

CENG364 SPRING 2009

QUANTITATIVE METABOLISM – WORKED EXAMPLES

Topic 1: Use of other electron acceptors other than oxygen

Topic 2 : Different carbon and energy sources

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

Topic 4: Mass and energy balances – alternative electron acceptor

Topic 5 : Y_{ATP} calculation

Topic 5 : Heat evolution during metabolism

Topic 1: Use of other electron acceptors other than oxygen

Worked Example:

Perchloroethane (C₂Cl₄) may be bioremediated to Trichloroethene (C₂HCl₃) and then to Dichloroethene (C₂H₂Cl₂) and then to Vinyl Chloride (C₂H₃Cl) and then to Ethene(C₂H₄).

In this process each successive compound is used as an electron acceptor.

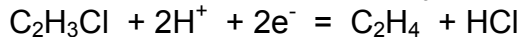
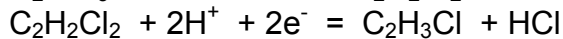
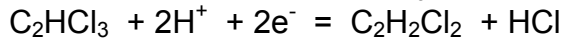
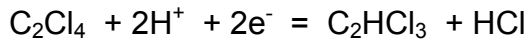
Write down the coupled reactions involving these compounds and NAD(H) which are taking place.

What are three possible sources of the NAD(H)??

Solution:



Coupled with



The NAD(H) either comes from a carbon source which is also a energy source or a non-carbon based energy source. It can also be made by using ATP.

Topic 2 : Different carbon and energy sources

Worked Example:

A microorganism uses Carbon Dioxide (CO₂) as its carbon source for the manufacture of cell material using the Calvin Cycle. It uses Ammonia (NH₃) as its energy source converting it to Nitrate (NO₃⁻).

Oxygen is used as the electron acceptor and the PO ratio (= ATP formed / ½ O₂ = ATP formed / mol NAD(H) regenerated) = 1.5

The specific growth rate of the cell is 0.4 h⁻¹ and the cell is composed of 48% carbon.

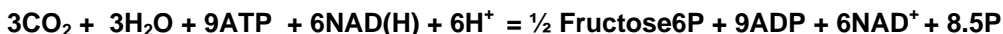
Assume that all carbon used in anabolism originated from Fructose-6-Phosphate, the end product of the Calvin Cycle.

Carbon dioxide uptake is limited to that sufficient to make new cell material only.

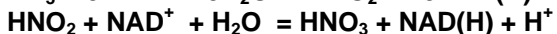
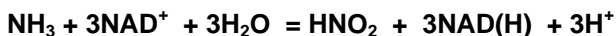
- (a) What is the rate of carbon dioxide uptake (mmol CO₂ / g biomass / h)?
- (b) What is the minimum uptake rate of ammonia (NH₃) (mmols NH₃ / g biomass / h) that must be achieved to allow the processes of energy generation and energy use to be balanced??

SOLUTION:

Calvin Cycle:



Ammonia Oxidation:



The production of new cell material occurs at the following rate :
0.4 g new cell material manufactured / g biomass / h

Assume that the new cell material has carbon in the form of Fructose 6 P (the carbon skeleton)

If 48% of new cell material is carbon, then 0.4 x 0.48 = 0.192 g new carbon used for cell manufacture / g biomass / h.

0.192g carbon is used to make (180/72) x 0.192 g Fructose 6 P

= 0.48 g Fructose 6 P / g biomass / h.
= 0.48 / 180 mols Fructose 6 P / g biomass / h
= 2.667 mmol Fructose 6 P / g biomass / h

This is equivalent to 6x 2.667 mmol of CO₂ / g biomass / h being uptaken
= 16.002 mmol of CO₂ / g biomass / h

This requires 3x16.002 mmols ATP / g biomass / h and 2x 16.002 mmols NAD(H)
to be available.

Since 2.0 mol ATP = 1.0 mols of NAD(H), this means the total NAD(H)
requirement is 3x16.002/2 + 2x16.002 mmols NAD(H) / g biomass / h
= 56.007 mmols NAD(H) / g biomass / h

Now 1 mol of NH₃ generates 4 mols of NAD(H), hence the minimum NH₃ uptake
necessary to allow the processes of energy generation and energy use to be
balanced is:

56.007/4 mmols NH₃ / g biomass / h = 14 mmols NH₃ / g biomass / h

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

Worked Example:

Acetobacter bacteria are able to use ethanol (C₂H₆O) as their carbon and energy source to form the end products and acetate (C₂H₄O₂) and carbon dioxide (CO₂).

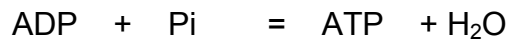
(1) Would you expect the formation of NAD(H) in any or all of the above reactions??

(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:



SOLUTION:

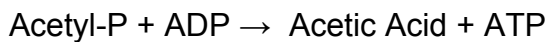
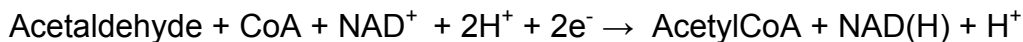
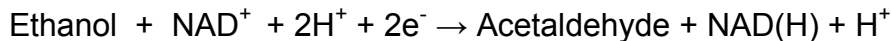
Balancing the reaction of ethanol to acetic acid:





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??



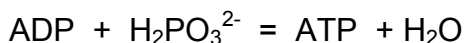
The finding of 2NAD(H) by a redox balance is consistent with the published pathway.



Using the thermodynamic data:

ATP:

The reaction for ATP formation is:



$$\begin{aligned}
Q &= \text{Heat Evolved} = \Sigma (\Delta H_{\text{formation}} \times \text{Products}) - \Sigma \Delta H_{\text{formation}} \times \text{Reactants} \\
&= (-3,619.91 - 191.17) - (-2,626.54 - 1,299) \\
&= (-3,811.08 - 3,925.44) \\
&= 114.36 \text{ kJ / mol}
\end{aligned}$$

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

For the reaction:



$$\begin{aligned}
Q &= (481.01 - 2 \times 31.94 - 3 \times 191.17) - (-288.30 - 2 \times 0 - 2 \times 0 - 2.5 \times 11.7) \\
&= -1124.4 - -317.55 = -806.85
\end{aligned}$$

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 also need to ensure that an enzyme capable of Substrate Level Phosphorylation (S.L.P.) is available for suitable reactions.

Topic 3: Mass and energy balances – alternative electron acceptor

Worked Example:

A microorganism is capable of using sulfate (SO_4^{2-}) as an electron acceptor forming sulfide. The sulfide is excreted.

Sulfate is also taken up by the cell to satisfy the sulphur requirements for growth (notably for protein synthesis).

The organism utilises NH_4^+ ions as a nitrogen source. It excretes one H^+ ion for each NH_4^+ ion utilised.

When growing on lactic acid ($\text{C}_3\text{H}_6\text{O}_3$), the organism converts the lactic acid to carbon dioxide for energy generation. The organism has the following characteristics when grown on ethanol:

Cell Composition:

Carbon: 46% ; Hydrogen: 9.2% ; Nitrogen: 9.9% ; Oxygen: 30.8% ; Ash: 4.1%

(The ash contains 50% sulphur)

Specific growth rate: 0.45 h^{-1}

Cell yield: $0.45 \text{ g biomass/g lactic acid}$.

The following atomic weights may be assumed:

Carbon 12, Oxygen 12, Sulphur 32, Hydrogen 1, Nitrogen 14.

- a) Calculate the cell formula in terms of carbon, hydrogen and oxygen only (on a 3 Carbon "basis").
- b) Calculate the specific rate of sulfate uptake ($\text{mmol SO}_4^{2-}/\text{g biomass/h}$) necessary for direct incorporation of sulphur (e.g into protein) within the microorganism.
- c) Write the equations for:
 - a. NAD(H) utilization to form NAD^+
 - b. Electron acceptance by oxygen to form oxygen ions
 - c. Water formation from oxygen and hydrogen ions

Write the equivalent equations using sulfate as the electron acceptor

Derive the relationship between oxygen and sulfate use for NAD(H) utilisation

- d) Calculate the difference in oxidation state between lactic acid and the cell. Hence, calculate the specific sulfate uptake rate ($\text{mmol SO}_4^{2-}/\text{g biomass/h}$) necessary for the manufacture of cell material from lactic acid due to redox differences between lactic acid and the cell material.
- e) Write an equation for the oxidation of lactic acid to carbon dioxide using SO_4^{2-} as the electron acceptor. Hence, calculate the specific sulfate uptake rate ($\text{mmol SO}_4^{2-}/\text{g biomass/h}$) necessary for the catabolic process within the cell.
- f) Calculate the overall specific sulfate uptake rate ($\text{mmol SO}_4^{2-}/\text{g biomass/h}$)

List ALL assumptions.

Solution:

(a)

$$\text{C:H:N:O} = 46/12 : 9.2/1 : 9.9/14 : 30.8/16 = \text{C}_{3.8333}\text{H}_{9.2}\text{N}_{0.7071}\text{O}_{1.925}$$

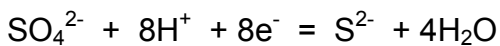
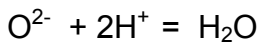
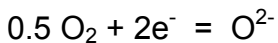
$$\text{Subtract N} : \text{C}_{3.8333}\text{H}_{9.2}\text{N}_{0.7071}\text{O}_{1.925} - \text{H}_{2.1213}\text{N}_{0.7071} = \text{C}_{3.8333}\text{H}_{7.0787}\text{O}_{1.925}$$

$$3 \text{ Carbon "basis"} = \text{C}_{3.0}\text{H}_{7.2}\text{N}_{0.5534}\text{O}_{1.5065} \text{ or } \text{C}_{3.0}\text{H}_{5.5399}\text{O}_{1.5065}$$

(b)

$$\begin{aligned} Q_{\text{SO}_4^{2-}} &= \\ & (0.45 \text{ g New Biomass / g Biomass / h}) * (0.041 \text{ g Ash / g New Biomass formed}) * \\ & (0.5 \text{ g Sulfur / g Ash}) * (1000 \text{ mmol Sulfate / mol Sulfate}) / (32 \text{ g Sulfur / mol Sulfate}) \\ & = 0.2882 \text{ mmol SO}_4^{2-} / \text{g Biomass / h} \end{aligned}$$

(c)



Sulfate accepts 8H^+ and 8e^-

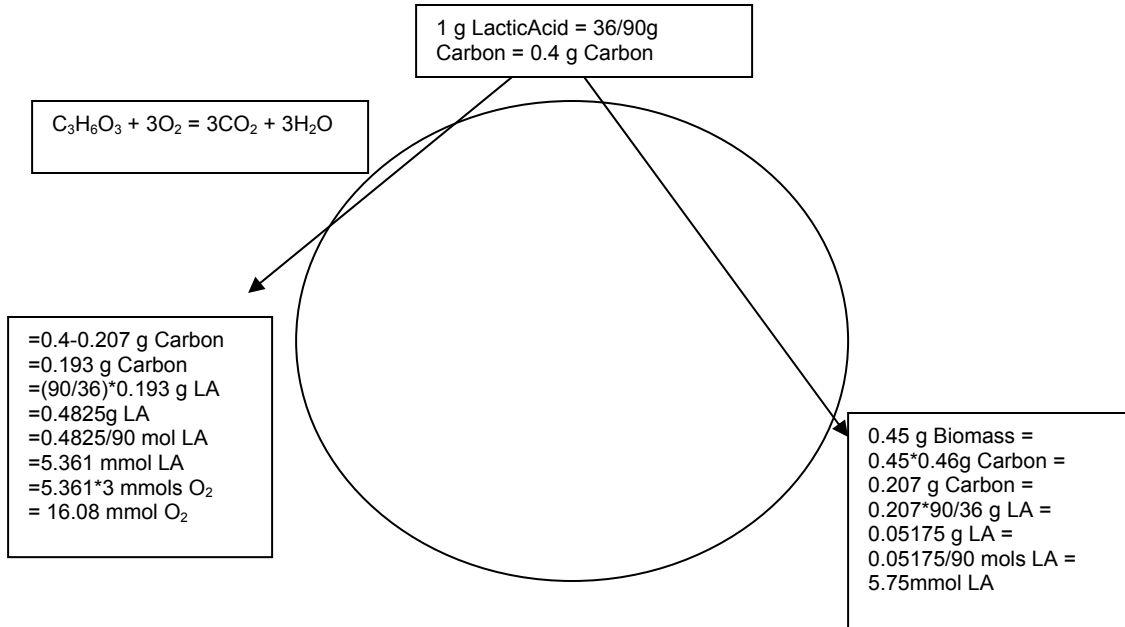
NAD(H) accepts 2H^+ and 2e^-

O_2 accepts 4H^+ and 4e^-

Therefore 1 mol Sulfate = 4 mol NAD(H) = 2 mol Oxygen

(c) and (e)

$$\text{Molecular Weight of Lactic Acid} = 3*12 + 6*1 + 3*16 = 90$$



(e)

$Q_{SO_4^{2-}}$ CATABOLISM

$$= 16.08 \text{ mmol O}_2 * (1 \text{ mmol Sulfate} / 2 \text{ mmol O}_2) * 0.45 \text{ h}^{-1} / 0.45 \text{ g Biomass}$$

$$= 8.04 \text{ mmol Sulfate} / \text{g Biomass} / \text{h}$$

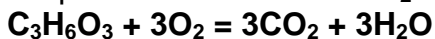
(d)

Anabolic Reaction is

$$0.5175 \text{ g LA} =$$

$$= 0.00575 \text{ mols LA}$$

Requires $3 * 0.00575 \text{ mols O}_2$



0.45 g Biomass

$$\text{Mol Wt} = \mathbf{C_3H_{7.2}N_{0.5534}O_{1.5065}}$$

$$= 3 \times 12 + 7.2 \times 1 + 0.5534 \times 14 + 1.5065 \times 16$$

$$= 75.0516 \text{ g}$$

$$0.45 \text{ g Biomass} = 0.45 / 75.0516 \text{ mols X}$$

$$= 0.005996 \text{ mols Biomass}$$

Requires 3.6317 mols O_2



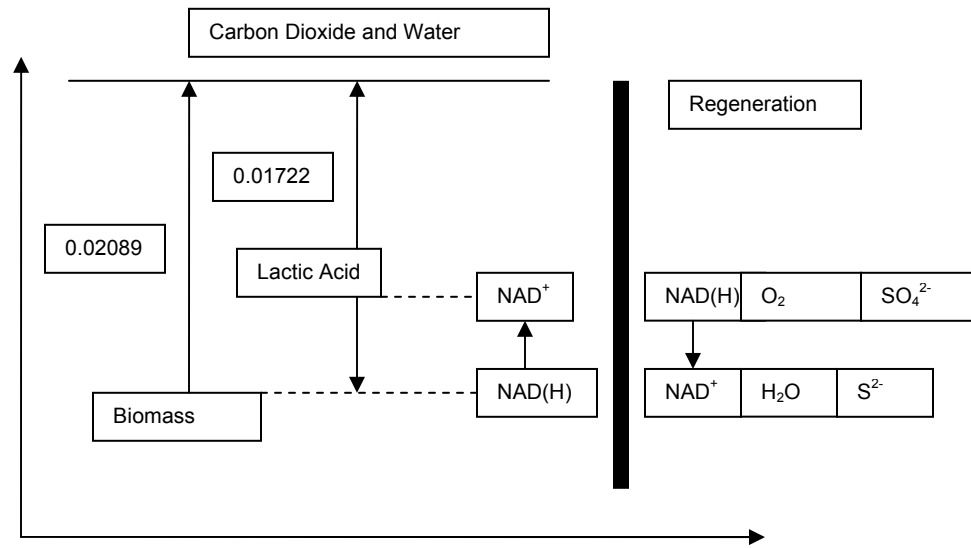
CELL – SUBSTRATE:

$$[(0.005996 \text{ mols cell} * 3.6317 \text{ mols O}_2 / \text{mol cell}) * (0.959 \text{ g Redox Contributing Cell} / \text{g Biomass}) - (0.00574 \text{ mols LA} * 3 \text{ mols O}_2 / \text{mol LA})]$$

$$= 0.02089 \text{ mols O}_2 - 0.01722 \text{ mols O}_2$$

$$= 0.00367 \text{ mols O}_2$$

$$= 3.67 \text{ mmols O}_2$$



Hence Sulfate is used in the anabolic process

$$\begin{aligned}
 & Q_{\text{SO}_4^{2-}} \text{ ANABOLISM} \\
 & = 3.67 \text{ mmol O}_2 * (1 \text{ mmol Sulfate} / 2 \text{ mmol O}_2) * 0.45 \text{ h}^{-1} / 0.45 \text{ g Biomass} \\
 & = 1.835 \text{ mmol Sulfate} / \text{g Biomass} / \text{h}
 \end{aligned}$$

(d) Overall Sulfate Uptake Rate = ASH + CATABOLISM + ANABOLISM

$$= (0.2882 + 8.04 + 1.835) = 10.16 \text{ mmol Sulfate} / \text{g Biomass} / \text{h}$$

Topic 4 : Y_{ATP} calculation

Worked Example:

A bacterium uses 3P-glycerate from glycolysis as its carbon skeleton for new biomass manufacture. It catabolises glucose using glycolysis - forming ethanol and carbon dioxide. If the cell yield is 0.1 g biomass / g glucose, what is the Y_{ATP} of this organism?

Undertake two calculations, one where the ATP used during the manufacture of the carbon skeleton manufacture is subtracted from the catabolic ATP production

and one where the ATP used during the manufacture of the carbon skeleton manufacture is NOT subtracted. The carbon content of the cell is 42%.

What do the relative sizes of these two Y_{ATP} estimates illustrate?

Solution:

Basis : 1 g glucose = 72/180 g carbon = 0.4 g carbon

Cell formed = 0.1 g = 0.42X0.1 = 0.042 g Carbon
= (180/72)X0.042 g Glucose = 0.105 g glucose = 0.5833 mmol Glucose

Carbon to catabolism = 0.4-0.042 g Carbon = 0.358 g Carbon = 0.895 g Glucose
= 4.972 mmol Glucose
mols ATP produced = 4.972X2 = 9.944 mmols ATP

Y_{ATP} (not including ATP used in manufacture of the carbon skeletons)
= 0.1 g Biomass / 0.009944 mol ATP = 10.06 g Biomass/ mol ATP

0.5833 mmol Glucose = 2 * 0.5833 mmol 3P Glycerate
This uses 2 * 0.5833 mol ATP = 1.1666 mmol ATP

If this is included in the Y_{ATP} calculation, then
 $Y_{ATP} = 0.1 \text{ g Biomass} / (0.009944 - 0.00117) \text{ mols ATP}$
= 11.39 g Biomass / Y_{ATP}

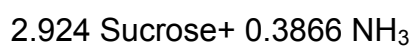
In “normal” metabolism, the glucose which enters the anabolic part of the cell enters as a carbon skeleton. This carbon skeleton is either a monomer in this form or is further converted to a monomer in a process that may or may not use energy (ATP). By convention, the energy associated with the transformation of carbon skeleton to monomer is considered part of catabolism.

In this example, the cell is manufactured from 3P Glycerate. This may be considered the monomer from which the biomass is made. When the correction is made for the manufacture of this monomer, the Y_{ATP} changes by 11.8%, which is relatively small. If 3P glycerate is considered a carbon skeleton but not a monomer, we would need estimate the number of monomers made from it and account for any energy usage/production during these processes. The ATP produced from catabolism would then need to be corrected accordingly.

Topic 5 : Heat evolution during metabolism

Worked Example:

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



=

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 $0.2h^{-1}$

Solution:

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

$Q = \text{Heat Evolved} = \sum (\Delta H_{\text{combustion}} \times \text{Products}) - \sum \Delta H_{\text{combustion}} \times \text{Reactants}$

$(1.4007 * (-21.82 * 256.06) + 3.913 * (-1,366.91) + 1.6667 * (-871.69) + 0.5556 * (-1,370) + 5.4545 * (0) + 2.2222 * (0)$

-

$(2.294 * -2,803 + 0.3866 * -438.77)$

$= (-7,826.03 - 5,348.72 - 1,452.85 - 761.12 + 0 + 0) - (-6,430.08 - 169.63)$

$= (-15,388.72) + 6,599.71$

$= -8,789.01 \text{ kJ}$

$Q_s = (-8,789.01 \text{ kJ} / (1.4007 * 256.06)) * 0.2 = 4.90 \text{ kJ / g Biomass / h}$

Homework 4:

Data from Topic 3 / Homework 1 should be used in this question

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