^-$	95.15	94.6[e]	99.4 ± 0.0
Succinate	$Na_2C_4H_4O_4 \cdot 6H_2O \rightarrow 2Na^+ + 4CO_2 + 14e^-$	51.82	51.0[d]	98.4 ± 0.4
Sulfur	$S_8 \rightarrow 8SO_4^{2-} + 48e^-$	187.13	51.5	27.5 ± 1.7
Threonine	$C_4H_9O_3N \rightarrow 4CO_2 + NH_3 + 16e^-$	134.32	132.3[e]	98.5 ± 0.5
Thymine	$C_5H_6O_2N_2 \rightarrow 5CO_2 + 2NH_3 + 16e^-$	126.86	141.2	111.3 ± 0.8
Tryptophan	$C_{11}H_{12}O_2N_2 \rightarrow 11CO_2 + 2NH_3 + 46e^-$	225.24	220.9[e]	98.1 ± 0.0
Tyrosine	$C_9H_{11}O_3N \rightarrow 9CO_2 + NH_3 + 38e^-$	209.72	205.6[e]	98.1 ± 0.0
Uracil	$C_4H_4O_2N_2 \rightarrow 4CO_2 + 2NH_3 + 10e^-$	89.21	98.8[d]	110.8 ± 0.9
Valine	$C_5H_{11}O_2N \rightarrow 5CO_2 + NH_3 + 24e^-$	204.87	198.5[d]	96.9 ± 0.5

[a] Water and H^+/OH^- components of water not included.
[b] Unless specified otherwise, numbers are means of triplicate analyses.
[c] Calculated as [(observed mean ± standard deviation) × 100]/theoretical.
[d] At least four to six total replicate analyses from at least two different runs.
[e] Duplicate analyses.

TABLE 3. *Effect of dichromate normality on the accuracy and precision of dichromate oxidation for determining the carbon-bound electron compositions of microbial components and substrates*[a]

Compound	Carbon-bound electron composition (me⁻eq/liter)[b] for dichromate normality of:						
	0.125 N		0.0625 N	0.03125 N		0.00625 N	
	Mean ± SD	% of theoretical	Mean ± SD	Mean ± SD	% of theoretical	Mean ± SD	% of theoretical
Theoretical	200.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	50.0 ± 0.0	100.0 ± 0.0	5.0 ± 0.0	100.0 ± 0.0
Acetate ($Na \cdot 3H_2O$)	190.1 ± 1.6	95.1 ± 0.8	97.9 ± 0.4[c]	47.7 ± 0.9	95.5 ± 1.8	4.7 ± 0.1	94.3 ± 2.0
Adenine	210.4 ± 3.3	105.2 ± 1.7	106.2 ± 4.3[c]	53.9 ± 0.9	107.9 ± 1.8	6.1 ± 0.3	122.0 ± 5.9
Alanine			99.2 ± 0.5[d]	49.1 ± 0.1[d]	98.1 ± 0.2	3.0 ± 0.1	59.7 ± 2.8
Butyrate (Na)	194.9 ± 0.0	97.5 ± 0.0	97.2 ± 0.3[c]	48.0 ± 0.3	96.0 ± 0.5	4.7 ± 0.1	94.0 ± 1.4
Cellobiose			95.7 ± 0.2	47.0 ± 0.0	94.1 ± 0.0		
Cystine	198.8 ± 1.4	99.4 ± 0.7	95.8 ± 2.9[c]	44.6 ± 1.1	89.1 ± 2.1	4.7 ± 0.2	93.7 ± 4.0
Cytosine	224.5 ± 1.4	112.3 ± 0.7	118.7 ± 0.8[d]	64.9 ± 0.6[d]	129.8 ± 1.2	5.7 ± 0.3	113.4 ± 6.0
Glucose	194.4 ± 0.5	97.2 ± 0.2	96.0 ± 0.8[c]	47.1 ± 0.0[c]	94.3 ± 0.0	4.7 ± 0.1[c]	93.6 ± 1.9
Glutamic acid			99.9 ± 0.0[d]	50.2 ± 0.0	100.4 ± 0.0	4.6 ± 0.3	91.2 ± 5.1
Histidine	217.1 ± 1.2	108.6 ± 0.6	105.5 ± 3.3[c]	51.4 ± 0.6	102.8 ± 1.2	5.1 ± 0.1	102.8 ± 1.8
Leucine	199.1 ± 0.5	99.6 ± 0.2	97.5 ± 0.2[d]	48.5 ± 0.1	97.0 ± 0.2	4.8 ± 0.2	96.6 ± 3.0
Lysine (HCl)			100.4 ± 2.3[c]	47.9 ± 0.3	95.6 ± 0.7	4.5 ± 0.2	89.3 ± 4.9
Mannitol	195.1 ± 0.0	97.5 ± 0.0	96.5 ± 0.8[c]	46.9 ± 0.1	93.7 ± 0.2	4.6 ± 0.1	92.9 ± 1.4
Phthalate (K.H.)	198.7 ± 0.5	99.3 ± 0.2	99.4 ± 0.4[c]	49.8 ± 0.1	99.6 ± 0.3	4.9 ± 0.1	99.0 ± 2.2
Proline	194.7 ± 2.0	97.4 ± 1.0	94.2 ± 1.0[c]	40.3 ± 1.3	80.6 ± 2.6	2.5 ± 0.4	49.7 ± 7.9
Ribose	194.2 ± 0.5	97.1 ± 0.2	95.7 ± 0.6[c]	46.8 ± 0.1	93.7 ± 0.2	4.6 ± 0.2	91.4 ± 4.1
Succinate ($Na_2 \cdot 6H_2O$)	197.7 ± 0.9	98.9 ± 0.5	98.4 ± 0.4[c]	49.0 ± 0.3[c]	97.9 ± 0.7	5.0 ± 0.0	99.8 ± 0.8
Uracil	213.0 ± 3.3	106.5 ± 1.6	110.8 ± 0.9[c]	54.4 ± 1.0	108.8 ± 2.0	5.5 ± 0.2	110.1 ± 4.1

[a] Reagents for the 0.125, 0.0625, 0.03125, and 0.00625 N dichromate oxidation systems were 0.5, 0.25, 0.125, and 0.025 N $K_2Cr_2O_7$ and 0.2, 0.1, 0.05, and 0.01 N (approximately) $Fe(NH_4)_2(SO_4)_2$, respectively.

[b] Unless specified otherwise, numbers are means of triplicate analyses ± standard deviation (SD). Percentages of theoretical values were calculated as [(observed mean ± SD) × 100]/theoretical.

[c] Based on at least four to six (total) replicate analyses from at least two different runs.

[d] Based on duplicate analyses.

TABLE 4. *Effect of dichromate normality on the carbon-bound electron composition of microbial cells*[a]

Species	Carbon-bound electron composition (me⁻eq/liter)[b] for dichromate normality of:					
	0.0625 N		0.03125 N		0.00625 N	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
E. coli	90.1	8.3 ± 1.0 (98.0 ± 1.1)	45.1	42.6 ± 0.5 (94.5 ± 1.1)	4.5	4.1 ± 0.1 (91.1 ± 2.2)
C. cartalyticum	107.6	105.4 ± 1.0 (98.0 ± 0.9)	53.8	52.3 ± 0.7 (97.2 ± 1.3)	5.4	4.7 ± 0.2 (87.0 ± 3.7)
A. vinelandii	139.6	136.8 ± 1.4 (98.0 ± 1.0)	69.8	67.3 ± 1.8 (96.4 ± 2.6)	6.8	6.2 ± 0.2 (91.2 ± 2.9)

[a] Reagents as for Table 2.

[b] Theoretical values are based on an assumed 98% recovery for 0.0625 N dichromate-oxidized cells. Observed are averages of duplicate analyses; numbers in parentheses are observed values expressed as percentages of theoretical values.

from declining accuracy for many biological materials (Tables 3 and 4) and are not recommended for general use. However, for C-bound electron levels of <10 me⁻eq/liter, use of 0.00625 N dichromate cannot be avoided (Table 6). Such levels are characteristic of the filtrates of energy-limited growth yield cultures, where the primary concern is validation of substrate exhaustion, and the 5 to 10% recovery decline associated with the use of 0.00625 N dichromate for many pure compounds (Table 3) and bacterial cell materials (Table 4) is quantitatively (e.g., for a typical 200-me⁻eq/liter growth system) not a major source of error, particularly if appropriate predetermined correction factors (e.g., see Table 4) are available.

Calculation of carbon-bound electron composition from empirical formulas and cell CHN data. The general half-reaction equation describing the C-bound electron content of an organic material containing nitrogen in the fully reduced, −3-valent, form is:

TABLE 5. *Theoretical versus 0.0625 N dichromate oxidation-derived carbon-bound electron composition of microbial cells*[a]

Compound	Amt in cells		
	Molar[b] (mmol/g)	Carbon-bound electrons (me^-eq/g)	
		Theoretical[c]	Dichromate oxidation-derived[d]
Protein			
Alanine	0.454	5.45	5.41
Arginine	0.252	5.54	6.83
Aspartate	0.201	2.41	2.41
Asparagine	0.101	1.21	1.21
Cysteine	0.101	1.82[e]	1.74
Glutamate	0.353	6.35	6.34
Glutamine	0.201	3.62	3.62
Glycine	0.403	2.42	2.36
Histidine	0.050	1.00	1.06
Isoleucine	0.252	7.56	7.46
Leucine	0.403	12.09	11.79
Lysine	0.403	11.28	11.33
Methionine	0.201	6.03[f]	3.96
Phenylalanine	0.151	6.04	5.94
Proline	0.252	5.54	5.22
Serine	0.302	3.02	3.00
Threonine	0.252	4.03	3.97
Tryptophan	0.050	2.30	2.26
Tyrosine	0.101	3.84	3.77
Valine	0.302	1.81	1.75
Polymer	(52.4%)	93.36	91.43 (98%)
Polysaccharide			
Hexose	1.026	24.62	23.64
Polymer	(16.6%)	24.62	23.64 (96%)
Nucleic acid			
Ribose	0.447	8.94	8.56
Deoxyribose	0.096	2.11	2.02
Thymine	0.024	0.38	0.43
Adenine	0.140	1.40	1.49
Guanine	0.140	1.12	1.30
Cytosine	0.140	1.40	1.66
Uracil	0.115	1.15	1.27
Polymer	(17.8%)	16.50	16.73 (101%)
Lipid			
Glycerol	0.140	1.96	1.87
Palmitate	0.280	25.76	25.04
Polymer	(9.7%)	27.72	26.91 (97%)
Total organic polymers	(96.5%)	162.2	158.7 (98%)

[a] Calculated from data of Morowitz (12) and Stouthamer (21).

[b] Numbers in parentheses are percentages of cell dry weight.

[c] Calculated from Table 2. Carbon-bound electron compositions of aspartate, cysteine, deoxyribose, and palmitate are 12, 10(+ 8 S-bound e^-), 22, and 92 e^-eq/mol, respectively.

[d] Calculated from Table 2. Percent recoveries for aspartate, cysteine, deoxyribose, and palmitate were assumed to be the same as those for asparagine, cystine, ribose, and butyrate, respectively. Percentages used for arginine and lysine were 123.3 and 100.4, respectively. Numbers in parentheses are percentages of theoretical values.

[e] Includes 0.81 S-bound me^-eq/g of cells (Table 2).

[f] Includes 1.61 S-bound me^-eq/g of cells (Table 2).

$$(C_aH_bO_cN_d)^v + fH_2O = aCO_2 + dNH_4^+ + mH^+ + ne^- \quad (7)$$

where v is the net ionic charge and n is the number of carbon-bound electrons per empirical mole of the material. Calculation of n is accomplished as:

$$n = 4a + b - 2c - 3d - v \quad (8)$$

Useful carbon-bound electron composition parameters calculated from n include: equivalent weight = formula weight/n; electron richness of C = n/a; average oxidation state of C = $4 - n/a$. Thus, for a typical bacterial cell composition of $C_4H_7O_{1.5}N$ + 10% inorganics (Table 1, line 11): n = 17 e^-eq/mol of cell N; formula weight = 93.11/0.9 = 103.46 g/mol; equivalent weight = 103.46/17 = 6.09 g/e^-eq; electron richness of C = 17/4 = 4.25 e^-eq/mol of cell C; average oxidation state of C = 4 − 4.25 = −0.25.

Knowledge of the inorganic content of microbial cells is necessary for calculation of C-bound electron composition from CHN data. Microbial cell inorganic content varies, sometimes widely, between species and according to nutritional conditions and growth rate. For example, assuming that cellular ribonucleic acid (9.7% P) constitutes about 65% of the total cell P (18), then growth rate-related differences in bacterial ribonucleic acid contents of 12 and 31% (19) would be reflected by cell P contents of 2 and 5%, respectively. Such variations, combined with potential complications arising from S, Cl, K, and possibly P volatilization on cell ignition (6), may explain the variable ash contents, e.g., 3% (10) and 10% (8), shown by microbial cells. In practice, nutritionally balanced microbial (bacterial) cells typically show an inorganic content of about 2 to 3% P, 1% S (12, 18, 19, 21), and about 4% other inorganics (19). Cell P exists solely in the fully oxidized, +5-valent, form and is present largely as polymerized ester-linked phosphate (i.e., essentially as HPO_3 or PO_3^-). Accordingly, the oxygen associated with microbial cell P typically comprises about 3 to 4.5% of the cell dry weight, giving a total cell phosphate content of 5 to 8.5% and a resultant total inorganic content of about 10 to 13.5%. In the absence of direct experimental validation of microbial cell inorganic composition, 10% inorganics (8) would appear to be a reasonable, conservative factor for estimating microbial cell "organic" oxygen content, information needed for calculation of the empirical CHON formula used for C-bound electron composition determination (Table 1). For example, cells of *Klebsiella pneumoniae* have a composition of about 47.5% C, 13.0% N, and 7.1% H (5), and this, assuming 10% inorganics, identifies 22.4% organic O and a resultant empirical formula of $C_{4.26}H_{7.59}O_{1.51}N$ with n = 18.61 e^- (Table 1, line 2).

TABLE 6. *Recommended volumes and reagent normalities for different ranges of sample concentration*

Dichromate oxidation normality	Sample		$K_2Cr_2O_7$ reagent		Ag_2SO_4/H_2SO_4 vol (ml)	Final vol before titration (ml)	$Fe_2(NH_4)_2(SO_4)_2$ reagent		
	Concn range[a] (me⁻eq/liter)	Vol (ml)	Normality (e⁻eq/liter)	Vol (ml)			Approx normality[b] (e⁻eq/liter)	Typical back-titration vol (ml)	
								Digested blank	Sample
0.125	50–375 (10–75%)	10	0.50	10	20	100	0.20	24.8 ± 0.1	22.3–6.1
	33–250 (10–75%)	15	1.00	5	20	100	0.20	24.8 ± 0.1	22.3–6.1
0.0625	25–188 (10–75%)	10	0.25	10	20	100	0.10	24.8 ± 0.1	22.3–6.0
	17–125 (10–75%)	15	0.50	5	20	100	0.10	24.8 ± 0.1	22.3–6.1
	10–17 (6–10%)	15	0.50	5	20	100	0.10	24.8 ± 0.1	23.3–22.3
0.00625[c]	2.5–10 (10–40%)	10	0.025	10	20	100	0.01	21.3 ± 0.2	18.8–11.3

[a] Numbers in parentheses are percent dichromate consumption.
[b] Changes with time.
[c] Not recommended for samples of >10 me⁻eq/liter (see text).

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