SUPPLEMENTAL MATERIAL

Text S1

Thermodynamic symbols and units

aq = aqueous state

$\Delta_r \mathrm{ATP}(l)$= change in ATP for reaction l (mol/mol)

$c_{i,t}$ = concentration of reactant i at time t (M)

$C^{\circ}_{pm}(j)$ = standard molar heat capacity of species j (J/mol/K)

$C'^{0}_{pm}(j)$ = standard transformed molar heat capacity of species j (J/mol/K)

I = ionic strength (M)

g = gaseous state

$\Delta_f G^0_j$ = standard Gibbs energy of formation of species j (kJ/mol)

$\Delta_f G'^0_j$ = standard transformed Gibbs energy of formation of species j (kJ/mol)

$\Delta_f G'^0_i$ = standard transformed Gibbs energy of formation of reactant i (kJ/mol)

$\Delta_r G'^0(l)$ = standard transformed Gibbs energy of reaction l (kJ/mol)

$\Delta_f H^0_j$ = standard enthalpy of formation of species j (kJ/mol)

$\Delta_f H'^0_j$ = standard transformed enthalpy of formation of species j (kJ/mol)

$\Delta_f H'^0_i$ = standard transformed enthalpy of formation of reactant i (kJ/mol)

$\Delta_r H'^0(l)$ = standard transformed enthalpy of reaction l (kJ/mol)

$\Delta_r H_t$ enthalpy of reaction at time t (kJ/mol)

$\Delta_r H(\mathrm{Buff})$ = enthalpy of dissociation of buffer (kJ/mol)

$\Delta_r H_t(\mathrm{cal})$ = enthalpy change in the calorimetric experiment at time t (kJ/mol)

N' = number of reactants

$N_{\mathrm{C}}(j)$ = number of carbon atoms in species j (mol/mol)

$\overline{N}_C(i)$ = number of carbon atoms in reactant i (mol/mol)

$N_{\mathrm{H}}(j)$ = number of hydrogen atoms in species j (mol/mol)

$\overline{N}_{\mathrm{H}}(i)$ = number of hydrogen atoms in reactant i (mol/mol)

$\Delta_{\mathrm{r}} N_{\mathrm{H}}(l)$ = change in binding of hydrogen atoms in reaction l (mol/mol)

q = heat flow into a system (kJ)

$\frac{dq_t}{Vdt}$ = rate of heat production (W/L)

$\frac{dq_t^*}{Vdt}$ = rate of heat production corrected for protonation of buffer (W/L)

$\frac{q_t}{V}$ = integrated heat production (kJ/L)

$\frac{q_t^*}{V}$ = integrated heat production corrected for protonation of buffer (kJ/L)

r_j = mol fraction of species j in a reactant (mol/mol)
T = temperature (K)

V = volume (L)

$v_i'(l)$ = stoichiometric number of reactant i in reaction l (mol/mol)
z_j = charge number of species

TABLE S1 Thermodynamic properties of species and linkages relevant to glucose use in rumen microbes (T = 25˚C, pH = 0, I = 0)[a]

Reactant	Species or linkage	z_j	$N_{\mathrm{H}}(j)$	$N_{\mathrm{C}}(j)$	$\Delta_{\mathrm{f}} G_j^\circ$	$\Delta_{\mathrm{f}} H_j^\circ$	Source	$C_{pm}^\circ(j)$	Source
Acetate	CH_3COO^-(aq)	-1	3	2	-369.32	-486.01	(1)	86.19	(1)
	CH_3COOH(aq)	0	4	2	-396.48	-485.76	(1)	169.70	(1)
Butyrate	$CH_2(CH_2)_2COO^-$(aq)	-1	7	4	-354.18	-538.19	(1)	133.05	(1)
	$CH_2(CH_2)_2COOH$(aq)	0	8	4	-381.62	-535.34	(1)	127.61	(1)
CO_2(g)	CO_2(g)	0	0	1	-394.36	-393.5	(2)	37.11	(3)
CO_2tot	CO_2(aq) + H_2O(l)	0	2	1	-623.16	-699.64	(2)	318.29	(4)
	CO_3^{-2}(aq)	-2	0	1	-527.81	-677.14	(2)	-55.00	(5)
	HCO_3^-(aq)	-1	1	1	-586.77	-691.99	(2)	-302.00	(5)
	H_2CO_3(aq)	0	2	1	-606.33	-694.91	(2)	69.00	(5-6)
Fructose	$C_6H_{12}O_6$(aq)	0	12	12	-915.51	-1259.38	(2)	369.00	(7)
Galactose	$C_6H_{12}O_6$(aq)	0	12	12	-908.93	-1255.20	(2)	319.00	(7)
Glucose	$C_6H_{12}O_6$(aq)	0	12	6	-915.90	-1262.19	(2)	336.30	(7)
H^+	H^+(aq)	1	1	0	0.00	0.00	(2)	0.00	(3)
H_2(g)	H_2(g)	0	2	0	0.00	0.00	(2)	28.82	(3)
H_2(aq)	H_2(g)	0	2	0	0.00	0.00	(2)	28.82	(3)
H_2O	H_2O(l)	0	2	0	-237.19	-285.83	(2)	75.29	(3)
Lactate	$CH_3CH(OH)COO^-$(aq)	-1	5	2	-516.72	-686.64	(2)	211.29	(8)
Mannose	$C_6H_{12}O_6$(aq)	0	12	12	-910.00	-1258.66	(2)	342.00	(7)
CH_4(g)	CH_4(g)	0	4	1	-50.72	-74.81	(2)	35.31	(3)
CH_4(aq)	CH_4(aq)	0	4	1	-34.33	-89.04	(2)	0.00	NA[b]
Propionate	$CH_3CH_2COO^-$(aq)	-1	5	3	-363.09	-513.08	(1)	110.88	(1)
	CH_3CH_2COOH(aq)	0	6	3	-390.99	-512.41	(1)	253.13	(1)
Reserve carbohydrate	Glucose-glucose (α1→4) linkage	0	0	0	-15.65	4.53	(9-10)	39.50	(9)

	Glucose-glucose (α1→6) linkage	0	0	0	-7.06	-5.80	(11-12)	0.00	NA
Valerate	$CH_3(CH_2)_3COO^-$(aq)	-1	9	5	-345.72	-562.25	(1)	160.25	(1)
	$CH_3(CH_2)_3COOH$(aq)	0	10	5	-373.38	-559.36	(1)	260.66	(1)

[a] See Text S1 in the supplemental material for symbols and units

[b] Not available and assumed to be 0

TABLE S2 Thermodynamic properties of species and linkages relevant to glucose use in rumen microbes (T = 39°C unless otherwise noted, pH = 6.8, I = 0.25)[a,b]

Reactant	Species or linkage	$C'^{0}_{pm}\ (j)$	$\Delta_f G'^{0}_{j}$	$\Delta_f H'^{0}_{j}$	r_j
Acetate	$CH_3COO^-(aq)$	75.48	-241.09	-485.93	0.995
	$CH_3COOH(aq)$	148.29	-227.46	-485.64	0.005
Butyrate	$CH_2(CH_2)_2COO^-(aq)$	100.93	-57.88	-539.71	0.994
	$CH_2(CH_2)_2COOH(aq)$	84.79	-44.62	-538.07	0.006
$CO_2(g)$	$CO_2(g) + H_2O(l)$	37.11	-394.43	-392.98	1.000
CO_2tot	$CO_2(aq) + H_2O(l)$	307.59	-537.31	-696.31	0.161
	$CO_3^{-2}(aq)$	-33.59	-524.34	-675.65	0.014
	$HCO_3^-(aq)$	-302.00	-541.29	-696.22	0.837
	$H_2CO_3(aq)$	58.29	-519.75	-695.07	0.000
Fructose	$C_6H_{12}O_6(aq)$	304.77	-399.30	-1260.51	1.000
Galactose	$C_6H_{12}O_6(aq)$	254.77	-395.31	-1257.32	1.000
Glucose	$C_6H_{12}O_6(aq)$	272.07	-405.06	-1264.25	1.000
$H_2(g)$	$H_2(g)$	18.12	82.45	-0.72	1.000
$H_2(aq)$	$H_2(aq)$	-10.71	101.09	-5.33	1.000
H_2O	$H_2O(l)$	64.59	-152.49	-285.90	1.000
Lactate	$CH_3CH(OH)COO^-(aq)$	189.88	-303.61	-685.94	1.000
Mannose	$C_6H_{12}O_6(aq)$	277.77	-390.75	-1260.02	1.000
$CH_4(g)$	$CH_4(g)$	13.90	115.32	-76.57	1.000
$CH_4(aq)$	$CH_4(aq)$	-21.41	133.17	-91.30	1.000
Propionate	$CH_3CH_2COO^-(aq)$	89.47	-150.84	-513.79	0.993
	$CH_3CH_2COOH(aq)$	221.01	-138.06	-512.24	0.007
Reserve carbohydrate	Glucose-glucose (α1→4) linkage	39.50	-16.62	5.08	NA[c]
	Glucose-glucose (α1→6) linkage	0.00	-7.12	-5.80	NA
Valerate	$CH_3(CH_2)_3COO^-(aq)$	117.42	34.55	-564.51	0.993
	$CH_3(CH_2)_3COOH(aq)$	207.13	47.52	-561.35	0.007

[a] See Text S1 in the supplemental material for symbols and units

[b] T = 25°C for $C'^{0}_{pm}\ (j)$

[c] Not applicable

TABLE S3 Thermodynamic properties of reactants relevant to glucose use in rumen microbes (*T* = 39˚C, pH = 6.8, *I* = 0.25)[a]

Reactant	$\Delta_\mathrm{f}G'^0_i$	$\Delta_\mathrm{f}H'^0_i$	$\overline{N}_\mathrm{H}(i)$	$\overline{N}_C(i)$
Acetate	-241.11	-485.93	3.01	2.00
Butyrate	-57.90	-539.70	7.01	4.00
CO_2(g)	-394.43	-392.98	0.00	1.00
CO_2tot	-541.45	-696.20	2.00	1.00
Fructose	-399.30	-1260.51	12.00	6.00
Galactose	-395.31	-1257.32	12.00	6.00
Glucose	-405.06	-1264.25	12.00	6.00
H_2(g)	83.05	-0.72	2.00	0.00
H_2(aq)	101.69	-5.33	2.00	0.00
H_2O	-152.49	-285.90	2.00	0.00
Lactate	-303.61	-685.94	5.00	3.00
Mannose	-390.75	-1260.02	12.00	6.00
CH_4(g)	116.52	-76.57	4.00	1.00
CH_4(aq)	134.37	-91.30	4.00	1.00
Propionate	-150.86	-513.78	5.01	3.00
Reserve carbohydrate	-268.60	-973.94	10.00	6.00
Valerate	34.53	-564.49	9.01	5.00

[a] See Text S1 in the supplemental material for symbols and units

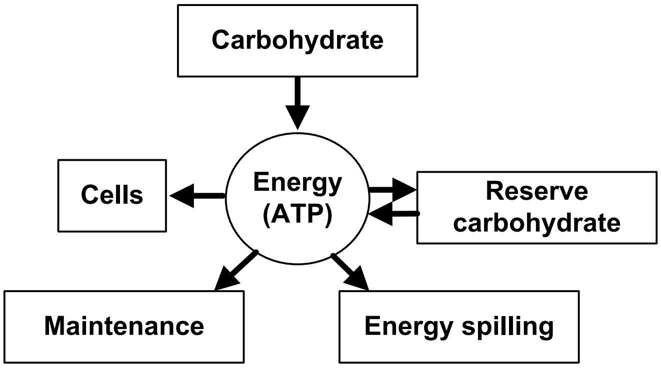


FIG S1 Use of carbohydrate energy by microbes. Adapted from (13).

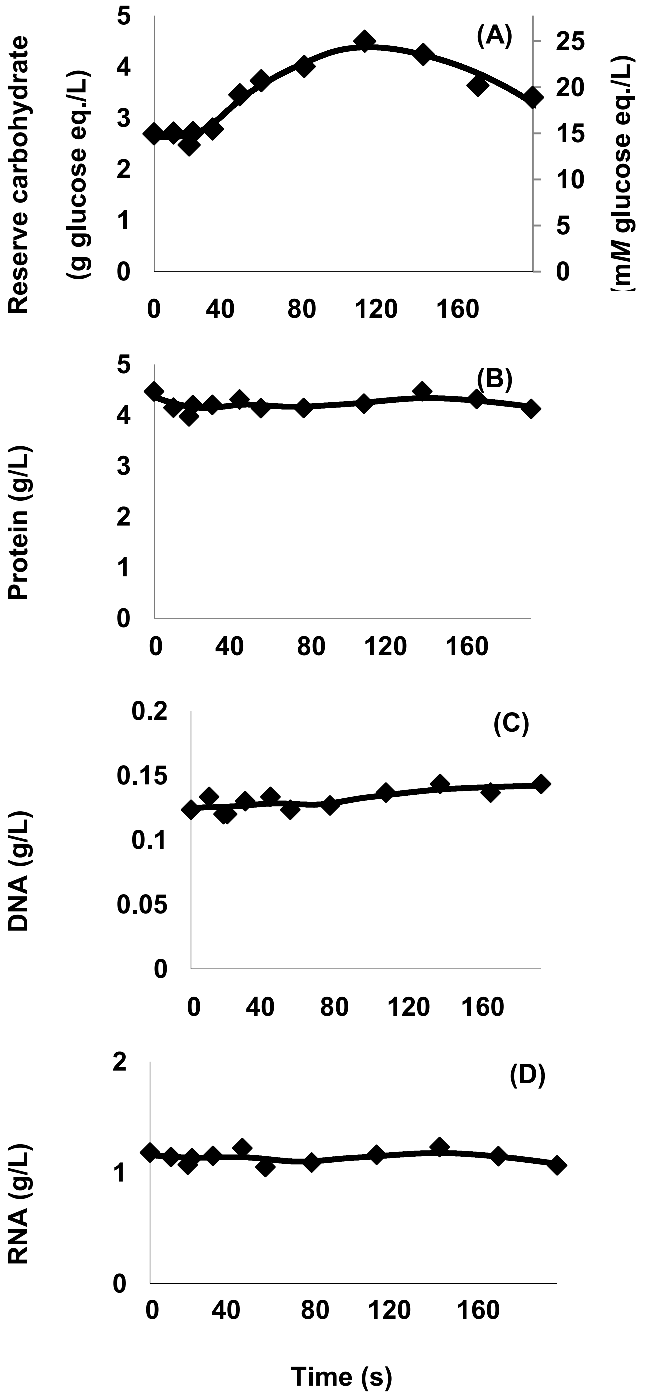
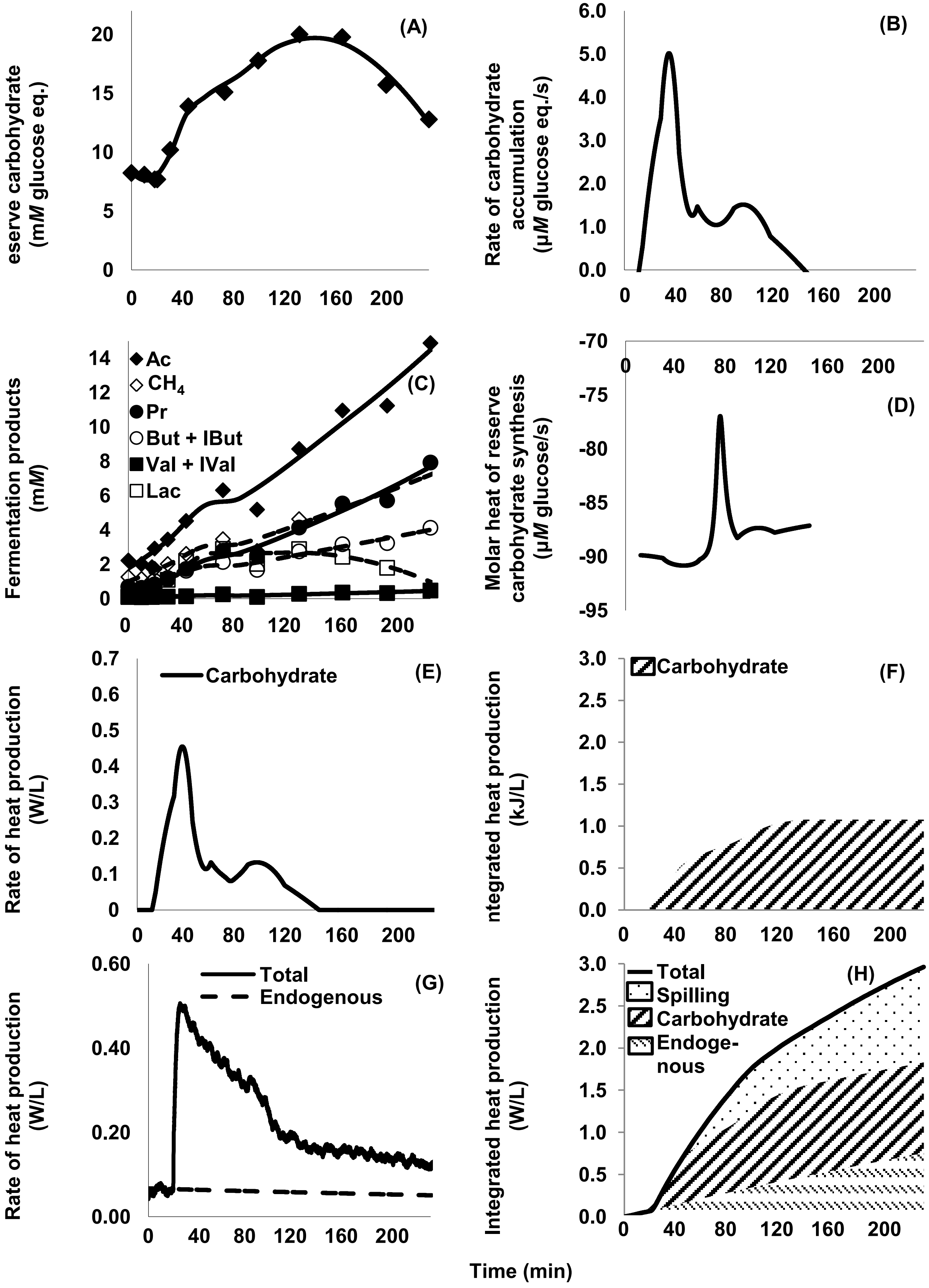


FIG S2 Cell components of mixed rumen microbes in response to 20 m*M* glucose dosed at 20 min. (A) Reserve carbohydrate. (B) Protein. (C) DNA. (D) RNA. Reserve carbohydrate was expressed in g/L (for comparison to other components) and m*M* (for comparison to concentration of glucose dosed), both in monomeric glucose equivalents. Data represent one experiment, and each datum point represents one sample that was analyzed in triplicate.

FIG S3 Example illustration of the calculations and overall approach used to quantify energy-spilling from mixed ruminal microbes from cow 472 dosed with 20 m*M* of glucose. Panel A (reserve carbohydrate) appears in the main text as Fig. 1D. The units of reserve carbohydrate concentration are expressed in monomeric glucose equivalents to be in same units as the glucose dose. From these data, the slope corresponding with each 1-s time increment was plotted in panel B and designated as the numerical derivative of reserve carbohydrate with respect to time. Fermentation products over time are presented in Panel C, which appears in the main text as Fig. 1H. The molar heat of reserve carbohydrate synthesis over time (Panel D) was calculated when individual measured values of end-products from Panel C and the parameter values in Table 1 were inputted into Equation 7 from the text. The rate of heat production accounted by reserve carbohydrate synthesis is shown in Panel E. At 1-s time intervals, these points were calculated by multiplying values illustrated in Panel B by absolute values illustrated in Panel D. The integrated heat production accounted by reserve carbohydrate synthesis (Panel F) was calculated using the rectangle method for each 1-s time increment. The rates of total heat production after glucose dosage and from endogenous metabolism are both shown in Panel G (which also appears in the main text as Fig. 1J). Total rate of total heat production is output from the calorimeter. The rate of endogenous heat production was set equal to the average rate of heat production prior to dosing. It was assumed that this would not remain constant over the incubation, but it would decline by 7.3%/h (see Results). At each 1-s time increment, these rates were integrated using the rectangle rule. These integrated heat production values over time are illustrated in Panel H (which also appears as Fig. 3B in the main text). The integrated heat production accounted by reserve carbohydrate synthesis (i.e., the area from Panel F) was stacked on top of endogenous heat production in Panel G. The estimate of energy spilling was determined by difference of the integrated total heat production minus the integrated heat production that was accounted by endogenous metabolism minus the integrated heat production that was accounted by reserve carbohydrate synthesis.

FIG S3



(A)
eserve carbohydrate (m*M* glucose eq.)
0
5
10
15
20
0
40
80
120
160
200
(B)
Rate of carbohydrate accumulation (µ*M* glucose eq./s)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
0
40
80
120
160
200
(C)
Fermentation products (m*M*)
◆Ac
◇CH_4
●Pr
○But + IBut
■Val + IVal
□Lac
0
2
4
6
8
10
12
14
0
40
80
120
160
200
(D)
Molar heat of reserve carbohydrate synthesis (µ*M* glucose/s)
-70
-75
-80
-85
-90
-95
0
40
80
120
160
200
(E)
Carbohydrate
Rate of heat production (W/L)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
40
80
120
160
200
(F)
▨Carbohydrate
ntegrated heat production (kJ/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
40
80
120
160
200
(G)
Total
Endogenous
Rate of heat production (W/L)
0.00
0.20
0.40
0.60
0
40
80
120
160
200
(H)
Total
Spilling
Carbohydrate
Endoge-nous
Integrated heat production (W/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
40
80
120
160
200
Time (min)

SUPPLEMENTAL MATERIAL REFERENCES

1. **Shock EL.** 1995. Organic acids in hydrothermal solutions: standard molal thermodynamic properties of carboxylic acids and estimates of dissociation constants at high temperatures and pressures. Am J Sci **295:**496-580.
2. **Alberty RA.** 2003. Thermodynamics of biochemical reactions. John Wiley & Sons, Hoboken, NJ, USA.
3. **Wagman DD, Evans WH, Parker VB, Schumm RH, Halow I, Bailey SM, Churney KL, Nuttall RL.** 1982. The NBS tables of chemical thermodynamic properties. selected values for inorganic and C_1 and C_2 organic substances in SI units. J Phys Chem Ref Data **11 Suppl 2**.
4. **Barbero JA, Hepler LG, McCurdy KG, Tremaine PR.** 1983. Thermodynamics of aqueous carbon dioxide and sulfur dioxide: heat capacities, volumes, and the temperature dependence of ionization. Can J Chem **61:**2509-2519.
5. **Larson JW, Zeeb KG, Hepler LG.** 1982. Heat capacities and volumes of dissociation of phosphoric acid (1st, 2nd, and 3rd), bicarbonate ion, and bisulfate ion in aqueous solution. Can J Chem **60:**2141-2150.
6. **Goldberg RN, Kishore N, Lennen RM.** 2002. Thermodynamic quantities for the ionization reactions of buffers. J Phys Chem Ref Data **31:**231-370.
7. **Goldberg RN, Tewari YB.** 1989. Thermodynamic and transport properties of carbohydrates and their monophosphates: the pentoses and hexoses. J Phys Chem Ref Data **18:**809.
8. **Wilhoit RC.** 1969. Thermodynamic properties of biochemical substances, p. 305–317. *In* HD Brown (ed), Biochemical microcalorimetry. Academic Press, New York, NY, USA.
9. **Goldberg RN, Bell D, Tewari YB, McLaughlin MA.** 1991. Thermodynamics of hydrolysis of oligosaccharides. Biophys Chem **40:**69-76.
10. **Tewari YB, Lang BE, Decker SR, Goldberg RN.** 2008. Thermodynamics of the hydrolysis reactions of 1, 4-β-D-xylobiose, 1, 4-β-D-xylotriose, D-cellobiose, and D-maltose. J Chem Thermodynamics **40:**1517-1526.
11. **Tewari YB, Goldberg RN.** 1989. Thermodynamics of hydrolysis of disaccharides. Cellobiose, gentiobiose, isomaltose, and maltose. J Biol Chem **264:**3966-3971.
12. **Tewari YB, Goldberg RN.** 1991. Thermodynamics of hydrolysis of disaccharides. Lactulose, alpha-D-melibiose, palatinose, D-trehalose, D-turanose and 3-o-beta-D-galactopyranosyl-D-arabinose. Biophys Chem **40:**59-67.
13. **Russell JB.** 2007. The energy spilling reactions of bacteria and other organisms. J Mol Microbiol Biotechnol **13:**1-11.