CENG364 SPRING 2009

QUANTITATIVE METABOLISM – WORKED EXAMPLES

Topic 3: ATP and NAD(H) production during metabolism

Topic 5 : Heat evolution during metabolism

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Worked Example:

Acetobacter bacteria are able to use ethanol (C_2H_6O) as their carbon and energy source to form the end products and acetate ($C_2H_4O_2$) and carbon dioxide (CO_2).

(1) Would you expect the formation of NAD(H) in any or all of the above reactions??

(2) R.A.Alberty in "Thermodynamics of Biochemical Reactions" (Wiley,2003) tabulates basic thermodynamic data for species at 298.15K in dilute aqueous solutions and has the following values for Heat of Formation for species relevant to the above reactions:

Lactate	-686.64
Ethanol	-288.30
Acetate	-486.01
Water	-191.17
NAD^+	0.0
NAD(H)	-31.94
Phosphate (Pi)	-1299.00
ADP	-2626.54
ATP	-3619.91
H^+	0.0
CO_2	-699.63

Which of the above reactions are capable of being able to form ATP ? What further information would be necessary to ensure that ATP formation occurred?

For the purposes of the calculation the formation of ATP should be regarded as a separate (coupled) reaction represented by:

$$ADP + Pi = ATP + H_2O$$

SOLUTION:

Balancing the reaction of ethanol to acetic acid:

C_2H_6O	$\rightarrow$	$C_2H_4O_2$
C: 2		C:2
H:6		H:4
O:1		O:2

$C_2H_6O + O_2 \rightarrow C_2H_4O_2 + H_2O$

C: 2	C:2
H:6	H:6
O:3	O:3

$2NADH + 2H^+ \rightarrow 2NAD^+ + 4H^+ + 4e^-$

$4e^- + O_2 \rightarrow 2O^{2-}$

$2O^{2-} + 4H^+ \rightarrow 2H_2O$

$2NAD^+ + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$

$2NAD^+ + 2H_2O \rightarrow 2NAD^+ + 2H^+ + O_2$

$C_2H_6O + O_2 \rightarrow C_2H_4O_2 + H_2O$

$2NAD^+ + H_2O + C_2H_6O \rightarrow C_2H_4O_2 + 2NADH + 2H^+$

C: 2	C:2
H:8	H:8
O:2	O:2
NAD:2	NAD:2
Charge +2	Charge +2

OK

This balance indicates that 2NAD(H) are produced in this reaction

Are your findings for NAD(H and ATP consistent with the following published metabolism??

Ethanol + $NAD^+ + 2H^+ + 2e^- \rightarrow$ Acetaldehyde + NAD(H) + H^+

Acetaldehyde + CoA + $NAD^+ + 2H^+ + 2e^- \rightarrow$ AcetylCoA + NAD(H) + H^+

AcetylCoA + ATP $\rightarrow$ Acetyl-P + ADP + CoA

Acetyl-P + ADP $\rightarrow$ Acetic Acid + ATP

The finding of 2NAD(H) by a redox balance is consistent with the published pathway.

$2NAD^+ + 2H^+ + 2.5O_2 + C_2H_6O \rightarrow C_2H_4O_2 + 2NADH + 3H_2O$

Using the thermodynamic data:
ATP:

The reaction for ATP formation is:

ADP + $H_2PO_3^{2-}$ = ATP + H_2O

Q = Heat Evolved = Σ ($\Delta H_{formation}$ x Products) - $\Sigma\Delta H_{formation}$ x Reactants)
= (-3,619.91-191.17) –(-2,626.54-1,299)
= (-3,811.08-3,925.44)
= 114.36 kJ / mol

This amount is the minimum energy that must be released in a reaction for ATP to possibly occur. This will not guarantee ATP formation sine we need to establish that the reaction is capable of Substrate Level Phosphorylation (SLP)

For the reaction:

$2NAD^+ + 2H^+ + 2.5O_2 + C_2H_6O \rightarrow C_2H_4O_2 + 2NADH + 3H_2O$

Q = (481.01-2*31.94-3*191.17) – (-288.30- 2*0 – 2*0 – 2.5*11.7)
= -1124.4 - - 317.55 = -806.85

This indicates that sufficient overall energy is released to make overall ATP formation a possibility. Each individual reaction would need to be investigated if we wanted to locate any specific reactions capable of ATP formation. We would laos need to ensure that an enzyme capable of Substrate Level Phoshorylation (S.L.P.) is available for suitable reactions.

Topic 5 : Heat evolution during metabolism
<u>Data from Topic 3 / Homework 1 should be used in this question</u>

An organism converts lactate to acetate and carbon dioxide. The cell yield from lactate is 0.07 g biomass / g lactate. The carbon content of the cell is 48% and the specific growth rate $0.25h^{-1}$.

Calculate the rate of heat evolution (kJ / g biomass /h)making the following assumptions:

1. Lactate is the only carbon and energy source
2. Water is used in the conversion of lactate to acetate and should be considered as a reactant
3. Ammonia is the only nutrient source (H^+ ions are excreted as a product during its use
4. Biomass, H^+, acetate, carbon dioxide and water are the only products
5. The cell formula is: $C_3H_{4.5}N_{0.4285}O_{0.136}$
6. The heat of formation of the biomass is unknown but can be considered to be identical to the heat formation of glucose on an equivalent mass basis. ΔH_f (glucose) = -1,262.19 kJ / mol.
7. The heat of formation of ammonia is -132.51kJ / mol

<u>Solution:</u>

The following molar reaction summarises mixed end product formation by a microorganism using sucrose as the carbon and energy source.

2.924 Sucrose+ 0.3866 NH_3

=

1.4007 Biomass + 3.913 Ethanol + 1.6667 Acetic Acid + 0.5556 Lactic Acid + 5.4545 Carbon Dioxide + 2.2222 Water

The heats of combustion (kJ/mol) have been obtained from a variety of sources:

Sucrose: -2,803
Ammonia: -428.77
Ethanol: -1,366.91
Acetic Acid: -871.69
Lactic Acid: -1,370

The heat of combustion of a cell is 21.82 kJ/ g Biomass

What is the heat evolved by this reaction (kJ / g biomass / h)

The cell formula is $C_{12}H_{19.672}O_{7.377}N_{0.276}$

The specific growth rate is $0.2h^{-1}$

<u>Solution:</u>

Molecular weight of the cell = 12*12+19.672+7.377*12+0.276*14 = 256.06

Q = Heat Evolved = Σ ($\Delta H_{combustion}$ x Products) - $\Sigma\Delta H_{combustion}$ x Reactants)

(1.4007* (-21.82* 256.06) + 3.913 *(-1,366.91) + 1.6667 *(-871.69) + 0.5556 *(-1,370) + 5.4545 *(0) + 2.2222 *(0)
-
(2.294*-2,803 + 0.3866*-438.77)

= (-7,826.03 – 5,348.72 – 1,452.85 – 761.12 + 0 + 0) – (-6,430.08 – 169.63)
= (-15,388.72) + 6,599.71
= -8,789.01 kJ

Q_S = (-8,789.01 kJ / (1.4007*256.06)) * 0.2 = 4.90 kJ / g Biomass /h