The Life System & Environmental &Evolutionary Biology II

Lab 11: Respiration and Nutrient Uptake

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This lab will have 3 parts and will examine many of the aspects of respiration and nutrient uptake we coved in this weeks lectures. You will need your lecture note to complete the lab.

Part A - Why does a chocolate bar give me energy? 

Warning: this portion of the lab contains real chocolate!

Part B - Why does my beer fizz?

Warning: this portion of the lab contains real beer!

Part C - Nutrient Uptake Kinetics 

Warning: this portion of the lab contains real data!

Part A - How much energy is in a chochocalte bar?

This Cadbury Dairy Milk bar has a net weight of 141g. How much energy is in it? Work all your calculations either in Excel or clearly on a piece of paper and turn in you work - not just your answer. May want to review the lecture notes on the theoretical yield of respiration. *ALWAYS put units on your calculations!*

To get started:

1. Convert g of candy bar to mols of glucose (for this first part of the lab lets assume the entire bar is sugar - not a bad approximation)

How do you do this?

If chocolate was made of water 1 mol of chocholate would have a mass of 18g

Hydrogen has an atomic mass of 1 and there are two hydrogens = 2

Oxygen has an atomic mass of 16 and there is one oxygen = 16

One mol of H_2O therefore has a mass of 18g

Of course we are assuming the chocolate is glucose - $C_6H_{12}O_6$

How many mols of glucose do you have?

2. Glucose contains 686 kcal mol^{-1}. How many kcal of energy does the dairy milk bar contain?

(ok now for the reward - show your *CORRECT* answer to your TA and consume the 38 kcal of energy they will give to you in form of a Hershey's Nugget - hopefully this should be enough energy to complete the remainder of the lab!)

3. If that was 38k cal, how much did it weigh?

4. Now back to the Dairy Milk - How many mols of ATP could you *THEORETICALLY* harvest from the Dairy Milk bar by completely oxidizing it in your stomach?

> If each mol of ATP contains 12 kcal of energy, how much energy is available?
>
> How many mols of NADH were produced and then converted to ATP?
>
> How many mols of $FADH_2$ were produced and then converted to ATP?
>
> How many mols of ATP were made directly from substrate-level phosphorylation?

5. Try as you might, none of you were able to gain the theoretical yield from the small chocolate your TA gave you.

> How much energy (kcals) did you harvest (*the actual yield*)?
>
> What percentage of the total energy available were you able to recover?

How many mols of CO_2 did you exhale from chocolate?

What was the mass of CO_2 that you exhaled?

What was the mass of C you exhaled? How does that compare to the mass of carbon you ate?

What was the mass of water formed in addtion to the CO_2? How many mols of water were formed?

6. For you over-achievers (*this means you don't have to answer this if you don't want to! - its a bonus question*) - of course this candy is not pure glucose. The "Nutritional Facts" of the Dairy Milk tells me there are 42g of fat! If you can correctly calculate the energy available (in kcal, and mols ATP) assuming the Fat is a six carbon fatty acid that is broken down by beta-oxidation (from the lecture notes) and the remainder of the mass is glucose you can have the candy bar (or at least enter a raffle for it if more than one of you gets the answer right). You'll need to assume the fatty acid is 120g mol^{-1}. Of course if no one tries this, your TA's get to eat the chocolate!

Part B - The regulation of Alcohol Fermentation

For the next part of this lab we will again utilize the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB). Go to the Expasy Home Page and search for "Ethanol" and answer the following questions:

1. Where does the alcohol in beer come from? Go to Expasy Home page and search for" Ethanol". Once you find it proceed to the biochemical pathway maps.
    - What enzyme produces Ethanol?
    - What is the direct substrate?
    - What type of reaction is this?
    - The substrate has 4 protons (H^+) while the product has 6. Where do the additional protons come from?
    - What is transferred with these protons?

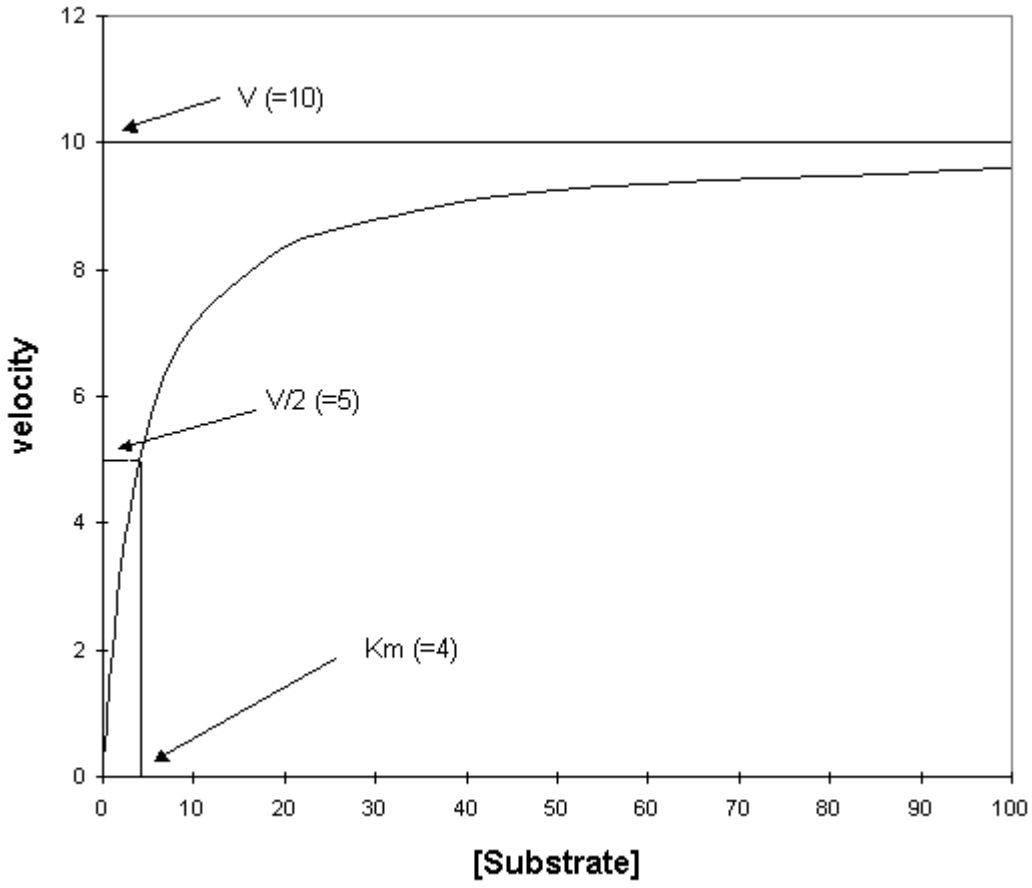
2. Now look up the enzyme catalyzing the reactions that produces Ethanol (the one you identified above)
    - What cofactors are used by this enzyme?
    - What is another name for this enzyme and how do you interpret it's meaning?
3. For you over-achievers (*this means you don't have to answer this if you don't want to! – it's a bonus question and please tell me your 21 so I can avoid being arrested*) - I mentioned in class that wine fermentation stops near 12% alcohol because the yeast die. Yet even a very strong home brewed beer is only 4 or 5 % alcohol - why? (of course I'm looking for a physiological answer and its related to enzyme kinetics) If you are the first to know the answer, your TA will give you a beer - or at least let you enter the raffle for one if you all know the answer.

Part C - Nutrient Uptake Kinetics

Nitrogen uptake by plants is an endergonic reaction that is enzyme mediated. Accordingly we should be able to describe reaction of the enzyme with its substrate with the simple Michaelis-Menton equation. Today lets work the problem from the other direction. I'll give you the data and find the constants to calculate the equation and plot the curve. Do all of your work in Excel and make sure you *ALWAYS LABEL THE AXES OF YOUR PLOTS!* the data we will use are real, and therefore not as neat and tidy as textbook plots. The data came from:

Bassirirad H., Griffin K.L., Strain B.R. & Reynolds J.F. 1996. Effects of CO_2 enrichment on growth and root $^{15}NH_4^+$ uptake rate of loblolly pine and ponderosa pine seedlings. *Tree Physiology* **16**, 957-962.

To Remind you:



Michaelis-Menton Equation

$$v = \frac{V.a}{K_m + a}$$

v = velocity

V= maximum velocity (V_{max})

a = [S]

Km = Michaelis-Menten Constant (curvature)

1. Plot these data in Excel:

Loblolly Pine		Ponderosa Pine	
$[NH_4^+]$ - µM	NH_4^+ Uptake Rate	$[NH_4^+]$ - µM	NH_4^+ Uptake Rate
10	2	10	1
50	9	50	7
100	14	100	11
150	21.5	150	12.5
250	21.7	250	14
500	25.0	500	17.5

note the uptake rate is µmol g^{-1} h^{-1}

2. From these plots determine V_{max} and K_m for each of the two trees

3. Now plot the Michaelis-Menton curve over the data points

In excel make two columns of numbers

Substrate from 0 to 500 µM in steps of 25µM

Velocity (from the equation)

4. How well does the theoretical curve fit the data? Why? Does this surprise you?

5. Which tree is able to take up more NH_4^+?

What level of NH_4^+ is needed to reach half the maximum velocity of the reaction? (Does this number look familiar?)

Loblolly pine is from the southeastern US, ponderosa pine is from the western US, from these uptake curves, how might you imagine the ambient NH4+ levels differ in the habitats these two trees are found in?

6. For you over-achievers - GO HOME, the lab is over!