CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

Laboratory Notes Series: Volume 6
July 2014 – December 2014

www.carnicominstitute.org
www.wikici.org

Carnicom Institute is a non-profit organization, 501(c)(3), working solely for the benefit of humanity and the public interest. Our goal is to provide the public with beneficial and responsible information concerning human health and the environment. The Institute is extensively active in conducting scientific research and public education relating to the consequences of geoengineering and bioengineering. Thank you for your support of Carnicom Institute.

PERFECT EDGE® MICRO PERFORATED FOR CLEAN TEAR OUTS

Chemistry Vol VI

Made in USA

Norcom Inc. Griffin, GA 30224
Item #77186

5 SUBJECT

180 Sheets

COLLEGE RULED

[redacted]

Chemistry Vol VI

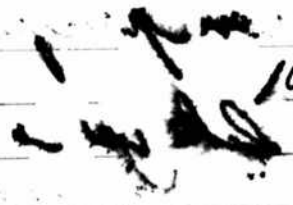
July 16 2014

Jul 16 2014.

The phosphoric protein approach has produced an interesting result.

When diluted in enough water & allowed to settle the solution has cleared & the whole protein has ended precipitated on the bottom

254 171
280 154



10 ml → 50 ml

It seems better to precipitate the protein to me. It can be purified this way.

We see that the protein has been found under extremely acidic conditions. It should be neutralized before testing. testing.

It passes Bradford easily w/ 1 drop 6.7 HCl after dissolved.

It precipitates cleanly under neutral pH conditions!!!

No need for VACUUM or heat.

It looks to me like

neutralization and dilution is bringing out the protein precipitate in droves.

Whether or not iron added or not was important or not. I do not know. But we are getting a dark green precipitate now.

Ya look at it on large quantities.

OK I have produced measurements of the protein. This time it is blue green (dark) vs light blue green.

The method?

CDB Prep:

Liquid:

1200 ml H_2O
20 ml Liq Iron
20 ml Glucose
1.1 ml Salt
CDB
Heat
Oxygen
VL[illegible] EF

Agar

210 ml H_2O
2.1 gms Agar
1 slice potato liquid
5 ml Liquid Iron
2.2 ml Glucose
0.6 mg Salt

Page 4

Pre Separation Bile Recipe:

600 ml H_2O
20 ml bile powder
60 ml CDB
NaOH to pH 9.5 - 10.0
Warm stir bile solution mildly
Incubate @ 5 days @ 85-90°F
Until pH drops to ~ 6.5

Bradford Reagent:

15 ml Coomassie
20 ml Phosphoric Acid
10 ml H_2O
5 ml Ethanol
Σ = 50 ml

Phosphoric Acid Recipe

35 ml Phosphoric
17 ml H_2O
8 ml Ethanol
Σ = 60 ml

Post Bile Base Solution

1. Take incubated bile solution
2. add 25% Xylene
3. Blend to light tan

Lipid Separation (A Separate Acting)

Take post bile solution & let settle for several hours.

Several layers will appear.
Separate & test bottom layer will have lipids in it.

Massive amounts have been produced.

Page 6

Protein Production.

1. Take tan, blended post bile base solution.

2. add 6.0 ml 8.7 M HCl / 600 ml blended bile

3. add 11 ml Phosphoric acid mix / 600 ml blended bile — Uncertain if required but I believe so — Not needed

4. Separate to liquid solution in sep funnel several generations if needed.

5. The water based solution will contain protein & be highly acidic in nature. Dilution is important.

6. Try two tests:

Alkaline only to neutral and dilute & separate precipitate

* This works

If no precipitate from left add liq iron then alkalize to neutral then dilute & separate.

Liq Iron is needed.

To prepare the protein for testing
(10 dilution)

1. add 5 ml protein to 50 ml H_2O (total = 55 ml)

2. add 2 drops 0.1M HCl.
This will turn the solution white.
Stir

3. add Bradford, a brilliant blue

4. on the 254/280 UV.

149 254
130 200

Ratio = $\frac{55}{5}$ = 11 dilution

Concentration = 23.5 mg/ml
perfect

DNA

Let's go get it.

pH of the milk is already 6.6

This is rather fantastic.

I did raise the temperature a lot and had it initially @ 9.3

In 24 hrs

Day 0 9.3

Day 1 6.6 excellent

In 200,00 3000 500

1600 day

AM

JUL 10 2014

1. Purification of DNA [redacted]

DNA failed so far with.

Fails
1. CDB + SDS
2. CDB Bile + SDS } + Pepsin
3. Protein + SDS

Fails
Currently:
1. Blood CDB Bile
2. Then SDS + Pepsin Fails

Fails
Next:
1. Blood CDB Bile
2. 7 drops NaOH + 7 drops KOH
3. Some heat + Liq Iron (precipitates)
4. SDS & Pepsin

Fails
Next.
1. Raw CDB
2. ~~7 Drops NaOH 7 drops KOH~~, Liq Iron, Strong KOH NaOH
3. ~~75°C~~ Water bath 85°C Low LC Nalog

Smelly curious.
You must have left some of the Dream Blend left over.

To this you have added SDS & placed in cold water & something is happening to it.

2nd test is w/ CDB w/ SDS + pep + Cold

3rd test is CDB + Conc KOH - NaOH + Pep + Cold water

Bile Xylene is next - sep funnel/ bottom layer

OK. We have Something important

1. Xylene - Bile Blend.
2. Sep funnel (There are two layers + lipids up top)
3. Take lower sep from layers
4. SDS + Enz. + blend after Conc. KOH - NaOH
5. Alcohol on top layer
6. Definitely a globular protein + DNA mix - insoluble in alcohol
7. Is it insoluble in water

Which of the two milky tan layer is producing it?

It actually appears to be almost a pure lipid! It actually fails the Bradford test but dramatically passes the alcohol emulsion test.

You can extract alcohol from the top layer. You mix it with water and it turns white in a vague way. This is LIPIDS.

One of the layers is lipids
The dock is proteins.
We expect the frothy upper layer to be the lipids.

You may not need the SDS stage @ all.

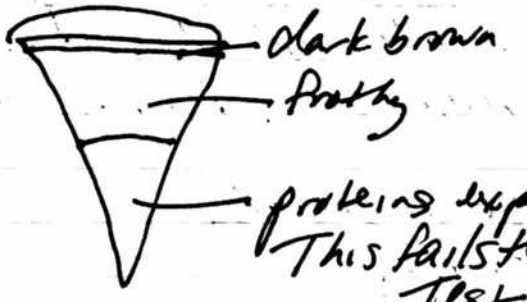
SDS not needed

1. Frothy layer
2. Add Conc. KOH-KAOH
3. Add Ethanol
4. Cool down if you can
5. The lipids will precipitate.

Lipid Extraction
Very Pure

Today

1. A mgn- success w/ separating precipitating lipids

1. Bile Solution
2. Xylene
3. Blended
4. Separate into 3 layers
5. 

6. Take frothy layer
7. add pale conc. KOH - NaOH
8. Add alcohol layer - do not mix
9. Lipids precipitate into alcohol
10. Lipids pass emulsion test when mixed w/ water
11. Lipids pass microscope exam not
12. Lipids fail Bradford Test.

SDS - Enzyme process w/ blender might result in a more pure lipid but this simple method is sufficient.

What we see here in both case

Proteins & Lips Lipids

They both have to be precipitated out of solution & the methods are different for each.

FROTH LOWER LAYER

To determine which layer has protein. We add 1 drop acid HCl to both Tubing

On this one, the adding of acid HCl seems sufficent to produce the lipid layer. but they didn't test weed it actually look fat lipids

Sure enough it is this layer that produces the precipitate.

When you add sufficent Fe^{+2} it precipitates out.
Failed a Bradford test on the precipitate.
Maybe you need both?

Protein Precipitate:

1. Tan bile solution post xylene blending
2. It separates into 3 layers but yu only want to remove the top one.
3. Yu add HCl only. Yu do not need phosphoric acid. It will separate into two layers.
4. Again remove only the top dark one.
5. This will be highly acidic & milky tan.
6. Dilute this considerably roughly 10 to 1 ratio
   It still will be highly acidic
7. add approx 1 ml liquid iron per 100 ml H_2O.
8. add NaOH until pH reaches @ least 5.5 It will go through 2 color changes. a dark green precipitate, very ~~soluble~~ that will form.

This looks very pure & it is a dark green. It actually will float.

Again ..

1. Take blended bile solution w/ only top layer removed. Shake it up well. as much as you want.
2. Take ~~50 mL~~. Add 8.7 M HCl
   Do not dilute yet
   This will form a dark top layer.
   Remove it w/ sep funnel
   Use 10 drops 8.7 M HCl for 75 mL blended bile
   separated.
3. Now sep the top layer off again
   You see that you have two sep layers
   to pull off, one under highly acidic conditions.
4. The separated layer is milky green (white w/ phosphoric acid added) Yes, this is better.
5. Now dilute it at least 5 to 1. Should be while
6. add 1 mL liq/iron per 100 mL solution. Remember this is highly acidic. (~ 1.5 pH) also a little more iron.
7. Now take pH w/ NaOH to @ least 5 8.
   Notice the color change.
   You will start to see it precipitate
   even as pH goes to 1.0. Tan precipitate.
   pH 3.5 produces even more, ~~dark~~ tan
   It starts turning green about pH 4.5.
   Bring to neutral. Lots of precipitate.

Notice this dark green prob floats instead of sinks

The protein formula not exactly the same and is not quite as pure.

Maybe the phosphoric acid does have a role of some kind.

Either way, to actually get the protein you must alkalize it even further, then bring it back to acidity to use the Bradford test. The test does not require that it be brought back to acidity.

Somebody about this as less pure but it is usable

Do not separate the whole solution into 3 layers, only two.

It is believed that the protein forms created on this day are not nearly so pure as on select previous occasions!

It is thought that this is due to the like solution incubating only one day instead of 5 days.

What is the difference between the Fe method of precipitation and the alkaloy method of precipitation?

07 20 14 Page 20

DNA Pursuit.

Remember your work with DNA was preceded by acetone

So:

~~Ace~~ CDB + Acetone + SDS? + enz

CBB + Acetone + KOH - NaOH + SDS + enz

CDB + Acetone + KOH - NaOH + Cig Iron + SDS + enz

CBB + Acetone + KOH + NaOH + Heat + SDS + enz?

1st Trial

CDB + Acetone + KOH + NaOH + Heat 75°C + SDS + Enz fails

CDB + Acetone + KOH + NaOH + Heat + Iron (Acetone boils) 80°C . $FeSO_4$ only
This is black!

It looks to me like I am getting closer again.
The solution is clear.

CDB + Acetone + KOH + NaOH + 80°C + Fe_2SO_4
Acetone boils for a few minutes..
It turns dark black.

Try Jan 20 notes a SDS approach

OK, you have an important discovery
Re: second protein form.

Page 22

2nd Protein Form!

07-21-14

The second protein ~~form~~ has made itself present again.

This is the ~~un~~[illegible], simple filament type. It has grown in alcohol after inculation for 24-48 hrs.

Let us try to recall the method. I believe:

1. CDB by themselves. 5 ml in a large test tube.

(might be best to blend)

2. added Acetone in equal volume.

3. Added KOH-NaOH Combo, a full eyedropper. This change its color to dark brown.

@132°F Acetone boils be careful

4. add Fe_2SO_4, a good eyedropper also.

5. Heated to 80° C (176°F) This turns it black.

6. ~~I believe I now subjected~~ this to the ~~SOS - Enzyme~~ method with the ~~Dremel~~.

7. I ~~then separated the~~ solution ~~from the acids~~.

B. ~~I now believe~~
Negative on 6 & 7.

What I believe I did was subject it to the notes of Jan 20 2014. No major blending took place. I was very gentle with solution.

S1:
1. Cold cold water for everything. 4°C & Cold ethanol
2. add the black mix
3. Truly a little salt (Salt causes it to boil over!)
4. Stir gradually over next 5-10 min while it cools down.
5. Add drops only of the full detergent (engyme blend)
6. Invert very gently every 5 minutes & let sit on the ice bath for 15-20 min.
7. Pour it into test tube
8. Add the Cold ethanol.
9. Nothing happens.
10. Let it sit for 24-48 hrs @ neutral temperature.

11. The protein forms in alcohol
THE PROTEIN IS water soluble
a positive Bradford blue green

The demonstrate the engyme detergent may be key to show you extracted DNA.

Jul 22 2014

I believed that I removed the acetone when I did all the DNA work.

But acetone might have had an important effect upon keeping the COB.

There might be a case where you distilled the acetone off.

Jul 31 2014

We need to reproduce the protein
(you actually have 2 different forms)
and the lipids
(you actually have 2 different methods)

[redacted]

You also have a separation in the previous
gels that were incomplete that
look like they need tendy to.

You have a nice white precipitate
that has formed on the top of the gels
after rotating for several days to a week.

The white gel precipitate that floats
slowly to the top does have proteins in
it but it does not exactly seem
very pure yet. Maybe after
rinsing?

Aug 1 2014

The easiest way to get this precipitate (presumed protein) is to extract it off the top w/ a pipette.

It is white & does look very soft.

Consider this as another viable method.

This allows for 3 populations of extracts phototrace on gels.

Get these documented & replicated!

It appears to be very pure looking.

It actually appears very similar to the 50 PSI extracts in the larger but take ? less reproduced

The very funnel method did not work likely; it required extensive settling for a week & then pipetting off.

The whole floaty precipitate easily passes the Bradford test.

No alkaline addition was required.
Only HCl to run the Bradford test.

Let's do a UV test:

254	~~145~~ 192		Dilution
280	174		2ml Pro #2
			10ml H_2O

Conc = 15.8 mg/ml good.

We have split the milk solution of ~ 600ml into 3 parts for three separate tests.

The pH of the white precipitate protein (protein #2) is 5 so it is not highly acidic.

The pH of protein #1 (the blue one) is 1.5 so it is highly acidic.

Therefore these proteins may not be the same.

The methods are different.

We may have 2 different forms.

Method 1 - Blue

June 25th seems to be the date. thru July 11.
add 25% Xylene. We have 100ml bike.
So add 25 ml Xylene.
Blend it? ~~shake it~~?
It looks like blending as advantageous.
We blend for abt 5 min. It turns tan.
We have 200 ml solution.
We add approx 20 drops 0.1N HCl
~~to~~ add 40 drops our Bradford reagent.
We pour this mixed solution into a sep funnel.
Let this sit for 2-3 hrs & determine layers.
You do indeed have 3 layers formed
as described on June 25.
Bile 80%
COB Sludge 3%
Xylene 17%

You actually had 4 layers start to form within
about 45 min.

3% - a dark layer
17% - a tan layer
40% - a more whitish bile layer (This layer interests me the most.)
40% - a blue bile layer (pH 1.1)

We are going to work with the whitish layer.
The pH of it is ~~.~~ 1.3

Take 10 ml of the whitish layer
Dilute to 100 ml
Split this into 3 different ~~ml~~ 35 ml solutions

35 ml (diluted white bile layer)	35 ml	35 ml
1	2	3
Fe_2SO_4 4-6 drops 1M NaOH to alkaline pH positively produced dark green floaty precip. tube. pH 10+	$FeSO_4$ limiting amount ~ 10 drops & pH 6.0 produces a higher volume light green precipitate.	

If we dilute this precipitate highly, add 0.1M HCl 1 drop & then Bradford it passes the Bradford test

This seems to be a target compound.

General Note on Protein Extraction all methods:

1. Let's learn how methods 1 & 2 differ from each other.
   Method 1 uses Coomassie Blue – ie, Bradford reagent. Has a part of the addition of HCl to the Xylene-bile blend.

2. Method 2 looks like JVI 16.
   You used HCl & Phosphomic Acid Instead of Coomassie Bradford reagent.
   Then you separated the water solution w/ several centrifugations.
   Then you added Liq iron
   Then you ~~added~~ alkalinized it.
   Then diluted & separated.
   Then let it set for a week.
   & pipette off top & rinse & separate.

Test also between liq iron & Fe_2SO_4

There are questions about adding iron to produce a precipitate VS alkalinizing to produce a precipitate.

Method 1 (cont) Page 33

We seem to have the method.
Take the lower bile solution.
Dilute it 10 to 1.
add $FeSO_4$ & NaOH to pH 6.0.

$FeSO_4$ (1M)
Estimate 2 ml per 100 ml
(Now you know you can use
liq iron & get the same result @ $FeSO_4$)
Yes 1 ml per 100 ml solution, either way
pH of diluted solution prior to liq iron is 2.3
So it is highly acidic.
We are going to add 2 ml / 100 ml
liq. iron this time.
This drops the pH down to 1.9
so it makes it slightly more acidic as expected.
Now bring pH carefully to 6.0.

It looks like a pH of 5.0 is sufficient.

The color will vary between dark green & brown.

What you are seeing is that this material is
partly proteinaceous but it is not pure.

It is the incubation of this precipitate which
produced the pure white form. The
white form is the TARGET, This takes
up to a week & ~~Cannot~~ be shaken
or disturbed. It floats on top.
Look @ the white under the scope.

Ok, we are of the opinion that the proteins are likely the same.

Notice that incubation was required in either method.

We appear to be forming a proteinaceous precipitate complex but it is not pure.

The incubation of the precipitate produces a lot appears to be a more pure protein. It is a whitish layer that appears on top but it took more than a week for this to actually appear. When we do so we pipette it off and remove & separate.

We have a good solid result w/ the precipitation growth method.
Now let's see if we get the growth again.

Large Test Tube Approach

The other method did not require the use of alkaline. What did it do?
NO Alkaline!!!

1. Take the bile. 200 ml (~~bile xylene blend~~)
2. Add 25 ml Xylene & blend. 5 minutes.
3. Add 2ml 0.1 ml HCl
4. add 4ml Bradford.
5. Let's blend it for 1 minute
6. Pour into sep funnel. It is light green.
7. You pull off the lower solution (it is bile based)
8. You do not use the upper darker layer 3 drops 1M.
9. You dilute it highly 10 to 1. add pure $FeSO_4$.
10. The pH does not need to be extreme.
    It will precipitate w/ only a couple of drops of 1M NaOH in a large test tube.
11. You get the precipitate you know about.
12. Dilute the precipitate 20 to 1.
13. Add 1 drops 0.1M ~~NaOH~~ HCl to prep the Bradford
14. Bradford test is highly successful.

No inoculation is required here.

We have a bold blue color.

I have it! The lowest sep funnel layer gave great results.

pH 2.70 is sufficient to precipitate!!

Ok, We have the method.

1. Bile Solution 200 ml.
2. add 25% Xylene (50 ml) & blend 5 min.
3. add 1ml 0.1M HCl per 100 ml blend.
4. add 2ml Bradford reagent per 100 ml blend
5. Blend for 1 minute
6. Pour into sep funnel & separate into layers. Use the water soluble layer.
7. Draw the lowest layer.

Not true. The precipitate by
~~~~ itself is only partially
& mildly protein.

It is the incubation process that
produces the protein & it is
White in color

~~~~

The precipitate by itself is not highly acidic and therefore why you do the prep for the Bradford lab you will see it generally stays at yellow color here was false because the solution was not properly acidified. pH of the precipitate in water ca. about 5.0.

←

The protein, however, by itself may indeed be very ACIDIC because of how you see that it reacts w/ NaOH.

You have a problem.

You do seem to be ok.
There is a question of the white protein is best ~~detected~~ by NaOH 1st (1 drop) then HCl or by HCl alone.

Ans HCl alone does appear sufficient.
1 drop ~~H2O~~ 0.4 M HCl.

The separated bile solution by itself, does have protein in it but you can tell ~~that~~ it is not very pure because of the cloudiness of the Bradford test.
Your growth of all protein is what's what's ~~[illegible]~~.

Our 2nd test method will be a non precip method.

1. Bile solution extracted from sep funnel (after HCl at Coonamble).
2. Diluted 5 to 1 in 5 jars.
3. Add one eyedropper 1M FeSO4
4. add 0.6 sugar
5. add 0.2 salt
6. add one eyedropper liquid soap to 2 jars.

Lipid Work

Fascinating.

1. Take the non polar lipid layer.
2. Mix 1 to 1 w/ acetone
3. We get a non immediate or distinct separation into 3 layers.
4. The middle one looks like pure lipids

Large scale.

50-50 mix w/ acetone looks perfect.

3 distinct layers

1. Blue on bottom — not clear
2. presumed CDB one in middle (should be yellow) — Should be Lipids
3. Acetone on top.

Rig 1

soluble — In acetone, olive oil sinks to the bottom.

soluble — In xylene, oil sinks to the bottom.

Lipid tests.

1. Insoluble in water
2. After mixing, Centrifuges into layers
3. Alcohol test
4. Bubbles visible in water after centrifuge & shake.

Aug 02 2014 Protein Generation Update.

1. After 12 hrs we do not see any evidence of protein generation with the precipitate method.

2. The non precipitate method using $FeSO_4$ & sugar and salt also does not appear to be productive @ this time.

3. It appears although we do have some minimal level of success of the non precipitating liquid lysis, sugar & salt method
the part is highly encouraging. It is directly taking the Coomassie Blue stain and it also is passing the Bradford test.

4. The key mystery is why & how can we produce this blue protein in large numbers? What caused this? Review notes carefully.

Great News! Page 41

It looks like we have major protein production w/ the precipitation method.

The Color of the protein seem to be highly variable from white to blue to rust but it appears to be of the same nature.

Incubator temperature was too high - approx 110°F.

Looks great, I think we have it.

The one 600ml that was brought to a higher pH is more productive.

The pH of this highly productive one is ~~8.7~~ 5.7
Remember it was dilluted in water 10 to 1.

So, what was the method?

Method of Protein Production:
200 ml

1. Have the bile solution ready gate incubator + pH down to approx 6.5.
2. 25% Xylene added.
3. Blend 5 minutes. (Hi Speed is ok too)
4. 1 ml 0.1M HCl per 100 ml
5. 2 ml Bradford reagent per 100 ml
6. Blend for 5 minutes (5 minutes)
7. Separate lower layer from sep funnel
8. Dilute it 10 to 1
9. Add 2 ml 1g/ml per 100 ml (1M $FeSO_4$ can be used)
10. Carefully bring the pH to ~ 7.0 to 9.0 w/ NaOH
11. This creates a waxy precipitate which is partly proteinaceous
12. Sep funnel & isolate the precipitate.
13. Divide it & incubate it @ 110° F for 12 hrs. No nutrient needed. Precipitate should be wet colored.
14. Precipitate forms as a top layer which can be pipetted off.
15. Rinse & Sep Funnel it to Final Isolation
16. Run Bradford & UV Tests.

Turns white

$FeSO_4$ 2 bottles?

When you blend it this more you ones get 2 layers.

We also noticed that a filamentous structure started growing on the top.

For UV test, also added some 6.7 M HCl
for reference

254 ~~153~~ 154 20 ml H_2O
200 129 2 ml Protein w/HCl
2 drops 6.7M

Conc = 18 mg/ml - very good.

[redacted]

Bile Prep again: 600 ml beaker

550 ~~600~~ ml H_2O 40°C
15 ~~20~~ ml bile powder Warm to dissolve (20 capsules)
100 ml ~~50~~ ml + CDB
NaOH to pH 9.0 - 9.5
Incubate until pH drops to 6.5, Cover w/ wax paper

Method of Protein Production
(Cont)

Step 7. The separated layer is very milky - it is very homogeneous Light green milky color. Probably would be pure white except for the Coomassie.

We are going to aplot this into 3 tests.
1. One for $FeSO_4$
2. One for Liq iron
3. One for acetone.

Try the acetone 1st in a test tube. Interesting that it seems to be "bubbly" by itself??

Very interesting of acetone added. Only a little bit is sufficient to seem to separate the lipids very clearly

Acetone caused a very clear separation layer of lipids up top.

Therefore it purified it further.

If you mix it up w/ acetone & shake it & then separate you get a green layer up top. Lipids not as obvious

The use of acetone seems to be a very helpful step in the purification process. It seems like it separates the lipids cleanly. They are a dark green color. I have them in a separate tube

The lipid layer is very greenish.

Now let go to work w/ dilutions & to the tests.
Lets split into 4 tests: 50 ml each.
Will use 10 to 1 for 500 ml. Total

Test 1: 50 ml Purified Bile Milky Layer
500 ml water
Total = 550 ml.
pH of this solution is: ~~2~~ 2.1
so it is highly acidic
add 6 ml 1 M $FeSO_4$
This drops the pH to 1.9 as expected.

Now add NaOH
Precipitate reading @ pH 5 to 6.
Greenish color.
add NaOH to bring pH to 12.5
just a darker green.
We are done. Separate the precipitate.
& Rinse in H_2O
This dark green precipitate FLOATS
Very clear layers.

test 2:

50 ml milky bile extracted
300 ~~250~~ ml H_2O
Total = 330 ml
Now add 6 ml liq iron
pH to 10.6
We get the same dark green color
So it does not seem to matter if
we use Liq Iron or $FeSO_4$.
I suspect pH level caused the color
change last time.
Try the next test w/ pH soln to 3 ~ 4 max.

This precip, although the same color
does not float & readily separate.
It is fairly uniform.

It is a slightly different color.

Test 3

1. 50 ml milkbile
2. 500 ml H_2O
3. 6 ml ~~H~~ $FeSO_4$
4. pH to ~~9~~ ~~4~~ very reactive. 4.6

Very clean light precipitate created here.

The method to produce a clean light white lighter looky precipitate.
We have it here immediately.
No need to incubate

Test 4: No Iron added, just pH to 4.6
It does not precipitate
The Iron is positively required.

When you add iron back in a increase the amt it turns it acidic again!
So it is a balancy act.

Test 4:
What if no $FeSO_4$?

Ok We have it.
Test 3 was the method
pH only needs to go to 4.0
w/ sufficient iron.

The method:

1. Milky bile purified w/ Acetone after the xylene layer.

So:
1. 200 ml bile (pH ~ 6.5)
2. 25% xylene
3. Blend 5 minutes (Hi speed OK)
4. 1 ml 6.7M HCl / 100 ml solution
5. 2 ml Bradford reagent per 100 ml
6. Blend for 5 minutes
7. Separate to lower layer.
8. Add Acetone & Shake
9. Separate the lower layer
10. Dilute it 10 to 1
11. Add approx 2 ml 1g iron per 100 ml
12. Carefully bring the pH to 4.0 - 4.2

Adding Iron Changes the pH
So be careful

13. Let it sit for 15 min to precipitate
It will be mostly white to light green
& massive amts.
This is protein

14. Separate, Rinse, Sep Funnel & Test w/ HCl!

No incubation is required but if you do it produces fluorescent protein.

DID IT

Let the protein set in acid for a while & it turns brilliant blue

adding a little 0.1 M HCl will turn the solution from milky white to tan. Tan color is high level protein.

We have minimum amount under these conditions

I set the brown form again.
I diluted it .15 to 2
I used 3 ml (1g iron) / 100 ml solution
I got pH to only 3.5 very slowly

The tan version sinks, the white & green versions float.

You do not need to incubate it you can hydrolyze it

UV test
12 → 150 254 101
200 75

Aug 03 2014

The pH of the bile solution after 8 hrs is 9.2.

Aug 04 2014

You have a method of getting the lipids pure.

1. Take top layer of bile. (This has lipid layer)
2. Mix w/ acetone
3. Separate off the layer w/ not solids in it. equal This is acetone based.
4. Mix it with water & shake
   (Acetone & water will mix, lipids float to top) large enough sample can use sep funnel.
4b Centrifuge.
5. Extract the lipids on top with a pipettor or s
6. Add to water. They will not mix & lipids can be seen.
7. Examine last w/ alcohol & microscope examination.

Aug 04 2014

Super work today Clifford.

You got the lipids today in pure form.

Successive separation was the method.

Bile → Xylene → Acetone → Water.

These last few weeks are history making.

Primary one left is DNA.

Aug 05 2014 Page. 52

1. We can apply on the DNA issue today if you would like to.

The DNA test method failed on 3 (Funscience.com) accounts so far:

1. Ethanol filtered (Conventional)
2. COB residue
3. Filtered ethanol mixed w/ equal acetone.

The test is based upon 2 day old milk without COB.

The protein fails also.

Now trying COB 20ml w/ 5 drops NaOH & 5 drops KOH & 1/2 acetone @ 60°C.

Two Cases.

CDB from partial life extraction	Same
2 eyedroppers Caustic KOH	Same
to 4g Iron	None
80°C	Same
15 min	Same

—

Next.

KOH Caustic & CDB
1/3 acetone
4g Iron
High Heat 96°C
10 min

This is most darker & look more kettle

Coursera - Bioinformatics Course

Initial Notes:

ncbi.com
Natl Center for Biotechnology Education
DNA sequence
Protein structure
Protein sequence

primary key - a unique identifier in relational database

PubMed search . . . pubmed.gov

greider[author] limits to papers by greider

AND NOT OR

free full text paper filter

* is the wild card

Sep 01 2014

The office has been moved!

It is reasonably functional again.
It is mostly work that needs to fall into place.

We need to see if we can get DNA.

The DNA methods by the kit are just not working @ all. A big zero but banana is very easy. So something is very different here. We are working on hulls treated CDB, w/acetone, KOH, $FeSO_4$, SDS, betaine HCl, salt & most all combinations. No go.

Let's review the successful paper again.

Jan 20 2014 has the primary notes.
This is Vol 4.

Sep 02 2014

(1)

KOH – NaOH – Heat addition

Caustic Form

20 Some COB

4 drops Caustic Form It does react – turns dark green

60–65°C is stated as the denaturing temp of DNA 65° is for hot spring bacteria which ours is not like . . . . . -15

Set oven @ 76°C Heat 10 mins It does turn black w/ heat.

An interesting question to ask it turn green-brown

Now cool down w/ salt added.

5% Salt = 5 gms per 100 ml of water. This is high.

It is 1.5%, so it is 1.5 gms /100 ml.

A plastic test tube holds approx 10 gms.

.015 (30 ml) = 0.45 gms (1/20)

.015 (20 m) = 0.3 gms = (1/30) Just measure it. I did.

Now add 5 drops Enzyme dot.

We place it back in ice water & let it rest for 15 min.

We also added enzyme directly.

Nothing appears to have happened.

Yesterday we had a strong reaction to the of enzyme added.

Wash why it w/ detergent fail

20 ml CDB
4 drops Caustic
Heat 15 min @ 76°C
Dump 3 Gms Salt
Cool down.
add, enzyme fagot to add soap.

Now add soap & swirl 4 drops
let set 15 min in ice bath

fade

(3) yesterday w/ enzyme.

20 ml CDB
Back to pink salt.
Cool down
add 2 drops soap + swirl
Remove pinch enzyme
Let sit in ice bath for 15 min

We are duplicating yesterday's work w/ this.

added enzyme afterwards also

It form a layer of some kind but it is not ONE.

(4) Prospect Herd

20 ml
2 drops Caustic
60°C

Salt
Cool down
4 drops soap
Cool down 15 min
Alcohol
Addy enzyme dependent produce a layer.
Color turn from green/black to brown
w/ use of current Ethanol HCl

We have a chance of success here -
Right on top of interface alcohol makes
are we likely working.
It is also miscible alcohol
soluble in H_2O
Very small quantities.

Looks like high absorbance at 280

$\frac{\Gamma^0}{110} = 1.55$ ~ 80% pro, 20% water

Scale up Proposal:

60 ml COB
6 drops Caustic Heat @ 60°C
60 (.015) = Ø.90 gms
lets use Ø.3 gms
Cool down 15 min.
12 drops soap & lightly swirl
Cool down 15 min.
larger test tubes (about 8 inches)
Added 1 full scoop enzyme
inverted) twice
added cold alcohol
Continue to cool.
Large dve form, what is it?

Salt ✓ This time I let it cool down by accident for 15 min before I added the salt

Filtered soluble water results, then UV

UV @ Ø.2

254 = 114 121 123 Ratio = 1.43
200 = 80 80

About 85% protein
15% nucleic acids

However,

It fails the Bradford Test.
This says that it is not 85% protein

It is green.

Sep 19 2014 Succeeds.
15 ml CDB
added salt next!
then 2 drops caustic
60°C for 10-15 min
Cool down
add 3 drops Enzyme Soap
added pinch of multi enzyme this time early in the process.
Cool down.
This time, we let it sit through the stall call

It appears to fail across the board.
1 out of 6 has a lesser list of activity that we just do not get to lucrative action that we know is productive.

Postscript: Sep 19 2014

We may have it!

Letting it ~~sit~~ sit overnight has produced a filament structure on the alcohol water boundary in 5 out of the 6 tubes.
We may have it!!! We do have it.
Comparison to Jan 'wonder' 5000x is identical.

It is important to realize that the work was done off a CDB culture that was under storage for close to a month. We do not know if this was a factor or not.

Sep 19 2014. Trial 2 - Seems to Fail

Everything says that we have DNA again. The gene may potentially include PCR & gel work.

Steps are:
1. 15 ml COB
2. Added salt next (small pinch)
3. 22 drops Caustic
4. 60°C for 10 - 15 min
5. Cool down in ice bath
6. Add 3 drops dishwashing soap
7. Add pinch of multi-enzyme
8. Swirl gently
9. Cool down for 1½ hrs in ice bath
10. add the alcohol ~~in~~ test tube separation.
(15 ml made 5 test tube w/ [illegible] alcohol is ice chilled.
11. Test appear to [illegible] at bottom.
12. Let sit overnight @ room temperature (maybe cool also?)
w/ the alcohol that has been added
13. Examine closely.

Now if you recall what happened last time, when you added the cold alcohol you use it to wash the additional sludge along the side of the tube. the material, regardless of how it looks, all settled w/in a few minutes to the bottom and essentially the results all looked negative. When you let it sit overnight in residual ice as when the result occurred.

It does look identical again.
They are settling when you let a few minutes & the solutions are almost entirely clear. A slight fuzzy interface between the alcohol and the water.

There is some ice remaining in the current test tube ice bath temperature is 5°C. Let it sit.

Let's test the previous set results for water solubility.

It does indeed pass the test for water solubility.

Ø	94	-44
-57	65	-61

Just not enough material for reliable results yet.

Sep 21 2014

DNA Search

This most recent test seems to have failed almost all, if not entirely.

Differences we note are:

1. The CBB post Caustic was not as dark. You added 2 drops. Let's double or tuple.

2. You left at 48hr instead of 12.

3. You left the water in the solution with the tube. might of a better to extract that to maximize surface at the alcohol interface.

Let's go ...

But here is UV ... al 2 with ethanol a the C...

		Back to Control
130	~~120~~ @ 0.1 @ 254	16
(1)	70 @ 280 @ 260	−10
(2)	135 @ 0.1 @ 254	14
	73 @ 0.1 @ 280	−14

Average

$\bar{X} = 132.5$ $\Delta = -7.5$ $254 = 125$

$\bar{X} = 71.5$ $\Delta = +6$ $280 = 77.5$

$\frac{125}{77.5} = 1.61$

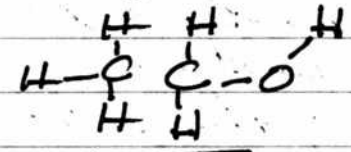
DNA Trial 3

1. 20 ml CDB solution, 20 g wheat in CDB.
2. Extract the top layer of water from the 50 ml tube.
3. Add salt grains.
4. added 5 drops Caustic. The past definitely made a difference it turned it green. 4 drops may have been sufficient(?)
5. 60°C for 15 min.
6. The color look more like the first set did, a darker brown color.
7. Am cooling down for 15 min.
8. Siphon off water.
9. Add 4 drops [illegible] soap
10. Add Enzyme paste (meat)
11. Stir mildly [illegible]
12. Cool down for [illegible] hrs.
13. You actually let it set for 3 hrs
14. Now pour into tubes & add the cold alcohol

What happened this time is that the material was very sludgy and heavy. It also had little water in it because of your prior extraction of the excess. You were able to put the alcohol in more forcefully because the filthy pretty much kept ready to the bottom. Now we wait overnight.

Question: What is the formula of ethanol?

C_2H_6O



Continuing our ratio estimate for Trial 2.
We had a 254/200 ratio for a total of 1.61

Ratio	% Nucleic Acid
0.57	0
1.06	5
1.32	10
1.73	30

Regression:

$$\%\ \text{Nucleic Acid} \cong 26.6\,\text{Ratio}^2 - 35.7 * \text{Ratio} + 11.9$$
$$r^2 = .996$$

for Ratio = 1.61, we get % Nucleic Acid = 23.4%

This result is:

1. With a very low volume of material
2. The control here is ethanol, not water.

This is not unreasonable.

We are now retracing the protein extraction process using 3 to 4 months old - 2 month old bile solution.
The pH of this solution is ~~6.3~~ 6.3
It does not have a real strong odor.

I used about 400 ml b/o solution + 100 ml xylene in this run with a 1000 ml beaker. This worked OK.

You need another 2 1000 ml beakers.
2 more 500s
2 more 250s.

Everything has gone fine. The color varies according to the combination of pH change & $FeSO_4$ (Ferrous) addition. You have representative examples now of dark green & light blue. It does look like this aged bile solution was very productive.

Now back to DNA, Trial 3

DNA Trial 3 Cont.

Wow also, this time, I have a much more clear separation between the alcohol & the water layer. The water layer below is yellowish in color and the alcohol is clear. It should be more obvious this time if there is any formation baby plants in the alcohol layer. We must wait and see.

After incubation : . . . 1/1

Next page

Sep 22 2014 (DNA Trial 3 Cont).

after incubation overnight, the results of DNA production @ the alcohol water interface are

Extremely successful
on all tubes. (5 out of 6)

I have now isolated the precipitated filaments into ethanol

They do indeed show up as a whitish precipitate so they are insoluble in ethanol as required.

Trial #3 worked very well.
Note the differences:

Notes:

1. Moderate salt pinch
2. Moderate to heavy moderate enzyme pipet
3. Incubated in ice water for 3 hrs before adding alcohol
4. NaOH was 5 drops in 2 ml CDB
5. The water was extracted every chance I got.
6. 4 drops soap
7. Stirred mildly, not swirled.

Trial 4. DNA Sep 22 2014

1. 38 ml COB
2. There is no water
3. Salt grind - moderate
4. 5 drops $\left(\frac{38}{20}\right)$ Caustic = 8 drops
5. 60°C for 15 min
6. 4 drops Soap $\left(\frac{38}{20}\right)$ = 6 drops Soap
7. Mod to full pint enzyme
8. Start Mixing
9. Cook down, Start @ 1940 1½ hrs min
   ~ 2hrs 3 preferred.
10. Add alcohol
    ~ 10 tubes this time
    Raw COB, no storage time.
11. Wait until AM

1. Additional Notes
   If you rinse protein enough, they float at least for a while, and stall well.
2. Lipids are next to pure but they are there.

Trial 4 Fails!

Sep 23 2014

Here are some lessons learned from the DNA trials, especially the failure of Trial 4.

1. The storage of the CDB for 2-4 weeks is an important factor in success. The texture of the culture must change to become more globular.

2. The recipe is:

1. 30 ml globular CDB culture
2. Extract any & all water after settling
3. Add salt pinch
4. add 5 droper caustic
5. Heat @ 60°C for 15 min
6. The will turn the color a darker brown
7. Siphon off water again
8. Add 4 drops enzyme soap
9. ~~Stir mildly not swirl~~
9. Add moderate pinch multi-enzyme
10. ~~Stir mildly~~, not swirl
11. Cool down for 3 hrs in ice bath
12. Pour into tube 1/3
13. Add 1/2 to 2/3 ICE cold alcohol with some vigor w/ the pipette.
14. Incubate overnight
15. Inspect the alcohol - water layer very carefully
16. Extract materials to alcohol

Cool down for 15 min!

The material will be more sludgy in this method

We also learned that the protein floats when it is small enough.

Actually it either floats or sinks it is right on the edge.

Sep30 2014

1. Today is a very significant day.

[redacted]

2. Many exciting things about happened here again.

[redacted]

4. DNA repeated
5. Samples prepared & sent.
6. DNA electrophoresis
7. Protein electrophoresis.
8. Resume writing of papers.
9. Glutamic acid prospect.

Sep 30 DNA Trial

1. 30ml COB layer
   We can see that a layer has indeed been formed on the top of the COB. This layer is one week old & it does look & act differently. It should be the character we need & eventually it can be examined microscopically to compare to the raw COB.

2. Extract the water (not much here)
3. Add the salt pinch. It remains moist.
4. Add 5 drops caustic
5. Heat @ 60°C for 15 min & after mildly. It has turned a dark olive color as the the caustic it does get darker after you heat it. It turns a muddy brown.

6. Cool it down for 15 min!
7. Add 4 drops enzyme soap
8. Add multi enzyme pinch
9. Stir mildly
10. Cool down for 3 hrs.
    I stirred lightly and intermittently (once in between)
    I went for 2 hrs.
11. Pour into test tubes 1/3
12. I had 11 tubes that are in a double strength ice bath
13. Will clear overnight.

This trial seems to have failed; maybe some last slight

Oct 2 Trial.

10. This time we let it sit approximately 8 hrs in the ice bath.

11. Pour into tubes: We get about 11 tubes which is more than we want. It is noticed that the material is not as "sludgy" or as dark as the successful test was. This has now been noticed twice. It is thought that storing the CO_2 for an extended period is going to make a difference. It is believed that soft proteins are being generated and that these are more likely to glaciate DNA. This means that we have a big significant lag issue involved that makes the process that much more difficult. But it has succeeded.

We have substantial material here: We are still trying to determine if it contains DNA. Favorable factors are that it does not appear to accept Coomassie blue stain as a protein would.

However, it does not appear water soluble upon Centrifuge.

It is a precipitation reaction that does take place in alcohol

@ 0.1 $\frac{\text{Li}\ 138\ 144}{\text{Na}\ 114\ 108} \rightarrow \frac{138}{144}$

$\frac{137}{107}$

We also acquired a

1. syphon
2. a pump that we will return
3. a thermometer
4. a blender
5. 6 large Camros jars

7. On the DNA problem you are going to heat it proportional (inversely) to the time of storage. You reference is 4 weeks you go 1½ weeks in.

$\frac{4}{1.5} = 2.7$ times (15 min.) = 40 min @ 60°C proposed.

DNA Trial Oct 03 2014

1. 30 ml CDB 25 ml
2. Salt
3. 5 drops Caustic
4. Heating for 45 min @ 60°C

W.r.t. Oct 02 trial we still see some potential filament production @ the alcohol - water interface. This has occurred after letting tile ~10 tubes set for another 24 hrs. There is after the second heat extraction. It looks like we have another opportunity to extract.

5. I went 45 min again for a total of 1½ hrs.
6. Cool down to room.
7. 4 drops soap, Enz. Purch. & Stor
8. Sit overnight!
9. Very interesting. This time we have a very clear water separation, approx 80% is water. We will draw it from the top.
10. Pour into tubes of alcohol
    Now, it was indeed cloudy & dark so this does seem to be more like the successful Trial #3.
    You heated 1½ week CDB for 1½ hrs.
    You left w/ enzyme & soap overnight.
11. Now let sit in alcohol overnight.

DNA

The Bradford test on Oct 02 Trial extract,
1st extract:

It fails the Bradford Test!
The is highly positive for DNA!

Both the Coomassie Blue stain & the Bradford test fail for protein detection.

Since it forms @ the alcohol - water interface & does have a semi stringy appearance, this is indeed highly suggestive of DNA. But why the poor result of the 260/280 ratio? Why the insolubility in water?
Nevertheless, these are highly encouraging results. Gel electrophoresis lab are coming up.

We ran a rapid test yesterday and it worked beautifully w/ the bubble solution. Each bubble session was only 5 sec long @ low speed.

Oct 04 2014

1. In COB culture, will add starting weighing to water & decreasing the sugar & salt.

2. We have created a sludgy DNA threat w/ water separation. Time periods are made extensive
   1. 6-8 hrs w/ enzymes & soap
   2. Heating 1½ weeks for 1½ hrs @ 60°C
   3. In alcohol overnight – to 24 hrs. – 36 hrs

3. We plant to try extracting protein w/ out Coomassie blue.

4. We want to keep examing the prospectra DNA.
   Positive:
      1. No Comm. Blue stain acceptance
      2. No Positive Bradford test
      3. Formal water/alcohol interface
   Negative
      1. Does not appear to be water soluble
      2. Does not look the same under scope?
      3. Fails 260/280 test

5. We want a DNA threat of fresh COB now.

6. When you pumped O_2 into the vessel it appeared to have created some very fine structured COB. It is taking a very long time to settle.

7. The cultures are <u>not</u> working w/ recycled water.

8. We have 2 cultures w/ $FeSO_4$ (II)

9. Oct 03 Trial! I believe we are seeing some DNA already & it is floating @ the top of the alcohol! It is very slight but I am seeing it!

10. A protein production test using only HCl failed! It is not the same for some reason! (We also used only $FeSO_4$ (II) not Fertilome.

11. Very interesting. You do have a precipitate but it is not protein based. This test has failed.

12. We figure that the consensus must bind to the protein as it is only partially protein. The lighter the color the more pure the protein appears to be.

Oct 04 DNA Trial.

1. Using 40 ml fresh CDB.
2. adjust salt & Caustic by $\frac{40}{28} = 1.42$

5 drops Caustic = 5 (1.42) = 7 drops Caustic
slightly more salt.

3. Heat @ 60°C for 1½ hrs = 90 min.
4. Cool down 20 min.
5. 4 drops soap (1.42) = 6 drops
   + Enzyme (a little more)
6. Let set overnight. But I advance one set. &

You may have spilled the advance set. You still have ½ remaining.

We have a very interesting observation on the protein precipitate. KOH break it down more successfully to reveal the protein better than Sat. NaOH & the Caustic formula.

Try NaOH in the DNA routines.

We seem to have a major precipitate forming on the advanced set

Oct 04 KOH DNA Trial 1820

1. 30 ml 2 wk aged CDB
2. Pinch salt
3. 8 drops 1M KOH
4. Heat 60°C ═══ boosted to 70°C

Clearly KOH is causing the protein to release staff from the protein precipitate complex. The protein is binding to Coomassie Blue in the Bradford test.

Therefore, to separate the protein further, mix the protein 1/2 w/ water.
add 6-8 drops 1M KOH. & Shake.
add 2 drops 0.1M HCl & then Bradford.
The protein now stained, will precipitate & bind to Coom. at the top.
This should be useful in electrophoresis.

Lessons

* last 2 days

We have learned quite a lot to day even though DNA retains unsuccessful @ this time.

1. The blender is a huge improvement.
2. Very succesful production of lipids by an extension to the use of alcohol for even further improvement.
3. It looks like the Coomassie may indeed be important in the creation of the protein precipitation complex.
4. It is necessary to break down the precipitate to test for proteins. KOH works much better.
5. You may not need liquid iron any more. Iron sulfate (II) looks to be more than adequate. This will save some expense (possibly).
6. You have radically changed & simplified the culture process. 5 half gallon mason jars now. Also regular sugar instead of fructose.
7. You have a slight aversion with gel electrophoresis now.
8. The sink does not work anymore!

9. DNA has some real difficulties. KOH / M vs Caustic??

Agarose gel electrophoresis of Proteins

1. Large pair 200 kDA.

Holes in agarose are not small enough to let small proteins move. Only rather large proteins.

Milk is 28-30 kDa — hopeless
Casein

This is why.

(Mole-Mass)

Red Dye #40 MW: 496
3 negative charge

Brilliant Blue MW: 793
net negative 2 charge

Oct 05 2014

! Stop the Presses !

I think we have the DNA w/ the advanced ½ set. Recall the steps as quickly as possible.

1. 40 ml Fresh CDB (great!)
2. Mildly Trace salt (about ½ spatula)
3. 7 drops caustic
4. Heat for 90 min @ 60°C
5. Cool down 20 min
6. 6 drops enzyme soap + Multi-enzyme (half heap spatula)
7. Let sit in ice bath about 5 hrs (1300 – 1800)
8. Now the ice bath sits & melts gradually overnight.
9. @ 1300 on the next day, see

alcohol here

3 out of 5 tubes have a clear formation of a filament based development @ the alcohol interface layer!

Great news is that this fresh CDB.

[redacted]

1. Tilt Scale
2. Gel work + Labs
3. [redacted] Dna + better all produce here
4. Leave on TVS
   (Provisional —

[redacted]

We are set to continue:

I think Success here.

1. ~~Try~~ good Try to look @ DNA project under scope
2. Get lab purchase + equipment purchase in place.

[redacted]

4. Watch Think well:
   Can we acquire a course for the tablet?
5. We have 2 more DNA treats to finish up and we can start another.
6. Gel electrophoresis investigation DNA? stains?
7. Amino acids other proteins

B. Continue writing of paper.

9. Glutamic acid prospect

10. Several projects:
    1. MRP
    2. DNA in labs
    3. Website
    4. Abstracts & Webinar
    5. NHFC advocacy

We have 2 more DNA tests to complete & another to initialize. KOH trial & the 2nd half of advanced

11. Also, if we recall, we were a little sloppy about how we poured the alcohol into the tubes. It was slightly disruptive to the process & you poured some out of a small beaker rather than pipetting it in carefully. You also tapped both & ya were concerned about water. Levy came in. You also had a note of some major precipitate occurring unusually early in the same.

The consistency of the advanced set (now the latter lab) was like, as like hot chocolate. It was smooth in consistency. Also I put plenty of alcohol in the tube directly from a small beaker & it pretty much took care of the residue on the inside of the glass tube.

Page 91

Oct 05 DNA Trial 1 (Labeled Repeat/St 1/2 Advanced)

1. 40 ml frozen CDB
2. Salt, 7 drops Caustic, 90 min 60°C
3. Cooling Down for 15 min. Ok
4. Add 16 drops soap + Enzyme 1/2 Spatula loaded
1615 oct05 5. Let this sit in ice water for 6 hrs min.
(We are going to leave overnight)

Cont from 2 page ago:

12. On the advanced set, now the latter half we have a major precipitate forming (almost immediately). So we have 3 layers.
    1. Chocolate below
    2. a major precipitate (bulk of tube)
    3. alcohol on the top.

So something is clearly different about the set and the process.
The implies that the 90 min heating @ 60°C in a highly caustic solution has made a big difference. Also the incubation time of the enzyme & soap (at least 5 hrs plus & now of the latter half 24 hrs. is also making a big difference). Incubation of both caustic influence & enzyme influence may be very important factors.

13. We have now also set up the KOH run & labelled it also. Repeat of the "advanced set" is also underway.

14. We have a major precipitate forming w/ the KOH run also. It is also separated some bubbles in the alcohol which is also a very good sign.

15. The KOH trial is already bubbling I think that it will end up being even more productive. The steps were:

✓ 1. 30 ml 2 week aged CDB
✓ 2. Pinch salt, 8 drops 1M KOH
OCT 05 1815 3. 70°C @ 90 min

1830 OCT 05 We have 4 tests running:
labeled 1. 2nd half of advanced set in alcohol
labeled 2. KOH trial in alcohol
labeled 3. Repeat 1st ½ advanced (even 4 soap incubating)
labeled 4. KOH repeat - Double bath heating)

t. Cool, dry. 5 drops Soap

Oct 06 2014

You are running out of time before you depart so we will have to summarize & conclude where we are.

1. The KOH trial looks very promising. I am seeing it twice now. It has been done w/ a fresh COB culture. It is possible that it will work even better w/ an aged culture. But for now, we may have minimal amount of a 24-36 hr. period & a fresh culture. Let's study this one first.

Oct 09 2014 Bass Creek

In the Field DNA

Use the KOH method of aged culture

1. 30 ml aged COB culture
2. Pinch salt
3. 8 drops 1M KOH
4. 90 min @ 70°C
5. Cool down for 20 min.
6. 5 drops Soap, ~~salt~~ enz. stir
7. let sit 5 hrs Start @ 1600 + 5 = 2100
   This is the smaller beaker
8. Alcohol added @ 2200 - sit 12-24 hrs

I see no success

~~Dupont COB~~

Ok we have an interesting case here
In all ways the KOH test has failed

Oct 09 2014 This finishes on Oct 11 2014

Now use the caustic method

1. 30 ml aged CDB
2. Pinch Salt
3. 5 drops Caustic
4. ~~[illegible]~~ 90 min @ 70°C
5. Cool down for 20 min. Timer on
   This is the larger beaker.
6. 5 drops soap, eg. 1800 + 5 = 2300
7. let sit 5 hrs.
   This is the larger beaker
8. Alcohol added @ 2200 Sit 12-24 hrs

I see no success.

~~Drop to~~ 60°

But the caustic test did not fail. But it took two days to develop! Recalling after 24 hrs wait w/ no results I decided to heat it in a water bath to max 35°C. Mostly it was between 30-35°C. You did this for about 2 hrs. You then left it to incubate overnight. You check it again the morning & you see success but only in the caustic case. 5 out 6 tubes ~~you~~ were successful & I spilled one

So we may have a modified method.
Mild heating & incubation over a 48 hr period.

You poured the alcohol in - it was definitely not subtle.

Oct 10 2014 - Oct 11

1. 30 ml fresh COB
2. Salt
3. 5 Drops Caustic
4. 60° C for 90 min

We are continuing w/ this trial on Oct 11

5. We let it cool down for 20 min
6. Then we add enzyme & soap but instead of letting it set for 6 hrs & add alcohol we forgot & let it set for 18 hrs and added more soap & enzyme on the morning of the 11th - Sat.
7. We have let it set for another 2 hrs of more enz & soap & now we are going to add alcohol (cold)
8. Now we added cold alcohol & placed it into the ice bath - speculated It was is a nice dark chocolate color. Even though we are not real delicate w/ the alcohol, we believe that it will settle out over the next hour or two
9. We have 6 tubes. We would like to split them up into two trials. 3 tubes stay in the ice bath until morning, approx 18 hrs. The other 3 we place into a water bath for 2 hrs @ 35° C & then incubate in the test trails where it is considerably warmer. We incubate until tomorrow morning.

Looks like success again!

A new algorithm is proposed.

1. 30 ml CDB (Hopefully fresh will work also).
2. Pinch salt (not too much)
3. 5 drops caustic
4. 90 min @ 70°C
5. Cool down for 20 min in ice bath
6. 5 drops soap & 1 mg (half scoop)
7. Let sit for ~ 6 hrs.
8. Add alcohol (cold)
9. Let rest for 12 hrs in ice bath.
10. Heat for 2 hrs @ 30-35°C. This did not help ???
11. Let incubate @ room temperature for 12 hrs.

You see it. You are not sure y'all the [redacted] required or not. But it did [redacted]. The cell test failed.

We continue to Oct 10-11 treatments on the next page.

Carry over Oct 10-11 Trial in Progress

10. We have taken three tubes from the alcohol set @ 1715 MT on Oct 11 and have placed them into a water bath of 35°C. The set is therefore advanced of the other set of 3 remaining in the ice bath until tomorrow. We will leave in the 35°C bath for 90 min & then incubate in the test tube heater.

11. It is Oct 12 now @ 0950 & we are going to compare the two sets: The advanced set is in the larger heater & the ice bath overnight set is in the smaller heater.

There is no obvious difference @ this time, but there is a lot of condensation so it is hard to say. Rubber bands will be placed around the advanced set & incubated all @ room temperature.

"Advanced" set w/ the rubber band fails.
The larger test in incubator set is better @ interface precipitate.
5. the 35°C water bath did not help matters

Oct 11 → Now to Oct 12 - A new trial carrying forward.

1. 30 ml CDB
2. 5 Caustic + Salt (not too much)
3. Apparently 70°C was OK, but let's drop it to 65°C for 90 min.
4. Well, at this, it has been in the cooler for about 2-3 hrs. How long should it be there? 20 min was enough! Add the enzymes + soap! Done @ 1115 MT.
5. This has incubated in the ice bath until 1030 AM on Oct 12 2014.
6. Lets add alcohol & Continue to incubate in the ice bath.

Summary of the DNA Situation.

Oct 12 2014

It is actualy doubtful that you have extracted DNA but it is possible that it has a partial Component!

What really seems to be happening is that when you pour the alcohol in it seems to be causing an enzymatic reaction. This reaction seem to take material upward & some of it adhere to a deposit stuck on the side of the glass tube.

Then after it circulates from 24-48hrs, the material settles. Some of the apparent remains @ the alcohol-water interface layer, but what it actualy is composed of is a separate question. There is duty to prove that it is DNA.

I think that the a summary of the numerous tests that have been done. These results are settled @ this time.

So from here we go on:

Do we have any alternate prospect for DNA work?

I need that as I could do better but I do not know where to come from:
The proteases is one idea.
Continued creativity of the COB is also helpful.

What is the function of the
1. Salt?
2. The Caustic?
3. The lysozyme?
4. The soap?
5. The temperature baths?
6. The cold alcohol

Research literature for this.

7. Does blending do anything? If so, how much?
Ultra sound do anything?
Bile do anything?

May combinations to explore here...

It seems like the lule solution should be a good candidate. Continued work by this stage?

Role of Salt: to neutralize the charge of the DNA's sugar phosphate backbone.

Salt: to dissolve the cell membrane (assumed to be a lipid bilayer)

NaCl: remove proteins that are bound to DNA, also to keep the proteins dissolved so they do not precipitate in the alcohol w/ the DNA

Alcohol - precipitation - indeed @ the interface layer.

1 liter water
15 gms NaCl
50 ml dishwasher detergent

But salt also destroys enzyme inhibitors so this would let a reaction go not very well

Oct 15 2015

We have an overflow of projects that need work. You need to sort them out.

CF:

1. DNA Lab Analysis - this is front & Center no matter what.
2. You are studying biology in general & that can be a bit overwhelming. You took an Course on Coursera, Think will be good but require power, and AP Course is good.
3. Advanced Biology Labs - these are fantastic & Coincide w/ AP
4. You have many gel work & DNA & protein lab work you would like to start with. Then does have a Cost, time & $.
5. You have many papers to write
   1. Mody intent to include glutamic acid project & the movie completed.
   2. CDB Characteristics Paper - a review paper
   3. Bass Creek Field Analysis - Spooky
6. You have two labs already purchased that need completion:
   1. Bacterial Fuel Cell
   2. Osmosis
7. You are no longer in the lab to work continuously & that is the way that it is going to be so get used to it.

[redacted]

[redacted] Page 107

Plants"

1. White berry

~~Bane berry~~ Snow berry
Baneberry — e
opposite, 3ft high Capifolier

2. Elderberry I was OK on these.
Compound —

3. Sitka Mountain-Ash
Sorbus - Genus
>>Sitchensis

4. Greenish gray lichen -unknown
on a grand fir

Common Name

5. Dark red brown berry Pipsissewa
Whorled leaf structure
Unknown Chimaphila umbellata ←
Genus | species

6. Light green & feathery lichen
Unknown —

7. Dark bright red berry
Nightshade
term. ternate (triangular)

8. Dogwood?
Capsule - a dried fruit · 4 sepals
Brown -
Opposite, Simple

9. Moss is unknown

10. Pine Ponderosa Cone Scale
Unit name

11. Anise - my reference only?
Biscuit root - first pass - unlikely?
A Challege

Page 109

[redacted] What are questions [redacted]
like to know?

1. Is methane really a problem?
[redacted]
3. Is the information genuine or a propaganda campaign?
[redacted]
5. Do you want to distract yourself from your work to clarify this issue?
6. Are there existing models of greenhouse gas influence? What are the greenhouse gases.

7. What is the balance of influence between specific heat & IR absorption?

CO_2
Water
Methane

"20 yrs Period
"Global Warming Potential"

Specific Heats

Air	1.01	
Ar	0.520	
CO_2	0.844	1
Methane	2.22	56
Nitrogen	1.04	
O_2	0.919	

400 ppm (1) vs. 2 ppm (56)

= 400 = 112

Weather Station Analysis.

We see now how the barometer works.
It is computing altitude from a reference with averaging.
1. Starting reference pt is 29.92 in (adj to sea level)
2. It takes differences in magnitude that you cannot see in absolute terms, eg 3600 ft ≈ 3.6 inches so it is taking readings in the area of 26.32 +/-
Then it also records the duration of given actual value centered around the region that you cannot see & averages all of them. Say it comes up with a avg of 26.5 in. Then it determines your altitude as approx 29.92 - 26.50 = 3.42" ≈ 3420' above sea level. Then as now adopted as the reference. So barometer will drop to 26.0 in actual = 3.92" So it works w/ local variations about your altitude.

The device will be the most accurate @ a near sea level with major weather variations.

If & when you change altitude that the device will be in error.

so as long as you do not change your altitude too quickly & too much it will still be reasonably accurate.

The device only uses barometric pressure to improve the results, use (~70% at best usually less)

1. Wind direction
2. Wind speed
3. Clouds
4. Humidity
5. Temperature
6. Barometer

So we really do have a lot of information @ our disposal to make a forecast with.

Example: ~1415 on Wed.
Humidity now is @ 56%
Temp is: 56°. (It warmed up quite nicely).
There are some clouds coming in.
Wind direction is from the NW. Gusts are increasing in frequency
Barometer = 29.85. & falling steadily
The suggests decent weather immediately ahead.
But never says a storm is coming in. Watch for it.

1830 - Somehow the Acurite has picked up rain. Very likely. I do not know how it has done this: Especially from the barometer alone.

We do have a slight fall in the barometer below 30.0 & fall slowly means unsettled
Element as a crucial piece of info.

Actually!
The signs are in place but they are much more subtle than imagined.

1. Barom. was slightly falling today. and slight is slight.

2. The wind has changed from northwest to southwest & the Clouds Vlefy at. The windspeed is almost non-existent but the tell tale reveals it.

3 It is, because of the wind shift, anticipated that the temperature will be quite moderate tonight. This notable warmer air w/ moisture coming in.

Believe it or not the forecast is indeed the increased possibility of rain tonight. Cloudy is early tomorrow or tomorrow.

Very Good! Pressure, Wind Temperature Change, Clouds, Change

Main Factors of Weather Forecasting

[redacted]

8L
8PM ET

Weather (cont) (relative to time frame)
a falling barometer coupled with warming temperatures
(might indicate rain

So temperature relates directly to winds.
Cold temperature = northerly winds
Warm temperature = southerly winds
Rising barometer w/ cooling temps (relative to time frame)
mean more fair weather.
Cold air = more dense, ie high pressure
Warmer air = less dense, lighter pressure.

So Wind direction, temperature and barometer
(i.e., changes in)
Can be used to great advantage in
predicting the weather.

MAIN FACTORS

Temp - warmer air from the south.
Colder air from the north

Pressure - Colder air is more dense, higher pressure
Warmer air is less dense, lighter pressure

Wind - Air from the north is colder, drier
Air from the south is warmer & more moisture
Clockwise trend: Fair weather
CC trend : Deteriorating weather

Oct 30 2014 Page 114

Let's get ourselves organized again:

1. We have an entire flow chart of things immediately on Step for the next week.

[redacted]

3. You have 3 textbooks to work with:

1. Adv Biology – there is an amazing course. You have an amazing Atlas exercise going on. Protein – disease relation. How relevant is this?

2. Osmosis lab
3. Bio fuel lab

4. You have some major protein – DNA electrophoresis work & labs coming in.

[redacted]

6. You want to study general Biology

1. Wolfe
2. AP (2 books + flashcards)
3. Bio. Workbook

7. Yr need to write papers!

1. NHFC Advocation
2. Status report update
   1. office moved
   2. projects outlined
   3. schedule
3. COB Characteristics Contenued.

8. Are yr going to take on the climate issue?

[redacted]

10. Immediate labs w/ DNA & protein electrophoresis.

Large amounts of banana DNA today!

Let the SDS sit for a half hour
filtrate

Oct 31 2014

We are quickly moving now to DNA production & testing. We have successfully produced large relative amounts of DNA from bananas.

Lets keep working the result. The standard method in our lab works very well.

Method
1. 7.5% SDS & 1.5% NaCl
2. Enzymes
3. Blender
4. Strain
5. Cold alcohol – wa la you are in.

However, you diluted the SDS by a factor of 4 (i.e. 1.875 ≈ 2%) and you maintained the salt concentration @ 1.5%.

Lets try a mix of our commercial soap.

100 ml H_2O + solid material combined total 3.5
2 ml soap (enzyme)
1.5 gms salt
blender.

(used 3 gms, want 1.5
total fluid abt 100 ml)

I forgot to add enzyme but the soap itself had some! But it still worked in an immediate mode!

Reference DNA Production

We are now in a reference DNA production mode w/ commonly available materials! Great!

We suspect the method to be.

1. 100 ml total fluid (sample & water combined.)
2. 3.5 ml Commercial eng. soap
3. 1.5 gms Salt
4. full Pinch of regular enzyme
5. Blend
6. Sample sits @ room temperature for 2 hrs if possible but not necessary required

7. Filter
8. add cold alcohol

Wa La!

GN Can try
Tomatoes
Potatoes — Need sugar — Soups for me
Banana — a source of iron?
Onion
Carrots — Banana Peel
yeast? — Alcohol

Ok, I have scaled the operation up with great success.

NOT necessarily true

1. Used 1 full banana
2. 4 ml Dawn soap
3. 2 gms salt
4. still about 100 ml liquid total
5. 2 full pinches of enzyme
6. blended approx 12 min @ low speed.
7. Filter - LET SIT IF POSSIBLE.
8. No matter what to precipitate, add 2 to 1 ratio of cold alcohol.

This batch did not work as well! Had less salt & more enzyme. More blending

9. We have immediate precipitation. DNA production.
DNA should eventually float in the alcohol
260/280 ratios now ready to read.
Methylene blue tests
A wire is a good extraction tool for test tubes

~~We did not produce as much~~. All is fine.

Recommend

1. 100 ml sample
2. 4 ml Enz. soap
3. 3 gms Salt
4. ½ pinch of enzyme only. FULL PINCH
5. 2 minute of blending w/ banana. per 100 ml
   * density / ~~banana~~

You are producing more than you ever could have imagined.

Pressure squeeze filter w/ water weights

SUPERB results here

Very high level of success w/ banana peel also!

1. Blending @ much higher speed for ~ 4-5 min
2. More water added - up to about 400 ml
3. same ratio of salt & Enz & Soap
   8 ml soap
   6 gms Salt
   2 full Scoops enzyme
4. Vacuum pump w/ Coffee filter worked like a Champ.
   only about 20 ml fluid extracted.
   Seem safe.

OK, a major faux pas w/ the vacuum pump.
You sat water in the pump and it mixed w/ the oil.
Absolutely bad news.
So you drained it successfully & put new oil in. But you have no spare oil now. You must get something.

Transmission oil?
You need some kind of screen or filter.
Never do this again.
Maybe a handi wipe?
Maybe a sponge material,

Potato was much harder for DNA but I believe I have it. It seems to be much smaller size than banana.
Genome size ≈ 844 ~~mb~~ Mb = Mbp
(what are these units?) mega base pairs

Genes per Mb appear to be on the order of 50+

844(50) = 42,200 genes.
How many base pairs would this be?
(Yes, actual genes is 39,031 (good)

Banana = 523 Mbp Genes = 36,542

1 picogram = 978 Mbp nucleotide base pairs (mass)

	Size	Genes
Potato	844 Mbp	39,031
Banana	523 Mbp	36,542
Tomato	900 Mbp	34,727
Celery	3000 Mbp	
Cranberry	~~470 Mbp~~ 570 Mbp	
Apple	742 Mbp	57,386
Carrot	473 Mbp	
Onion Yellow (5 times larger than humans)	16,000 Mbp	
Human	3000 Mbp	21,000

Oct 31 2014 Start Potato Cultures
With the vacuum you must use a
hand wipe and a screen filter!

Let's go for mushroom! Looks good!

The GEL Fails @ last @ this point.
NO The last small Concentrated tube succeeds
We have set up 12 potato gel cultures.

1. Slice Potato
2. 3 drops CDB
3 4. Smallest scoop powdered iron $FeSO_4$
4 5. Pinch salt (flat spatula)
5. Incubated @ ~ 90° F.

Nov 01 2014

Our Sal DNA prospect samples have gone through two changes.

Our primary sample seems to have settled and destroyed the separation that was apparent @ the onset.

One of the other 5 tubes has developed a separate prospect.

You should have separated this first one when you saw it.

Try to salvage the second one.

DNA Prospect II has succeeded. Small but very clear samples. This was the bleeder residue.

OK, upon closer inspection you have some surprises here.

You must pour the alcohol gradually!

It looks possible that most everything succeeded here. Incubation overnight does seem to have made a difference.

You now have 3 (microscopic) candidates from the Gel test for DNA that have been extracted & labeled. They are all candidates for testing. They also set a precedent for what is to come. Your work yesterday on reference DNA from various samples has really paid off.

We are now...

1. Following through w/ additional reference DNA
2. Headed towards preliminary gel electrophoresis work
3. Can we extract efficiently directly from the COB??? ??? ?

Isopropyl alcohol does not work..!

Celery is very hard, it appears.

The apple seems even harder - why?

There are some questions that I have
about.

Is the DNA from allium always stringy?

Does pulverizing too much damage the DNA?
Think of potatoes as they are cheaper &
they seem to demonstrate the issue.

Try 30 seconds on the potato

This will be a blender exp.
w/ potato.

Potato is thrown in with big cubes
Pulse it to break it down 300ml

Low Speed

1. 1 min. Appear to be the most successful.
Most distinct, larger chunks

2 2 min

3 4 min

4 6 min less separation of color
finer materials

More blendy produces more fluid. Makes sense.
15 ml each well

So the big lesson here

IS DO NOT OVERBLEND!

Pulsing may be adequate.
Keep time short; 1 min was
fine for a potato
you might even try 30 seconds

It is more stringy and more white
with the bowl pulsing.

The time you used

1. 1 good sized potato.
2. 12 ml soap (should have been 40 ?)
3. 1.5 ml salt
4. 5 scoops soy yue.

Blending for 2 min after pulse breakdown.

Looks very good.

None of the samples from 1-4 minutes
are bad. They are actually
all productive.

I have to say 4 min actually seemed to be
the most productive, even if it was
broken down a little more.

What you did or use a little more of everything
in the process, soap, salt, soy yue

The Human Saliva Test

Even the saline human saliva test which looked quite hopeless has produced a visible result. The alcohol layer has become cloudy over the last time. It looks digested to me but it takes a microscope pretty much to see it. The alcohol has turned cloudy. Lets look under the big scope on a well slide

Also methylene blue
You can see the material w/ a mag glass

Lets start working on the gel

1 ✓ We want meth blue w/glycol
red food dye w/ glycol
? Why did 2 it so? 3 ✓ old Stained DNA w/Glycol (banana)
4 ✓ fresh Stained banana DNA w/Glycol
5 ✓ fresh tomato. Stained banana DNA
6 ✓ banana alone w/Glycol
7 ✓ tomato alone w/Glycol
8 ✓ fresh Stained onion w/Glycol

	lane
	1 D
500	2 D redone w/ a duplicate of track 4 but on NOV 02 vs NOV 01 (replaced banana stained overnight)
	3 D disappeared, so we have eliminated it. (it has moved already)
	4 D
	5 D
	6 D
900	7 D replaced with #5 (tomato, stained overnight)
	8 D
	~~9~~

Watch tracks 2 & 7 today.

The dye did move...
but the wrong way!?

3, 4, 5, & 8 have moved toward the negative terminal - why & how?

The anode is positive in a device that consumes power, and negative in a device that provides power.

So what happens in a battery?
In a battery, the anode is negative
the cathode is positive.

So for our voltage supply,

Remember these have been sitting in the dye overnight so they may end up looking better. It seems to change the dye's color cast

Nov 02 2011
Gel Electrophoresis

We have some very interesting results that have taken place

Lane
1 Moved moderately toward flipped pole (negative)
2 Replacement now towards positive
3 Is weak, apparently toward negative
4 moderate strength, towards negative
5 " " "
6 Clear and cannot be seen
7 Sharply towards positive
8 Towards both neg. & positive

2 & 7 are interesting cases. They are replacements that are now going in the expected direction. This says that stem overnight appear helpful.

Let rearrange terminals. Use existing stem overnight

Page 130

1 Something changed when you increased the voltage to 50V vs 10.
2
3 They flipped direction.
* 4 overnight banana Overnight stains were alone important
* 5 overnight tomato
6
7
* 8 overnight onion

Move these now to lanes 1, 2, 3 & reverse terminals.

4 1 overnight banana
5 2 overnight tomato
8 3 overnight onion
4 4 banana
5 5 tomato
8 6 onion Done @ 1500 MT
7 Stained Banana] 1500 MT
8 Stained Tomato]

Nothing moved! Our DNA is much too large! We need to break it into fragments & use loading food color dye!

Several problems have been identified.

We need a tracky dye that
matches about 2000 ~~100~~ 100 bp

Humans have ~ 3.2E9 base pairs

= 3200 Mbp

~~Tags~~ Fragments in gel electrophoresis
need to be between 1000bp to 25,000 bp
& 4000 to 1000 bp looks common.

Starch content (like a potato)
affects the result or ease
of extraction.

One of the papers we found has
~~different~~ variations.

You need shorter fragments to
use w/ a tracky dye.

Maybe the melt blue locked up the DNA.

Page 132

SUMMER RESEARCH PROGRAM FOR SECONDARY SCHOOL SCIENCE TEACHERS

LESSON PLAN

Edwin Klibaner

Extraction of DNA from White Onion

The procedures involved in biotechnology implementation are predicated on the isolation of DNA from a tissue sample. [Content Standard E- Understandings about science/technology] This laboratory exercise is designed to give you the opportunity to extract DNA from onion tissue. The technique used is quick and easy for both you and your students. The DNA that is isolated can be digested using various endonucleases, followed with an electrophoresis of the digest. We use an onion because of its' cost, abundance and low starch content. You will make the onion filtrate from onion treated with salt, distilled water, and dishwashing DNA to be more clearly seen. The SDS detergent causes the cell membrane to break down by emulsifying the lipids and proteins of the cell and disrupting the polar interactions that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins, causing them to precipitate out of solution. The use of NaCl salt shields the negative phosphate ends of the DNA which allows these ends to come closer so they can precipitate out of a cold 95% ethyl alcohol solution. You will be altering the filtrate so that you can "spool" DNA out when it precipitates. The DNA is soluble in the detergent solution but is insoluble in the alcohol. [Content Standard B- Structure and properties of matter]When you add the chilled alcohol, the DNA will come out of solution and easily spool on a glass rod. At the end of the exercise you will find a flow chart summarizing the steps and solutions required.

The procedure has three basic steps:
Homogenization which involves heating and blending the onion tissue in order to break down the cells. [Content Standard C- The cell] The heat treatment softens the phospholipid in the cell membrane and denatures the DNAse enzymes which if present, would cut the DNA into small fragments so that it would not spool. The onion tissue is mixed in a blender with homogenization media, which breaks down the cell wall, cell membrane and nuclear membrane allowing the release of DNA.

Deproteinization which involves adding a protease enzyme Papain - a common enzyme used to clean soft contact lenses. This will denature the proteins clinging to the DNA making the molecule flexible and easy to spool. Precipitation of DNA which involves adding ethanol alcohol which causes every component in the filtrate to stay in solution except DNA. The DNA will gather at the interface of the filtrate and ethanol and can be spooled out with a glass rod.

Homogenization media
SDS (Sodium Dodecyl Sulfate) is a biological detergent which causes the cell membrane to break down further and emulsifies the lipids and proteins of the cell by disrupting the polar interactions that hold the cell membrane together. The detergent forms complexes with these lipids and proteins causing them to precipitate out of the solution. SDS is the major ingredient in laundry detergent.

EDTA (Ethylenediamine tetracetic acid) weakens the cell by binding the divalent cations (Mg^{++} and

Ca++) which are needed for membrane stability. This further aids in breaking open the cells of the onion.

NaCl (Sodium chloride) enables nucleic acids to precipitate out of an alcohol solution because it shields the negative phosphate end of DNA causing the strands to come closer together and coalesce.

1. DNA is found in the nucleus of membrane-bound cells. The membranes are lipid and protein in composition. The cell membranes must be lysed in order to release the DNA.
2. DNA is a polymer made up of repeating chains of nucleotides. The sugar and phosphate components of DNA (the backbone) are both readily soluble in water.
3. The phosphate groups on the outside of DNA carry a negative charge. These negative charges are attracted to and are neutralized by cations such as sodium. When sodium is added to DNA it forms a protective "shell" around it. On the other hand, protein molecules precipitate from solution in the presence of salt.
4. DNA is insoluble in ethanol (ethyl alcohol). As ethanol is added to a solution containing DNA, the DNA will come out of solution and stick to whatever is around.
5. At room temperature DNA begins to denature by the action of DNase (present in cell extracts). DNA extraction procedures must be carried on in ice.

Procedure:

1. Place 100 ml of homogenizing solution in a beaker heat the solution in a water bath until it reaches 60 C
2. Mince the onion and add to the solution when it has reached 60 C. Stir and let sit for 15 minutes. Try not to let the temperature go much above 60 C. The temperature is intended to denature proteins that would break up the DNA into small segments.
3. After the heat treatment, immediately place the beaker into an ice bath for 5 minutes. Swirl the solution gently to allow even cooling throughout. This step slows down the break down of DNA.
4. Pour the contents of the beaker into the homogenizer and blend as per the flow chart.
5. Filter the homogenate through cheesecloth draped over a clean beaker.
6. Pour some of the filtrate into a large test tube. Hold your test tube with filtered homogenate at an angle, gradually pour twice the volume of ice cold alcohol down the wall of the test tube as there is homogenate present.
7. Watch what happens. You should see some stringy substance precipitate out. This is DNA. When it looks very stringy, place a glass rod into the tube so that the end of the rod is just bellow the upper layer of liquid(alcohol) and try to spool the DNA. It should look very clear and glistens around the glass rod. Using a glass stirring rod, gently but quickly twirl the rod into and out of the 2 layers. Gently lift the rod out of the tube and observe any substance attached to the rod.
8. This DNA represents all the DNA found in the onion cells. The chromosomes were broken in the process and the DNA precipitated due to the chemical treatment.

To use DNA for cloning or restriction digests, wash with 95% ethanol, then 70% ethanol. Air dry and resuspend in 500ul TE buffer. Heat in a 60 C water bath for 10 minutes to denature potential DNases. Store at -20 C. **Tips**DNA clings to glass - negative charge of DNA is attracted to positive charge in the silica of glass. Therefore, use plastic tubes for the spooling part of this lab You can make simple glass rods by heating the ends of glass pasteur pipettes and pushing the end to make a small hook. These make dandy rods to spool and hook up the DNA. A similar procedure to this one can be used to extract DNA from animal tissue such as calf thymus. Thymus glands from calves are sold in butcher shops and gourmet grocery stores as "sweetbreads". You can use either fresh or frozen.

Protocol

Extraction of DNA from Onion

Dice an onion into small pieces

weigh out 50g of onion

place onion into a 250 ml beaker

+

add 100 ml of homogen. medium incubate in a 60 C water bath for 15 min.

chill quickly in an ice bath (15-20 C) handle the DNA gently, not rough

pour chilled preparation into blender homog. for 45 sec. at low speed homog. for 30 sec. at high speed

pour into a 1000 ml beaker allow to stand in ice bath for 15-20 min.

pour through 4 layers of cheesecloth over a 500 ml beaker in ice

slowly add cold 95% ethanol down the side of the beaker 80 ml

spool out DNA in one direction only

dry the DNA with paper towel and suspend in TE buffer, store in freezer

Homogenization Medium
5% SDS (50g/L) ---------- 0.15M NaCl (8.8g/L)
0.15M sodium citrate (43.7g/L) ---------- 0.001M EDTA (0.5M stock, 2ml/L)

TE Buffer pH 8.0
0.01M Tris-HCl pH 8.0 ---------- 5 mls of 2M stock
0.001M EDTA pH 8.0 ---------- 2 mls of 0.5M stock

Return to Biology Menu

This method seem to be
unnecessarily difficult to me.
OK

Actually it does look decent after sitting a while. I would still skip the high speed blend though

SUMMER RESEARCH PROGRAM FOR SECONDARY SCHOOL SCIENCE TEACHERS

Page 133 A

On a potato test our method worked even better than this

LESSON PLAN - it is a lot simple. No temp control was needed. Out of blender appears to be a primary factor

Edwin Klibaner

Extraction of DNA from White Onion

The procedures involved in biotechnology implementation are predicated on the isolation of DNA from a tissue sample. [Content Standard E- Understandings about science/technology] This laboratory exercise is designed to give you the opportunity to extract DNA from onion tissue. The technique used is quick and easy for both you and your students. The DNA that is isolated can be digested using various endonucleases, followed with an electrophoresis of the digest. We use an onion because of its' cost, abundance and low starch content. You will make the onion filtrate from onion treated with salt, distilled water, and dishwashing DNA to be more clearly seen. The SDS detergent causes the cell membrane to break down by emulsifying the lipids and proteins of the cell and disrupting the polar interactions that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins, causing them to precipitate out of solution. The use of NaCl salt shields the negative phosphate ends of the DNA which allows these ends to come closer so they can precipitate out of a cold 95% ethyl alcohol solution. You will be altering the filtrate so that you can "spool" DNA out when it precipitates. The DNA is soluble in the detergent solution but is insoluble in the alcohol. [Content Standard B- Structure and properties of matter]When you add the chilled alcohol, the DNA will come out of solution and easily spool on a glass rod. At the end of the exercise you will find a flow chart summarizing the steps and solutions required.

It is not necessary to do all of these steps.

The procedure has three basic steps:

Homogenization which involves heating and blending the onion tissue in order to break down the cells. [Content Standard C- The cell] The heat treatment softens the phospholipid in the cell membrane and denatures the DNAse enzymes which if present, would cut the DNA into small fragments so that it would not spool. The onion tissue is mixed in a blender with homogenization media, which breaks down the cell wall, cell membrane and nuclear membrane allowing the release of DNA.

Deproteinization which involves adding a protease enzyme Papain - a common enzyme used to clean soft contact lenses. This will denature the proteins clinging to the DNA making the molecule flexible and easy to spool. Precipitation of DNA which involves adding ethanol alcohol which causes every component in the filtrate to stay in solution except DNA. The DNA will gather at the interface of the filtrate and ethanol and can be spooled out with a glass rod.

Homogenization media

SDS (Sodium Dodecyl Sulfate) is a biological detergent which causes the cell membrane to break down further and emulsifies the lipids and proteins of the cell by disrupting the polar interactions that hold the cell membrane together. The detergent forms complexes with these lipids and proteins causing them to precipitate out of the solution. SDS is the major ingredient in laundry detergent.

EDTA (Ethylenediamine tetracetic acid) weakens the cell by binding the divalent cations (Mg^{++} and

Ca++) which are needed for membrane stability. This further aids in breaking open the cells of the onion.

NaCl (Sodium chloride) enables nucleic acids to precipitate out of an alcohol solution because it shields the negative phosphate end of DNA causing the strands to come closer together and coalesce.

1. DNA is found in the nucleus of membrane-bound cells. The membranes are lipid and protein in composition. The cell membranes must be lysed in order to release the DNA.
2. DNA is a polymer made up of repeating chains of nucleotides. The sugar and phosphate components of DNA (the backbone) are both readily soluble in water.
3. The phosphate groups on the outside of DNA carry a negative charge. These negative charges are attracted to and are neutralized by cations such as sodium. When sodium is added to DNA it forms a protective "shell" around it. On the other hand, protein molecules precipitate from solution in the presence of salt.
4. DNA is insoluble in ethanol (ethyl alcohol). As ethanol is added to a solution containing DNA, the DNA will come out of solution and stick to whatever is around.
5. At room temperature DNA begins to denature by the action of DNase (present in cell extracts). DNA extraction procedures must be carried on in ice.

Procedure: Notice no enzyme added

1. Place 100 ml of homogenizing solution in a beaker heat the solution in a water bath until it reaches 60 C How much onion? It can be up to about 200 ml minced.
2. Mince the onion and add to the solution when it has reached 60 C. Stir and let sit for 15 minutes. Try not to let the temperature go much above 60 C. The temperature is intended to denature proteins that would break up the DNA into small segments. It takes 10 min to read
3. After the heat treatment, immediately place the beaker into an ice bath for 5 minutes. Swirl the solution gently to allow even cooling throughout. This step slows down the break down of DNA.
4. Pour the contents of the beaker into the homogenizer and blend as per the flow chart blender
5. Filter the homogenate through cheesecloth draped over a clean beaker. Cool down the blender also
6. Pour some of the filtrate into a large test tube. Hold your test tube with filtered homogenate at an angle, gradually pour twice the volume of ice cold alcohol down the wall of the test tube as there is homogenate present.
7. Watch what happens. You should see some stringy substance precipitate out. This is DNA. When it looks very stringy, place a glass rod into the tube so that the end of the rod is just bellow the upper layer of liquid(alcohol) and try to spool the DNA. It should look very clear and glistens around the glass rod. Using a glass stirring rod, gently but quickly twirl the rod into and out of the 2 layers. Gently lift the rod out of the tube and observe any substance attached to the rod.
8. This DNA represents all the DNA found in the onion cells. The chromosomes were broken in the process and the DNA precipitated due to the chemical treatment.

To use DNA for cloning or restriction digests, wash with 95% ethanol, then 70% ethanol. Air dry and resuspend in 500ul TE buffer. Heat in a 60 C water bath for 10 minutes to denature potential DNases. Store at -20 C. **Tips**DNA clings to glass - negative charge of DNA is attracted to positive charge in the silica of glass. Therefore, use plastic tubes for the spooling part of this lab You can make simple glass rods by heating the ends of glass pasteur pipettes and pushing the end to make a small hook. These make dandy rods to spool and hook up the DNA. A similar procedure to this one can be used to extract DNA from animal tissue such as calf thymus. Thymus glands from calves are sold in butcher shops and gourmet grocery stores as "sweetbreads". You can use either fresh or frozen.

Page 133 C

Protocol

Extraction of DNA from Onion

Dice an onion into small pieces

weigh out 50g of onion

place onion into a 250 ml beaker

+

add 100 ml of homogen. medium incubate in a 60 C water bath for 15 min.

chill quickly in an ice bath (15-20 C) handle the DNA gently, not rough

pour chilled preparation into blender homog. for 45 sec. at low speed homog. for 30 sec. at high speed

pour into a 1000 ml beaker allow to stand in ice bath for 15-20 min.

pour through 4 layers of cheesecloth over a 500 ml beaker in ice

slowly add cold 95% ethanol down the side of the beaker 80 ml

spool out DNA in one direction only

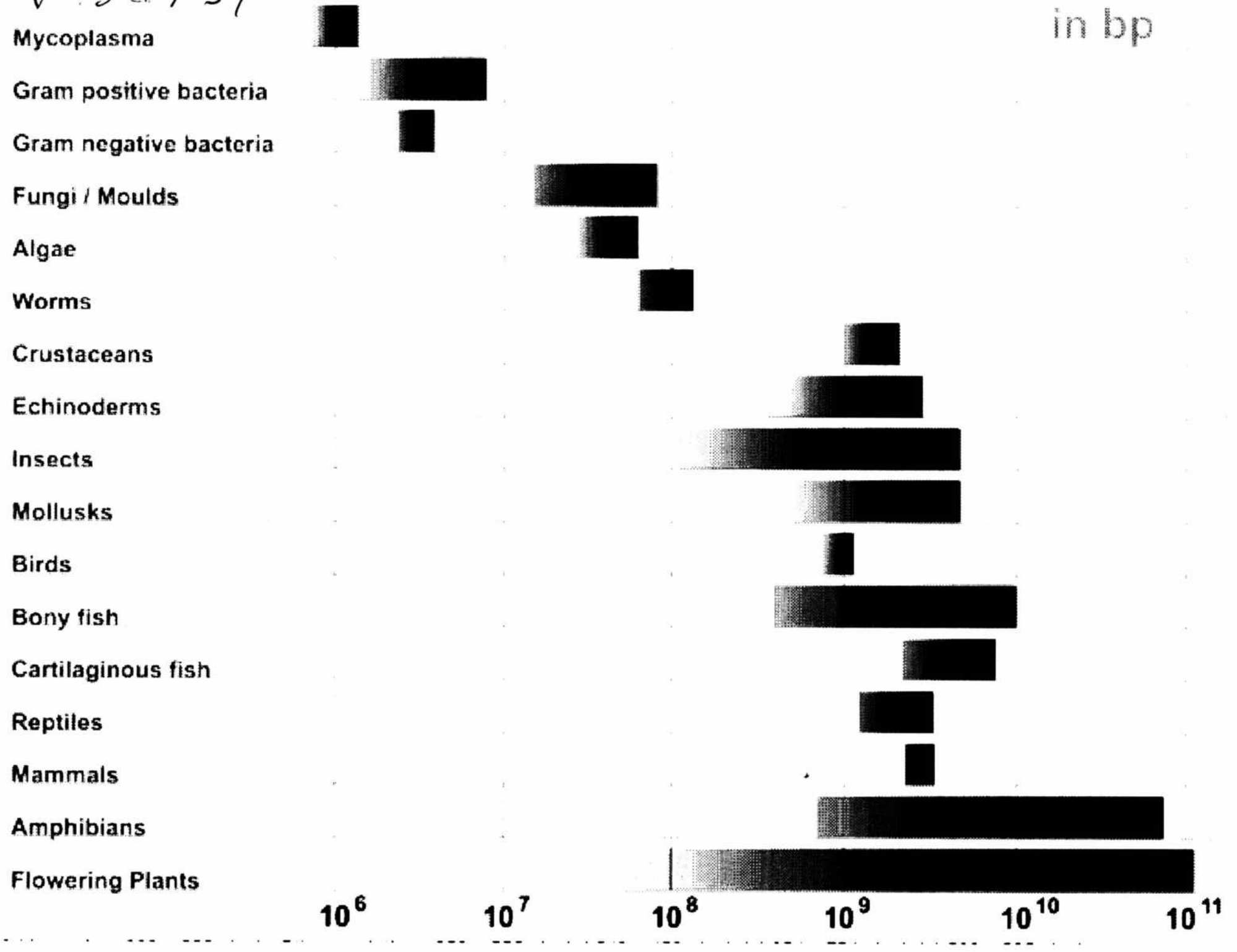
dry the DNA with paper towel and suspend in TE buffer, store in freezer

For get the high speed blending

Homogenization Medium
5% SDS (50g/L) ---------- 0.15M NaCl (8.8g/L)
0.15M sodium citrate (43.7g/L) ---------- 0.001M EDTA (0.5M stock, 2ml/L)

TE Buffer pH 8.0
0.01M Tris-HCl pH 8.0 ---------- 5 mls of 2M stock
0.001M EDTA pH 8.0 ---------- 2 mls of 0.5M stock

Return to Biology Menu


in bp
Mycoplasma
Gram positive bacteria
Gram negative bacteria
Fungi / Moulds
Algae
Worms
Crustaceans
Echinoderms
Insects
Mollusks
Birds
Bony fish
Cartilaginous fish
Reptiles
Mammals
Amphibians
Flowering Plants
10^6
10^7
10^8
10^9
10^{10}
10^{11}

Page 134A

Genome Sizes

The genome of an organism is the complete set of genes specifying how its phenotype will develop (under a certain set of environmental conditions). In this sense, then, **diploid** organisms (like ourselves) contain two genomes, one inherited from our mother, the other from our father.

The table below presents a selection of representative genome sizes from the rapidly-growing list of organisms whose genomes have been sequenced.

Table of Genome Sizes (haploid)

	Base pairs	Genes	Notes
φX174	5,386	11	virus of E. coli
Human mitochondrion	16,569	37	
Epstein-Barr virus (EBV)	172,282	80	causes mononucleosis
nucleomorph of *Guillardia theta*	551,264	511	all that remains of the nuclear genome of a red alga (a eukaryote) engulfed long ago by another eukaryote
Mycoplasma genitalium	580,073	517	two of the smallest true organisms
Mycoplasma pneumoniae	816,394	679	
Rickettsia prowazekii	1,111,523	834	bacterium that causes epidemic typhus
Treponema pallidum	1,138,011	1,039	bacterium that causes syphilis
Pelagibacter ubique	1,308,759	1,354	smallest genome yet found in a **free-living** organism (marine α-proteobacterium)
Helicobacter pylori	1,667,867	1,589	chief cause of stomach ulcers (not stress and diet)
Methanocaldococcus jannaschii	1,664,970	1,783	These unicellular microbes look like typical bacteria but their genes are so different from those of either bacteria or eukaryotes that they are classified in a third kingdom: **Archaea**.
Aeropyrum pernix	1,669,695	1,885	
Methanothermobacter thermoautotrophicus	1,751,377	2,008	
Streptococcus pneumoniae	2,160,837	2,236	the pneumococcus
Pandoravirus	2,473,870	2556	A **virus** (of an amoeba) with a genome larger than that of the bacteria and archaea above and about the same as that of some parasitic **eukaryotes** [Example].
Listeria monocytogenes	2,944,528	2,926	2,853 of these encode proteins; the rest RNAs
Synechocystis	3,573,470	4,003	a marine cyanobacterium ("blue-green alga")
E. coli K-12	4,639,221	4,377	4,290 of these genes encode proteins; the rest RNAs

Page 134 B

E. coli O157:H7	5.44×10^6	5,416	strain that is pathogenic for humans; has 1,346 genes not found in E. coli K-12
Schizosaccharomyces pombe	12,462,637	4,929	Fission yeast. A **eukaryote** with fewer genes than the three bacteria below.
Agrobacterium tumefaciens	4,674,062	5,419	Useful vector for making transgenic plants; shares many genes with *Sinorhizobium meliloti*
Pseudomonas aeruginosa	6.3×10^6	5,570	Increasingly common cause of opportunistic infections in humans.
Sinorhizobium meliloti	6,691,694	6,204	The rhizobial symbiont of alfalfa. Genome consists of one chromosome and 2 large plasmids.
Saccharomyces cerevisiae	12,495,682	5,770	Budding yeast. A eukaryote.
Neurospora crassa	38,639,769	10,082	Plus 498 RNA genes.
Thalassiosira pseudonana	34.5×10^6	11,242	A diatom. Plus 144 chloroplast and 40 mitochondrial genes encoding proteins
Naegleria gruberi	41×10^6	15,727	This free-living unicellular organism lives as both an amoeboid and a flagellated form. 4,133 of its genes are also found in other eukaryotes suggesting that they were present in the common ancestor of all eukaryotes. The great variety of functions encoded by these genes also suggests that the common ancestor of all eukaryotes was itself as complex as many of the present-day unicellular members.
Drosophila melanogaster	122,653,977	~17,000	the "fruit fly"
Caenorhabditis elegans	100,258,171	21,733	
Humans	3.3×10^9	~21,000	[Link to more details.]
Tetraodon nigroviridis (a pufferfish)	3.42×10^8	27,918	Although Tetraodon seems to have more protein-encoding genes than we do, it has much less non-coding DNA so its total genome is about a tenth the size of ours.
Mouse	2.8×10^9	~23,000	
Amphibians	10^9–10^{11}	?	
Arabidopsis thaliana	0.135×10^9	27,407	a flowering plant (angiosperm) with one of the smallest genomes known in the plant kingdom.
Picea abies	19.6×10^9	28,354	the Norway spruce, a conifer (gymnosperm). Even though it has only ~900 more genes than Arabidopsis, it has 145 times as much DNA. Most of this appears to be derived from transposons.
Psilotum nudum	2.5×10^{11}	?	Note

Even though ***Psilotum nudum*** (sometimes called the "whisk fern") is a far simpler plant than Arabidopsis (it has no true leaves, flowers, or fruit), it has 3000 times as much DNA. No one knows

Page 134C

why, but 80% or more of it is **repetitive DNA** containing no genetic information. This is also the case for some amphibians, which contain 30 times as much DNA as we do but certainly are not 30 times as complex.

The total amount of DNA in the haploid genome is called its **C value**. The lack of a consistent relationship between the C value and the complexity of an organism (e.g., amphibians vs. mammals) is called the **C value paradox**.

How many genes does it take to make an organism?

The scientists at The Institute for Genomic Research (now known as the J. Craig Venter Institute) who determined the ***Mycoplasma genitalium*** sequence have followed this work by systematically destroying its genes (by mutating them with insertions) to see which ones are essential to life and which are dispensable. Of the 485 protein-encoding genes, they have concluded that only 381 of them are essential to life.

Welcome&Next Search

---

19 April 2014

Page 134 D

Staining your Agarose gel to reveal DNA

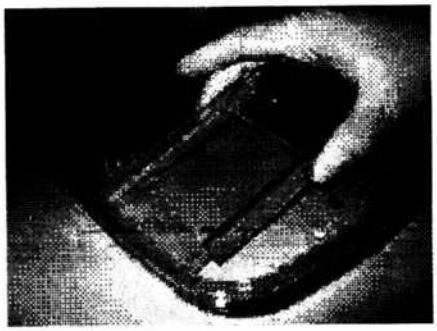
SOUTHERN biological

"a world of learning"

Electrophoresis of DNA requires a staining step to make the separated DNA fragments visible. The safest and easiest method is to use methylene blue solution. As methylene blue carries a positive charge, it binds to the negatively charged DNA fragments and stains them blue. We have found two suitable methods for staining agarose gels.

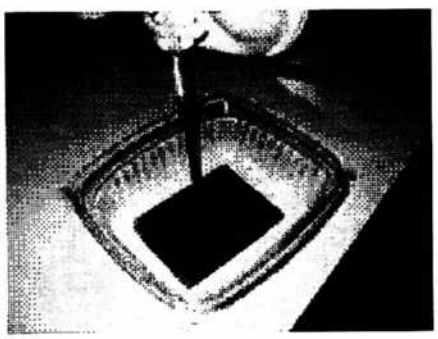
Method One – using dilute Methylene Blue solution

Carefully slide the agarose gel into a small shallow container and pour in dilute methylene blue solution (0.025%) until the gel is submerged. Allow to stand for up to 16 hours so the stain diffuses into the gel and binds with the DNA fragments.



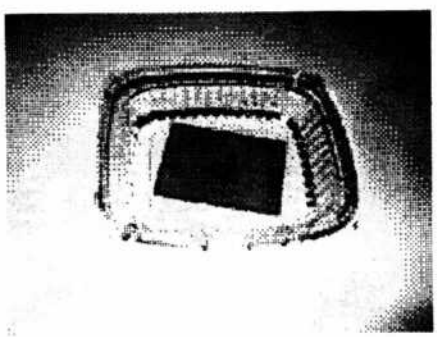
Method Two – using concentrated Methylene Blue solution

Carefully slide the agarose gel into a small shallow container and use a pipette to cover with a few millilitres of concentrated methylene blue solution (0.1%). Add just enough to cover the surface of the gel with the concentrated stain. Allow to stand for 5 minutes, then rinse off the excess stain. Leaving the gel in the container, seal it with cling wrap to prevent any evaporation and allow it to stand for up to 16 hours so the stain diffuses into the gel.



Destaining

Once staining is achieved by either of the methods described above, you can heighten the contrast between the stained DNA fragments and the background gel by destaining. This involves soaking the stained gel in clean water to leach out unbound methylene blue. Change the water occasionally until no further stain can be seen in the rinse water or until the DNA fragments can be plainly seen in the gel.



Note

- Wear gloves and a protective laboratory coat to avoid staining your skin and clothing.
- Methylene blue stain may be reused, so retain excess stain in a labeled bottle.

Southern Biological - A Division of Cogitamus Pty Ltd
ACN 097 468 954 ABN 25 838 926 933
19-21 Worrell Street or PO Box 57 Nunawading Victoria 3131 Australia
Phone: +61 3 9877-4597 Fax: +61 3 9894-2309
Email: sales@southernbiological.com Web: www.southernbiological.com

Nov 03 2014

Our method of DNA extraction seems simple, straightforward & reliable.

1. Assume 100 ml of sample/water combined. Adjust proportionally.
2. 4 ml Oxi Clean ~~detergent~~ (4 gms)
3. 3 gms salt
4. ~~or~~ Two full scoops of enzymes
5. Pulse the sample to break it up in the blender. Keep this to a minimum.
6. Blend @ low speed for a 30 sec.

In other samples adjust blend time by ratio:

$$\frac{\text{Volume of sample}}{100\text{ ml}} * 30\text{sec} \times \frac{\text{Density of Sample}}{\text{Density of Banana}}$$

Do not overblend. It breaks up the DNA into smaller filaments & eventually particulate level.

Question:

Can we try DNA on the COB today? ?

I think we have enough to try.

Maya success today!
COB DNA Extraction
Verified
Nov 03 2014

Method:

1. 50 ml COB (approximately a 3-4 week old culture)
2. ~~2 ml~~ 2 ml OxiClean detergent (2 gms)
3. 1.5 gms salt
4. 1 full microscoop general enzyme.
5. Blend on low speed for 2 minutes
6. Strain
7. Cold alcohol
   Let it sit for a half hour
   Maya success !!!

2nd batch not as successful as the 1st batch but still ok and usable
Suspect more scoop & more enzyme?
Maybe blend less - 30 sec
It is building up ok eventually.

Try:

1. 50 ml COB
2. ~~3~~ ml soap
3. ~~1.5 gms~~ 2 gms salt
4. 2 Scoops enzyme
5. Blend ~~30 sec~~ ~~45~~ sec or 1m ~~30~~ 45
   Next time blend 1m 15 sec

I am not sure that straining is actually necessary. A mga complex is forming w/ the raw filtrate.

We have ~~the~~ total consumption taking place. There is essentially nothing left except for what is floating.

It might be a DNA Complex vs pure DNA.

It seems to me that the egg area has a very high DNA Content.

Whatever we pour off essentially appears to be transforming ~~into~~ to a DNA Complex.

Even the 30 second first run is producing volcanoes of a nice light color.

Page #138

First run w/ ~~30~~ 45 sec heater is
Very filamentous & light color
but takes an hour to form.
This ~~was only one~~ scoop enzyme 2 scoops
& ~~2 gms~~ soap. 3 gms.

DNA - Tracky Trial

1 Red
2 Green
3 Blue
4 Yellow
5 Banana w/ Green
6 Banana w/ Red
7 Banana alone
8 ~~Bananana~~ Tomato alone

The next lesson is that we must have a separation of DNA lengths w/in the sample in order to be form separately visible on the gel.

Heaty is the approach to use for now. Maybe further Wendy.

100°C for 10 min 30K → 1K bp
120°C for 30 min 30K → 0.3K bp

So Heat for

5 min
10 min
20 min

Today we are attempting to create different size of DNA from raw banana DNA extract.

First goal is to find the boiling point of our isopropanol. We want to get as close to 100°C as possible. The will also save our ethanol.

Use a principle of time x temp = Constant.

5 min (100°) = 500 min-deg.

or

$\frac{500}{T^\circ}$ = min a me simply $\frac{100^\circ}{\text{BP of alcohol}}$ = Factor

The isopropanol boiling @ 79°C ⇒ Factor = 1.27

(1) 5 min (1.27) ≈ ≤ 6 m 21 s @ 79°C +10 m gradual

(2) ~~10 min (1.27) ≈ ≤ 12 m 42 s~~ @ 79°C ~~19 m 20 m~~

(3) ~~20 min (1.27) ≈ ≤ 25 m 24 s~~ @ 79°C 40 m

(4) ~~0 min~~ = 0 m @ 79°C 60 m

(5) ∅

DNA Run Page 141

Lane
1 6m
2 20m
3 40m
4 60m
5 Øm.
1=6 6m
2=7 20m
3=8 40m

I see no band formation. Why?

Lets look on 260/280 ratios.

possible problems.
1. Too much RNA, too little DNA
? 2. Too much salt
3. Contaminated w/ protein

TOS is 960. This is high. It states to use ethanol precipitation to remove salts but the sample is in the ethanol.

Major problem is concentration of sol. Should be 0.3% instead of 2%.

Here is a question. What is the TDS of ethanol by itself?
It is Ø so there is salt in there.

Nov 08 2014

I have made a 5 way air valve for the cultures! Very good.

I can now get 3 out of 5 jars. I just need a stronger pump.

DNA Trial:

1 Whipped Banana w/ Glycerol
2 Whipped Banana whipped w/ red dye
3 " " " w/ methylene blue
4 Whipped Tomato w/ Glycerol
5 w/ red dye
6 w/ methylene blue
7. Whipped Onion w/ Glycerol
8. Whipped onion w/ red dye

0.5 gr Banana

Does not look good again.

1. We used approx 0.5 g Banana
2. The wells looked very clear
3. The two lane w/ methylene blue (3,6) went backwards again.
4. No bands appear to be showing up again - why?

[redacted] 11/00/14

It does indeed say that methylene blue is positively charged.

So the backwards migration does make sense here.

Cation (positive) Cat on a hot tin roof
Anion (negative) Cat means means up top
a means away

So it will eventually run off the DNA & then maybe that is what we saw more.

But why don't we see the clear bands accept a dye?

Food dye must be negatively charged.

Reload

1 = #3
2 = #6
#3 * 3 ~~= #3~~
4 ~~= #3~~ #3
5 = #6
#6 * 6
#3 broke 7 = #3
#6 0 = #6

reverse the leads!

Very interesting. After adding the dye for about 2 hr (next day)

All four of these bands ARE MOVING AS A UNIT! BUT THE WRONG WAY!!!

What does this mean???

Sitting in the methyl blue dye for 2 hr means that it still has a net positive charge. But it does not look like a very strong charge because the bands are moving very slowly. But they are moving!

Moving towards the negative terminal remember.

So how does DNA depend upon pH?
Migration

They are moving for the first time ever!

It looks like it is forming a broader band than 3.
It may be the buffer??

DNA is negatively charged @ neutral pH!
But we are not @ neutral pH!

A strong base causes DNA strands to separate!! ie, denature

We apparently do need TRIS buffer @ 7.8

Our pH is 10.3, we also added methyl blue, which further neutralized the negative charge of DNA. So we are ending up w/ a slightly positive charge in an alkaline pH.

Gel Work - Progress!

There is pretty amazing.

My buffer is distorting the results.
Unbelievably enough, I had 2 supplies of buffer from 2-3 yrs ago, one of them from ELVOTEK.

#607 is for DNA.

I have TAE #607 this was perfect! 50x
15 ml (49) = 735 + 15 ml = 750 ml

I now purchased Tris - Glycerine
which is for protein agarose, so save it!

15.29 (@49) = 749 ml to add ⇒ Total = 765 ml
ml

I am learning about buffers now!

Ya made some progress!

1. Blending the DNA in a small blender. w/ glycerol & methylene blue even
2. Methylene needs to sit on sample for an hour or so
3. Gel is 0.5% or maybe even less.
4. The buffer matters a lot
It can not be alkaline!
5. We may have too much salt in our sample.

Nov 09 2014

The bands moved across the entire gel for the first time!

But the wrong direction!

We accomplished by

1. Using a 0.5% agarose instead of 2%
2. Staining w/ methylene blue & let sit for 1 hr before charging system.
3. Learning that the buffer pH is all wrong.

Next trial

1. Use proper buffer
2. Grind & glycerol & dye & no dye
3. Use methylene blue to test if dye is the problem.
4. Consider 0.4% agarose next. You are making progress.

Nov 12 2014 Back from Missoula

I now have a good air pump that is more than sufficient for the 5 culture jars. This represents a great improvement. It is quite feasible that culture production will increase w/ this modification. Very good work.

We would now like to:

1. place an order
2. set up modified set (1000)

Nov 13 2014

Today we start a modified sel mm.

1. Using TAE buffer!
2. 0.4% Agarose w/ TAE buffer!
3. Now set up the Lanes:

1	Bananaaa w/red	1 hr wait. Blended
2	Banana clear	
3	Banana w/ marble blue	
4	~~Tomato~~ Red Potato	
5	~~Tomato~~ clear Potato	
6	~~Tomato~~ blue Potato	
7	Onion red	
8	Onion blue	

Methane issues.

US Methane emissions ~650

Specific heat of a mixture = (means @ constant pressure or volume) $\sum_{i=1}^{n}$ mole fraction $_i$ * specific heat $_i$

Assume we have
78% Nitrogen by | weight
21% Oxygen

C_v = specific heat for a constant volume

C_p = specific heat for a constant pressure

Molar heat capacity is heat capacity per mole
specific heat capacity is heat capacity per unit mass

	C_v	Mole % (This is by volume!)
Air	= 1.01 KJ/Kg·K°	
Methane	= 2.22	.00017
CO_2	= Ø.844	.0350
Argon	= Ø.52Ø	Ø.934
Oxygen	= Ø.919	20.947
Nitrogen	= 1.04	78.084

.781(1.04) + .209(.919) + .009(.520) = 1.01 Very good.

Mole fraction = Volume fraction. $\frac{KJ}{Kg \cdot K^\circ}$

Specific Heat of Air = $1.01 \frac{kJ}{kg \cdot K^\circ}$

Heat capacity per unit volume

Heat capacity has units of $\frac{\text{Joules (heat)}}{K^\circ \text{ (or } C^\circ\text{)}}$ (actually $1^\circ K$)

(i.e. Joules per Kelvin)

So assume w/ specific heat, we have

1 kg of air raised by 1°C then that energy required is 1.01 kJ (heat)

We could assume a constant amount of energy but a variation in volume

$$C_{V_{mix}} = \sum (\%\ \text{Volume}) \cdot C_{V_i}$$

$$C_{V_{mix}} = V_1C_1 + V_2C_2 + V_3C_3 + V_4C_4 + V_5C_5$$

(Terms 1–4 are essentially a constant)

$$C_{V_{mix}} = K + V_5C_5$$

$$\frac{dC_{V_{max}}}{V_5} = C_5$$

A Methane Evaluation

on $\Delta CV_{max} = C_5 \cdot \Delta V_5 = \frac{KJ}{Kg \cdot K^o} \cdot kg$

$.00017\% = .0000017 \cong 1.7 ppm. = 1.7E\text{-}6$

Assume we doubled it. to 4 ppm $= 4E\text{-}6$

$\Delta V = 4E\text{-}6 - 1.7E\text{-}6 = 2.3E\text{-}6$

Cv methane

$\Delta C_{V_{mix}} = \frac{2.22\ KJ}{Kg \cdot K^o} (2.3E\text{-}6) =$ ~~5.11 KJ~~ ~~5.11E~~ KJ

$\Delta CV_{mix} = \frac{5.11E\text{-}6\ KJ}{Kg \cdot K^o} \cdot \frac{KJ}{K^o}$

Mass of earth $= 5.972 E24$

Mass of atmosphere $\cong 5.1E18$ kg
Assume we raise it $\underline{1}^o K$

ERROR !!

$= 2.61E13$ Joules. @ any moment. This was KJoules! Not Joules!

~ Change per year $= 15.9E22 / [(2003\text{-}1961) + 1]$ Joules

$=$ ~~3.70~~ 4.2 E21 Joules per year

A watt = 1 Joule per second
in a year there are 3.154E7 seconds

ERROR! This is !!! Watts.

So we have $(2.61E13)(3.154E7\ sec) = 8.23E20$
Total Joules Change in a year.

So if methane were then doubled
we'll have

$\frac{8.23E20}{\sout{3.70E21}\ 4.2} = 0.222 =$ ~~22.2~~ $19.6\% \cong 20\%$

The release of methane were to immediately double in concentration, it would still be about 1/5 of the total heat change that is now occurring on an annual basis.

The is a very interesting perspective.

Nov 14 2014

On the gel, lane 1 may have had a little bit of activity.

Lane 7 is onion w/ red stain.

The suggest that a very large blue pair may have had some motion.?
What happened w/ everything else?

Another very interesting observation.
There is clearly a difference in the dyed gel prior to the addition of the red dye.
But there are no bands.
Clearly something has moved through the gel but it is diffuse, not banded.

Maybe it is because they are not different sizes.

Page
154

Size
1K
moss spec

[exterior
keratin or cellulose

metals

The emphasis might be upon
restriction enzyme now.
Maybe the pieces are all the
same length

meth blue Trials:

1 ~~#3~~ meth blue alone
2 #3
3 #6
4 ~~#3~~ #8
5 ~~#6~~ #3
6 #6
7 #8
8 meth blue

] Middle position this time.

Choosing a middle position was smart.
There is a split separation taking place.
One set light colored & moving relatively
quickly at a uniform rate & migrating
toward the negative terminal.
They are uniform & I suspect they're
what it is for meth blue dye which
we know does have a positive charge.

The other side is dark & moving very slowly toward the positive terminal.

Size

523 #2 & 1 = #3 Banana w/ meth blue is moving the quickest & is matchy the control of meth blue alone. These the samples you had to add water to to reconstitute it.

844 #6 Potato Blue is not moving @ all.

16,600 #0 Onion blue is moving very slightly

These samples have been dyed in meth blue for 24 hrs.

But now the meth blue control separated into two so I am not sure anything means anything.

[redacted]

Nov 16 2014

The climate model approach we have taken is interesting.

1st question: why do high & low clouds behave differently?

Ok, High Clouds do warm the earth.

We have a model that we have developed.

There is confusion on the way that the computations are stated. Some people say mass, some people say volume. There is a huge difference.

I believe that an error is commonly circulated

Mixture: from Mech Eng Page

$$C_p = \sum X_i C_{pi}$$

X_i: mass fraction!

C_{pi}: specific heat @ Constant pressure mass basis

$$C_{p_{air}} = .7552(1.04) + .2315(.919) + .0128(.520) + .000035(.844)$$

$$= 1.00484 = 1.005 \text{ Good}$$

This is correct

Mass fractions:

Molar mass of air = 28.967 gms/mol

Mass fract of air
= .20948 (31.990) = 6.703
(Mole fraction, or volume fraction)

$\frac{6.703 \text{ gms}}{28.967 \text{ gms}}$ = 23.14% Very good. Randall gets 23.15%

Si now look @ methane and CO_2

Methane: .0000017 (16.043) = $\frac{.00003 \text{ gms}}{28.967 \text{ gms}}$ =

~~CH_4: .000315 (44.0099) = 0.946 = 0.946-4 %~~

≈ .0104% = .000104 ratio

CO_2 = .000315 (44.0099) = $\frac{.0165 \text{ gms}}{28.967 \text{ gms}}$ = .000570 = .057%

This does not agree w/ Randall.

So mass of CO_2 is about 60 times as great as methane.

Now lets look @ methane concentration

Over last 20 yrs $\Delta \approx$ 7.5 ppb per year.

$= \frac{7.5}{1700} = 0.44\%$

Our actual difference over last 36 years is 0.85 ppb

So % diff is

$\frac{0.85}{1644} = .000415 = 0.05\%$

This is giving no significant change

It comes out as .009%

or ~ 1/10% of a percent.

Over the last 200 years it has risen ~ @ 5.45 ppb each year.

This represents $\frac{5.45}{1245} = .0030$ .0044

$= .30\%$ 0.40

The temperature right now is increasing at approximate .15 to .20° C per decade

.175 deg C per decade = .0175° C per year

1 - 5°

little ice age

ice age

Our effect of 1% increase in methane leads to .029° C change per decade = .003° C per year.

This would be 33K year to be one degree.

Nov 17 2014 Climate Model

Now that we have a better understanding of mass fractions, mole fractions, specific heat ratios accordingly we need a better practice to check our work & logic.

Then in a quarter of global heat state vs atmosphere state & its relationship to the heat budget. Remember only a small portion of the heat budget goes into the atmosphere.

When we finish, we want to go to CO_2. Then aerosols!

(We also want to do our gel labs.

With scratch, we would like to be able to:

1. Resize the windows
2. Print the code
3. Print text on a screen

Now let's go after the model & verification of the logic.

		mf_i	Cp_i
1	N	.7552	1.04
2	O	.2315	.919
3	Ar	.0128	.520
4	CO_2	5.7E-4	1.02
5	CH_4	1.04E-4	2.22

$\frac{KJ}{kg \cdot K} = \frac{J}{gm \cdot K}$

We now know..

$Cp_{mixture} = \sum mf_i \cdot Cp_i$

and now you know what this means.

$Cp_{mixture}$ = specific heat (mass based) of the mixture.

mf_i = mass fraction of the i^{th} component

Cp_i = specific heat (mass based) of the i^{th} component

For immediate gases of interest we have

Nitrogen, Oxygen, Argon, CO_2, Methane

$Cp_{mix} = \sum_{i=1}^{5} mf_i \cdot Cp_i = mf_1 Cp_1 + mf_2 Cp_2 + mf_3 Cp_3 + mf_4 Cp_4 + mf_5 Cp_5$

Now what varies if the composition of the atm. changes?

Mass fraction!

Mass fraction = Molar Fraction (Volume fraction) * Molar Mass $\left(\frac{gm}{mol}\right)$ (i.e. molecular mass)

So.

$\Delta Cp_{mix} = Cp_1 \Delta mf_1 + Cp_2 \Delta mf_2 + Cp_3 \Delta mf_3 + Cp_4 \Delta mf_4 + Cp_5 \Delta mf_5$

(Δmf)

But in our case, N, O, & Ar are going to remain constant.

Therefore $\Delta mf_1, \Delta mf_2 \& \Delta mf_3 = 0$.

Therefore:

$\Delta Cp_{mix} = Cp_4 \Delta mf_4 + Cp_5 \Delta mf_5$

So

$\Delta Cp_{mix} = 1.02 \Delta CO_2 + 2.22 \Delta CH_4$ where Δ are mass fraction changes in mass fractions.

Where mass fraction numbers are higher than volume fractions.

Methane volume fraction $\cong 2E-6$ but mass fraction $= 1.04E-4$

Notice this, so ΔCp_{mix} will be a greater change.

Lets double for kicks.

$\Delta Cp_{mix} = 1.02(5.7E-4) + 2.22(1.04E-4) =$

$= 8.12E-4$ What are units?!!!: $\frac{Joules}{gm \cdot C^\circ} * \Delta Ratio$

So units are the same as specific heat.

We have a hypothetical case of Clouds. mass fractions

So $\Delta C_{p\,mix} = 8.12E-4 \frac{\text{Joules}}{gm \cdot C^\circ}$ = $\frac{\text{KJoules}}{kg \cdot C^\circ}$

$= .000812 \frac{KJ}{kg \cdot C^\circ}$

Now the mass of the atmosphere is $5.1E18\ kg$

So for 1° Change: $5.1E18\ kg \cdot \frac{8.12E-4\ KJoules}{kg \cdot C^\circ}$

$= 4.1412E15 \frac{KJoules}{C^\circ}$ per degree Centigrade!

Heat Capacity

In the particular gases & my components CO_2 & methane.

My numbers before were in error.

You had Joules vs KJoules and an arbitrary rise of 1°C. Maybe it is a fraction of a degree.

For instance $\Delta = 1/2^\circ C = 1/2(4.1412E15\ KJoules) = 2.07E15\ KJ$

So keep track of units.

The next piece of information we have is the global warming budget. This is the century plan

21E22 Joules in 50 years This is actually the oceans only

Now look at raw data...

We all that approx 7.5% of the total heat global is land + ice + Air

Total now is 234E21 Joules in 2008.
or 23.4E22
or 2.34E23 Joules in 2008.
This is over a period of 2008 − 1961 = 47 + 1 = 48 yrs

This means the annual change is approx $\frac{2.34E23 \text{ Joules}}{48 \text{ years}}$
$= 4.875E21 \frac{\text{Joules}}{\text{per year}}$ (Earlier we had 4.2E21 OE)

Now, of this, only approx 7.5% is atmospheric + air so this means .075(4.875E21) = 3.656E20 Joules is atmospheric + land + ice.

The atmosphere is actually only about 2%
This would be:
.02(4.875E21) = 9.75E19 Joules per year.

For our 1° hypothetical change it would be a ratio of
$\frac{4.1412E15 \text{ KJ}}{} = \frac{4.1412 \text{ Joules E18}}{9.7E19 \text{ Joules E19}}$
$\approx .043 = 4.3\%$ of the total atmosphere change which is now occurring.

Trying to put this into perspective.

If we immediately doubled the Concentration of CO_2 & CH_4 in the atmosphere it would change the specific heat of air of ~ 1.005 KJ / Kg·C° by .000812 KJoules / Kg·C° per degree Change

The appear to be a small amount & we can adjust it by the actual temperature change of the earth and air.

We have a value of

$\Delta C_{p\,mix} \cong 8.12E\text{-}4$ KJOULES / Kg·C° per degree

~~The atmosphere will therefore change by~~
The atmosphere heat capacity will therefore change by:

Change

Air Heat Capacity (double CO_2, CH_4) = 8.12E-4 KJ / Kg·C° (5.1E18 Kg)

= 4.1412 KJ E15 / C° per degree

Energy Change Degree Centigrade

This is still a hypothetical construct.

Yes Heat Capacity is expressed in units of Joules per degree.

Good.

= 4.1412E18 Joules / C° per degree

What we have is heat capacity change
Not total heat capacity.

Total heat capacity of air = $\frac{1.005\ KJ}{kg \cdot C^\circ}(5.1E18\ kg) = \frac{5.125E18\ KJ}{C^\circ}$

So the heat capacity of the atmosphere has changed by:

$$\frac{\frac{4.1412E15\ KJ}{C^\circ}}{\frac{5.125E18\ KJ}{C^\circ}} = .8.1E-4$$ exactly as you suspect
or $\approx .0.081\%$ very reasonable

Now, what do you want to do with this?

We have the atmospheric annual heat change of 2^{nd} of the global heat change.

You see your situation. To equate the change, you have to assume a change in temperature.
We need the annual temperature change.

Heat Capacity:

$(4.1412E18)\ \frac{Joules}{C^\circ} \cdot (\text{Temperature Change!}) = Joules$

and then the units cancel!

How much is the earth's temperature changing now? on an annual basis?

Current temperature change is .0175° C per year.
Where did we get this? It is in our notes.

Scenario:

100% increase in CO_2
100% increase in Methane
Earth is now rising @ .5°C per year
(.5/.0175) = 30 times normal

The increase rate of atmosphere heats by 2% yearly.

so .5(1.02)

$.5(1.02)^n$

in 100 year the earth will be rising by 3.6° per year

$.5(1.02)^n = 1°$ = Little ice age

There is an integration problem here.

When would a little ice age occur @ 1° total change

$\frac{1°}{\text{current change}} = \frac{1}{.5} = 2$ years.

What if it was .1° C?

$\frac{1°}{.1°C} = 10$ years but we would also have .42% each year

so at the end of 10 year it would be rising @ $.1(1.0042)^{10} = .104°$ per year

so @ that rate it would only take $\frac{1^\circ}{.104} = 9.6$ years.

See how this works.

Now lets go @ .0175 per year
It increases the rate @ .014% per year.

So in 100 years:
$.0175 / (1.00074)^{100} = .0188$
So it will be rising @ .0188 deg/year.

So instead of a little ice age taking
$\frac{1}{.0175} = 57.1$ years it will take: $\frac{1}{.0188} = 53.2$ years
yrs

So the equivalent of a little age ice age is already upon us.

So now temper the results w/ realistic CO_2 & methane charges.

Methane: Last 40 years 0.05°
Last 200 years 0.40°

CO_2 ~~ppp~~ 2.1 ppm per year out of 400 ppm

$\frac{2.1}{400} = 0.52^\circ$

The current conditions are increasing the rate of heating by approx

3.5E-4 % per year.

or. 3.5E-6 in terms of a rate per year

so $\frac{1^\circ}{.007} \cdot \frac{1}{.0175} = 57$ years.

already for a new little ice age.

$\frac{5^\circ}{.0175} = 285$ full ice age.

CO_2 is now increasing @ ~ 0.5% per year.
Methane 0.4% per year.
Temperature now. .0175 per year.

~~.0005~~ % = .0175°/year

So increase current level by 100% / 100% and divide to temperature to .035°C per year

We get .15% ~~to~~ = .017526° per year

but we already said it was .035 °C year and now it is ~~changing~~ increasing by .15% per year.

$\left.\begin{matrix}300\\300\\.5^\circ\end{matrix}\right] = 6.4\%$ annual rate

Certainly interesting to get a 3 way graph here.

2/3 of Ø.8° C has occurred since 1980

$2/3(.8^\circ) = .533^\circ C$ in $(2014-1980)+1$ yrs = 35 years

$\frac{.533^\circ C}{35\text{ years}} = \frac{X}{1}$ $X = .015\ ^\circ C$ per year

If we hold temp change constant & increase CO_2 & methane by 100%, rate of heating will increase by Ø.07%

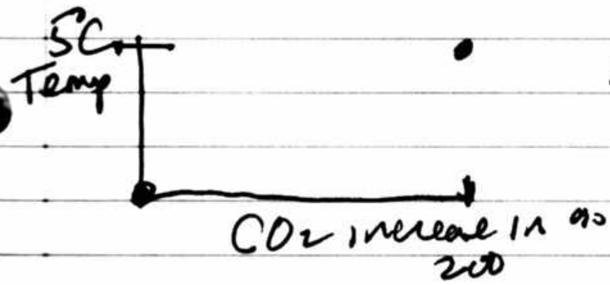
$\frac{1}{.0175} = 57.14$ years $\frac{1}{(.0175)(1.0007)} = 57.10$ years

Two weeks earlier..

Yes there is something missing here.
We ignored the fact that methane will cause temp to increase.

There is a correlation between CO_2 & temp but causation cannot be stated.

Δ Temp = $.05(CO_2)\%$ increase



We now have a projected relationship between CO_2 Change & temp change
Not Causation.

Δ Temp °C = .05 CO_2 increase

Global Warming Potential of Methane

15.72 for a 20 year period.
25 " 100 yrs
7.6 500 yrs

$\frac{1.0}{.0175} = 57.143$ years

1% 3.1% 51.6 Reduction
5% : 3.9% 55.0 years −10%

[illegible] Δ=45.5% 49.5 years −10%

20% Δ=62.1% 35.2 years −36%

50% Δ=387% 11.7 years −78%

Increase in CO_2

1% / 1% → .06%

5% / 5% → 1.4%

10% / 10% → 5.5%

15% / 15% → 12.4%

20% / 20% → 22.1%

30 / 30 → 49.7%

50% / 50% → 138%

100% / 100% → 552%

88% reduction

.5% / .5% → 0.014%

= .00014

= 57.134 g

Reduction .017%

Methane is currently charging @ .05%

You have a Cat model in place now.
Good work

So now the question is, if body of the air change by a certain amount, what can we predict from the system?

1° Little ice age

5° Major ice age

Say it increase by 2.4°:

1°

1° rise = 2.3 meters rise in sea level

0.2°C per decade

= ~~0.F~~°C per year 0.02 per year

You need to think how to translate.

5% / 10% → 1.77%

$.5\%$ / $.5\%$ → 0.01%

5% / 5% → 1.38%

20% / 20% → 22.1%

Let's translate what it means have an "increase in the rate of change of atmospheric heating".

You might want to say something about causality.

Assume we have a 2% increase, what does this mean.

~~0.1~~ 0.02°C per year.
.02040 2nd year
.02081

$1.02\left(\frac{1.02}{\cancel{2.0}}\right)^n = 2.04$

~~2.0~~ =

$1.02^n = 2$
$n \log 1.02 = \log 2$

$n = \frac{\log 2}{\log 1.02} = \log 2 - \log 1.02 \quad n = 35$

$1.02 \cdot .02 = .02040$
$1.02 \cdot .02040 = .02081$

Eg
Current heating is .0175°C per year
Assume we have a 2% increase in place for 5 years

.0175
$1.02(.0175) = .01785$
$1.02(.01785) = .01821$
$1.02(.01821) = .01857$
$1.02(.01857) = .01894$
$1.02(.01894) = .01932$ $\quad \Sigma = .092$

$X_{n+1} = (.1 + \%\text{ increase}) \cdot X_n$ $\quad X_n$ = Current Annual Increase in C°

$= 1.02(X) + 1.02(1.02)X + 1.02(1.02)^2X + \dots$

$= 1.02(X) + 1.02^2(X) + (1.02)^3 X + (1.02)^4 \cdot$

$= X(1.02 + 1.02^2 + 1.02^3 + \dots)$

~~$= X^n(1.02)$~~

$= 1.02 \cdot X \cdot 1.02^2(X) \cdot 1.02^3 X + 1.02^4 X + 1.02^5 X$

$= 1.02^{1+2+3+4+5} \cdot X$

$\sum_{k=1}^{n} n = \frac{n(n+1)}{2}$

$(X \cdot 1.02) + (1.02)(1.02)$

$= X \cdot 1.02^5$ This is it

Current Annual Rate ~~Increase~~ $\cdot (1 + \text{Rate of Increase})^n =$ Desired Factor Increase

$\frac{\log(12)}{\log(6)} \stackrel{?}{=} \frac{\ln(12)}{\ln(6)} = 1.387$ 1.387

yes

so

Annual Rate $(1 + \text{Rate of Increase})^n = \text{factor}$

Let factor $= 2$

Annual Rate $(1 + \text{Rate of Increase})^n = 2$

$(1 + \text{Rate of Increase})^n = \frac{2}{\text{Annual Rate}}$

$n \cdot \log(1 + \text{Rate of Increase}) = \log\left(\frac{2}{\text{Annual Rate}}\right)$

division ok here

$n = \frac{\log\left(\frac{2}{\text{Annual Rate}}\right)}{\log(1 + \text{Rate of Increase})}$

$\log\left(\frac{2}{.0175}\right) \div \log(1.02) = 239.3$

yes, this works

At this rate, the heat in the earth's atmosphere will double in ___ years

$\log\left(\frac{3}{2}\right) = .176 = \log 3 - \log 2$

$\log\left(\frac{a}{b}\right) = \log a - \log b \neq$ ~~$\log c - \log b$~~ !!!

$\frac{\log(a)}{\log(b)} \neq \log(a) - \log(b)$!!!!

$\sum_{k=1}^{n} a^k$

No of years for the rate to double

So we have

$$n = \frac{\log(\text{Multiple Factor})}{\log(1 + \text{Current Rate of Increase})}$$

eg (2.0)

annual Rate

eg (.0175°C)

eg $2^n = 1.02$

For Factor = 2:

$$n = \frac{\left(\frac{\log(2)}{.02^0}\right)_{\text{Annual Rate}}}{\log(1 + \text{Current Rate of Increase})}$$

What is doubling?

??? $1.02^1 + 1.02^2 + 1.02^3 = 3.1216$

NO! $1.02^{\frac{n(n+1)}{2}}$

$$= \sum_{k=1}^{n} a^k = \left[\frac{a^{n+1} - 1}{a - 1}\right] - 1$$

Another way of looking at the problem is when does the sum = 1°C.

$x + 1.02(x) + 1.02^2(x) + 1.02^3(x) \ldots = 1$

$x(1 + 1.02^{\sum n}) = 1$ $\quad \sum n = \frac{n(n+1)}{2}$

$1 + 1.02^{\sum n} = \frac{1}{x}$ $\quad 1.02^n = \frac{1}{x} - 1$

$x = .02$

$n \log 1.02 = \log\left(\frac{1}{x} - 1\right)$ $\quad n = \frac{\log\left(\frac{1}{x} - 1\right)}{\log 1.02}$ $\quad n = 196.5$

Page 178

Since $\Sigma n = \frac{n(n+1)}{2}$

We now have:

$$X\left(1+(1+.02)^{\frac{n(n+1)}{2}}\right) = 1$$

This is actually true

appears to be formulated incorrectly

or

$$1+(1+.02)^{\frac{n(n+1)}{2}} = \frac{1}{X}$$

$X = .02$ = Rate of Increase

$$(1+.02)^{\frac{n(n+1)}{2}} = \frac{1}{X} - 1$$

$$\frac{n(n+1)}{2}\log(1.02) = \log\left(\frac{1}{X}-1\right)$$

$$\frac{n(n+1)}{2} = \frac{\log\left(\frac{1}{X}-1\right)}{\log 1.02}$$

$$n(n+1) = 2\left(\frac{\log\left(\frac{1}{X}-1\right)}{\log 1.02}\right) \qquad X = .0175$$

$$n(n+1) = 406.8$$

$n = 20$ for has this is a valid solution so Something must be wrong in the formula

$1.02^{\frac{n(n+1)}{2}} = 56.14$

The answer is indeed about 20. It is a quadratic equation.

Our formula is:

$$n(n+1) = 2\left(\frac{\log\left(\frac{1}{\text{Annual Increase}} - 1\right)}{\log(1 + \text{Rate of Increase (decimal form)})}\right)$$

or $n(n+1) = b$

$n \quad n^2 + n = b$

$\therefore \quad n^2 + n - b \doteq \emptyset \quad \therefore \quad ax^2 + bx + c = \emptyset$

Quadratic

1	.0175	11	.02133
2	.01785	12	.0276
3	.01821	13	.02219
4	.01857	14	.02264
5	.01894	15	.02309
6	.01932	16	.02355
7	.01971	17	.02402
8	.02010	18	.0245
9	.0205	19	.02499
10	.02091	20	.02549
	.1916		.425

We are close

I love it.

The actual relationship is: ~~Something wrong here~~

$$X\left(X+\left((1+.02)\left[\frac{1.02^{n+1}-1}{1.02-1}\right]-X\right)\right)=1$$

or

$$1+(1+.02)\left[\frac{1.02^{n+1}-1}{1.02-1}\right]-1 = \frac{1}{X}$$

or

$$(1+.02)\left[\frac{1.02^{n+1}-1}{1.02-1}\right]-1 = \frac{1}{X}-1$$

or

$$\left(\left[\frac{1.02^{n+1}-1}{1.02-1}\right]-1\right)\log(1+.02)=\log\left(\frac{1}{X}-1\right)$$

$$\left[\frac{1.02^{n+1}-1}{1.02-1}\right]-1=\frac{\log\left(\frac{1}{X}-1\right)}{\log(1+.02)}$$

$$\left[\frac{1.02^{n+1}-1}{1.02-1}\right]=1+\frac{\log\left(\frac{1}{X}-1\right)}{\log(1+.02)}$$

or

$$1.02^{n+1}-1=.02\left(1+\frac{\log\left(\frac{1}{X}-1\right)}{\log(1+.02)}\right)$$

$$1.02^{n+1}=1+.02\left(1+\frac{\log\left(\frac{1}{X}-1\right)}{\log(1+.02)}\right) \qquad X=.0175$$

closer should be about 40.

$$n+1=\frac{\log\left[1+.02\left(1+\frac{\log\left(\frac{1}{X}-1\right)}{\log(1+.02)}\right)\right]}{\log 1.02}$$

$n \approx 81.15$

$n=f(y)-1$??? $\qquad n=40.6$??

historical heat rate

1° Change in years

$$X\left(\frac{1.02^{n+1}-1}{1.02-1}\right) = 1^\circ C$$

Page 181

$$\frac{1.02^{n+1}-1}{1.02-1} = \frac{1}{X}$$

$$1.02^{n+1}-1 = .02\left(\frac{1}{X}\right)$$

$$1.02^{n+1} = 1 + \frac{.02}{X}$$

$$n+1 = \frac{\log\left(1+\frac{.02}{X}\right)}{\log 1.02}$$

.02 = new rate of increase (decimal form)

X = current heat rate

.02 = Rated Increase

X = .0175° C

Current heat rate

$$n = \left[\frac{\log\left(1+\frac{.02}{X}\right)}{\log 1.02}\right] - 1$$

↑ new rate

n = 375

Got it

~~Got it.~~ This is for 1° Change Accumulated.

Fascinating result.

We could easily do this for 5° C now.

Nov 18 2014

We now have a javascript version that is giving the numbers very efficiently.

Let's get the doubly and 1° jacks in.

400 parts CO_2
2 ppm methane.

So what if GWP = 25
If there is 200 times less

Page 183

Nov 21 2014 Where are our papers?
We have to review to model from scratch again.

1. We have an expected temperature change of

This is a problem!

$.05 \cdot CO_2^{\%} + 25(.05) CO_2^{\%}$

CO_2 influence — methane influence — GWP is ~25 times that of CO_2. But does this factor in concentration?

This says that

Double CO_2 = 5°C increase of the ocean.
Not the whole earth.

NO!

So water this. They are at home.

$2 \cdot (CO_2^{\%}) = 5°$

So how is the relationship?

CO_2 Temp
.04 .02°C
100 5°C

but this is ocean!

You used $0^{\%}$ $0°$
$100^{\%}$ $5°$ $= 1/20 = .05$
but this is the ocean! That is still legitimate.

We know that the atmosphere heats in a fraction of pure carbon heating.

One source says $16°F = 300$ ~~ft~~ of sea level

$1°C = (5/9) 1°F$ or $\frac{8.9°C}{300 ft} = \frac{1°}{x}$

$x = 33.34$ ft
not 6 feet

So, now because the GWP is a function of equivalent mass.
But they hardly are of equivalent mass.

The mass fractions tell us the ratio.
Mass fractions are
Methane 1.0E-4
CO_2 5.7E-4

so there is a ratio that should be used.
It is not ~~2ppm~~ which must be volume
400ppm not mass

So we must multiply the GWP of methane by the factor $\frac{1.0E-4}{5.7E-4}$
which is about 1/6
We had multiplied it by 25
Now it will be about $\frac{25}{6} \approx 4$
instead of 25.
Then be very conservative and reasonable roundy.

Let's check this number.

$.02°C. + .05(.4) + 25(.05)(.17)(.4) =$

$.02 + .02 + .085$

$= .125°C.$ per year.

Let's call this annual additional temperature change
DS total annual temperature change

	CO^2 Concentration	Temperature
My rate was	~~100~~ 0%	0°C
	~~100~~ 100%	5°C

$y = .05x$

$0°C = .05(100\%)$ ← current annual rate

This works, because $.05(0.4^{\circ}) = 0.02$ deg per year which is exactly what it is doing.

To change it by $.1^{\circ}$ you are really changing it by about 25% since it currently rises @ about .4% per year.

So we need to modify this

$25\%(.4)\% = .1\%$

Points

1. Evidence indicates rising temperature induced CO_2 & methane increases may not visa versa
2. Changes in climate can occur very quickly.
3. The geological record indicates that we are currently overdue and headed toward another ice age.
4. Temperature changes in the geological record have been explosive, in large part, with natural variation in the earth around the sun.
5. The current temperature, CO_2 & CH_4 levels are now outside the range of the geological record.
6. Any climate studies must use the geological and records of ice, pollen, & sediments as records

Nov 22 2014

It is starting to fall into place. The climate program

You had a problem the CO_2 charge had to be in place in addition to have a methane effect. That has not reasonable

You chose to evaluate methane in this way

Methane effect = 25 * ((CO_2 no effect) Current methane mf / Current CO_2 mf

This is the GWP multiplier proportional to MBS

* methane %

This is seeming reasonable

Now lets go onto the alternative thing for we have a model.

$Cp_{mix} = \sum_i mf_i \cdot Cp_i$

we consider

$Cp_{mix} = mf_1 Cp_1 + mf_2 Cp_2 + mf_3 Cp_3 + mf_4 Cp_4 + mf_5 Cp_5$

Nitrogen Oxygen Argon CO_2 methane

we took

$\Delta Cp_{mix} = Cp_1 \Delta mf_1 + \ldots Cp_4 \Delta mf_4 + Cp_5 \Delta mf_5$

but we assume that $i = 1$ to 3 that there are no change. This leads to

$\Delta Cp_{mix} = Cp_4 \Delta mf_4 + Cp_5 \Delta mf_5$

or

$\Delta Cp_{mix} = 1.02 \Delta mf_4 + 2.22 \Delta mf_5$

So

$\Delta Cp_{mix} = 1.02\,\Delta MF_{CO_2} + 2.22\,\Delta MF_{CH_4}$

Now lets look at units.

$Cp = \frac{KJ.}{kg.C^\circ}$ now $\frac{\Delta Cp}{\Delta mf} = \frac{\Delta Cp}{\Delta}$ Δ is a unitless number as mf is a ratio.

So the units of ΔCp remain the same as $\frac{KJ}{kg.C^\circ}$

The mass fractions (ratios are known). We can certainly change them by a percentage. The most that we know is the mass of the atmosphere 5.1 E18 kg.

So if we multiply ΔCp_{mix} · mass of atmosphere then equals

The Heat Capacity of the atmosphere.!!

Lets play with this.

Could we not assume the heats impact is proportional to the concentration of the gas.?

Ie

CO_2 mass fraction change = relative CO_2 change $\times$ mass fraction

100

We have this number as "Carbon dioxide change in percent already. and as "methane change in percent"

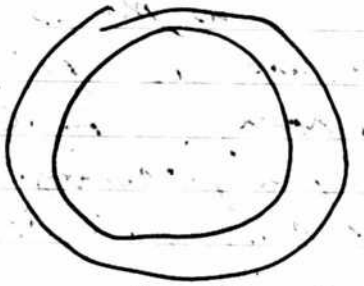
So now we do have the heat capacity change in the atmosphere. = atmospheric heat capacity change. $\frac{\text{Joules}}{kg.C^\circ}$

It is a big number. ~ 4.6E20 Joules.

Page 188

Now we also know the ~~intrinsic~~ Change in the heating of the current atmosphere in Joules

Some how



and

The Heat Capacity of the atmosphere per deg C based upon a specific mass fraction change that affects

Cp

Units are

Joules
C°

Joules

So you need to multiply this by the degree per year to see if it matches

Fascinating results.
We have an observation
We have a model based on
theoretical change of specific heat

We could combine the two.

Let's go on to ~
one degree years.
We talked of a series but I do not think that
it is necessary.

BUT our series form a more accurate sense
... it is not linear!

Our "heat energy ratio is the Δ in heat. (decimal)

Nov 23 2014

We see that a local minimum is reached.

Example

CO_2: $+6^{\%}$

CH_4: $-13^{\%}$

leads to a max decrease of $-4.4^{\%}$
w/ lots of decimals - why?

We have a problem with our series:

$X_{n+1} = (1 + {}^{\%}\text{ increase}) \cdot X_n$

~~$X_1 = X_n$~~ $X_1 = 1.02$

~~$X_2 = 1.02$~~ $X_2 = 1.02^2$ 1.04 $\varepsilon \overset{?}{=} 2$

$X_3 = 1.02^3$ 1.061

So when does $\sum \frac{X^n}{X} = 2$

$$\frac{X^1}{X} + \frac{X^2}{X} + \frac{X^3}{X} \cdots = 2$$

$$= \frac{1}{X}\left(X^1 + X^2 + X^3\right) = 2$$

Theorem $\sum_{k=0}^{n} a^k = \left[\frac{a^{n+1} - 1}{a - 1}\right] - 1$

Page 191

So

$$\frac{X}{X}\left(\left[\frac{X^{n+1}-1}{X-1}\right]-1\right)=2 \qquad \text{solve for } n$$

$$\left[\frac{X^{n+1}-1}{X-1}\right]-1=2X \quad \sim \quad \frac{X^{n+1}-1}{X-1}=2X+1$$

or

$$X^{n+1}-1=(2X+1)(X-1)$$

or

mistake $X^{n+1}=(2X+1)(X-1)+1$

or

$$n\log X=\log\left[(2X+1)(X-1)+1\right]$$

or

$$n=\log\left[(2X+1)(X-1)+1\right]-\log(X)$$

Test for $X=1.02$ $\quad n=.61$??? NO

$$\left[\frac{X^{n+1}-1}{X-1}\right]=3$$

$$X^{n+1}-1=3(X-1)$$

$$X^{n+1}=3(X-1)+1=3X-3+1=3X-2$$

$$(n+1)\log X=\log(3X-2)$$

$$n+1=\log(3X-2)-\log(X)$$

Nope

Again.

Our series:
$X_{n+1} = (1 + \%\ \text{increase})\, X_n$

$X_1 = 1.02 = 1.02$ $\quad 2^{nd}$
$X_2 = 1.02^2 \quad 1.04$
$X_3 = 1.023 \quad 1.061$
$\varepsilon = 3^{rd}$ NO !!!

$X_1 = .02 = .02$
$X_2 = .02^2$
$X_3 = .02^3$

$X_1 = .02$
$X_2 = (1.02)(.02) = .02040$
$X_3 = (1.02)(.02)^2 = .02081$ NO
$X_4 = (1.02)(.02^3) = .02122$
$X_5 = 1.02\,(.02)^4 = .02164$

Series is

$n=0$	.02	.02
1	.02040	$= 1.02(.02)$
2	.02081	$= (1.02)(1.02)(.02)$
3	.02122	$= (.02)(1.02)(1.02)(.02)$

$$= \sum_{k=0}^{n} (1.02)^k \cdot .02 = .02 \sum_{k=0}^{n} 1.02^k = \text{A}$$

$$= .02 + .02 \sum_{k=1}^{n} 1.02^k$$

$= (x-1)(x-1)$

$= .02 + .02 \sum_{k=1}^{n} \left[\frac{x^{n+1}-1}{x-1}\right] - 1 \quad = 1^0$

$(x-1) + (x-1)\left[\frac{x^{n+1}-1}{x-1}\right] - 1 = 1^0$

$(x-1)\left[\frac{x^{n+1}-1}{x-1}\right] = 1^0 + 1 + (x-1)$

$\left[\frac{x^{n+1}-1}{x-1}\right] = (x-1)\left[2 + (x-1)\right]$ $= 2(x-1) + 1$
$= 2x - 2 + 1$
$= 2x - 1$
$= 2(x-1) + (x-1)^2$

$x^{n+1} = \left[(x-1)^2\left(2 + (x-1)\right)\right] + 1$

$(n+1)\log(x) = \log\left[(x-1)^2(2+(x-1))\right] + 1$ $\quad x = 1.02$

$n = \log\left[(x-1)^2(2+(x-1)\right] - \log(x) - 1$ NO
try it:

$= -2.40$

Try again:

Again:

$1(.02) = .02$

$1.02(.02) = .02040$

$(1.02)(1.02)(.02) = .02081$

$(1.02)(1.02)(1.02)(.02) = .02122$

$(1.02)^4 \cdot .02 = .02165$

$= .02\left(1 + \underbrace{1.02 + 1.02^2 + 1.02^3 + 1.02^4 + \ldots}_{\text{we know this series}}\right)$

$$\sum_{k=1}^{n} a^k = \left[\frac{a^{n+1}-1}{a-1}\right] - 1$$

Therefore

$= .02\left[\text{(crossed out)} + \left[\frac{x^{n+1}-1}{x-1}\right] - 1\right] = 1 = 100\%$

increase not $1^{\circ}C$!!

or

$$.02\left[\frac{x^{n+1}-1}{x-1}\right] = 1 \quad \text{or} \quad \frac{x^{n+1}-1}{x-1} = .02$$

or

$$x^{n+1} - 1 = (x-1)^2 \quad \text{or} \quad x^{n+1} = (x-1)^2 + 1$$

or

$$(n+1)\log(x) = \log\left((x-1)^2+1\right)$$

or

$$n = \frac{\log\left((x-1)^2+1\right)}{\log(x)} - 1$$

$x = 1.02$

NO

why.

Again:

$.02(1) + .02(1.02) + .02(1.02)^2 + .02(1.02)^3 + \ldots$

$= .02(1 + X^1 + X^2 + X^3 + \ldots)$

$= .02\left(1 + \sum_{k=1}^{n} X^n\right) = .02\left(1 + \left[\frac{X^{n+1} - 1}{X - 1}\right] - 1\right)$

$(= X-1)$

$= .02\left(\frac{X^{n+1} - 1}{X - 1}\right) = 1$

Seems ok

If $n = 40$

$1.21 \approx 1.0$

$\sim$

$\left(\frac{X^{n+1} - 1}{X - 1}\right) = \frac{1}{(X-1)}$

$\sim X^{n+1} - 1 = 1$

$\sim X^{n+1} = 2$ Still ok

$n+1 \cdot \log(X) = \log 2$

$n+1 = \frac{\log 2}{\log(X)}$

No, not $\log\left(\frac{a}{b}\right) = \log a - \log b$

not $\frac{\log(a)}{\log(b)}$!!

$\sim n = \log(2) - \log(X) - 1$

$n+1 \cdot \log(X) = \log(2)$

$\frac{\log(a)}{\log(b)} = \log(a-b)$?

$n+1 = \frac{\log(2)}{\log(X)}$

$X = 1.02$ for example

$n = \frac{\log(2)}{\log(X)} - 1$

$X > 1$ Condition

← this was your problem

if X is negative

I have it.

A very sharp minimum @

$\Delta CO_2 = +4.5\%$
Δ Methane -2.1%

time = 2414 years
instead of 50

They are perfectly balanced

We have some type of problem.

We add CO_2 585 ppm
CH_4 31.5 ppm.
Heating is increased by 1639 %
but:
Heating by 1 year is 50.3 years? Why why?

$\frac{\log(2)}{\log 2.639} - 1 = .286$ years
$(1 + 163.9\%)$

We get 50.3 years

Check this

Decrease temperature

Next problem:

CO_2 61.5%
CH_4 -33.5%
Heat is -0.4%

No. of years = 0. This is not true.

n is wrong:

It means the ratio is $1 - .004 = .996$

So we have

$-.004$

$.996(-.004) = -.00398$

$.996(.996)(-.004) = -.00397$

So we take this out.

$-.004(1 + .996^1 + .996^2 + .996^3 + \dots)$

$= -.004\left(1 + \sum_{k=1}^{n} .996^n\right)$

$= -.004\left(1 + \frac{x^{n+1} - 1}{x-1} - 1\right) = -.004\left(\frac{x^{n+1}-1}{x-1}\right)$

$-.004 \text{ or } \frac{x^{n+1}-1}{x-1} = \frac{1}{x-1}$

$x^{n+1} - 1 = 1$

$x^{n+1} = 2$

$n = \frac{\log(2)}{\log(x)} - 1$

So we need a block

g. to $c = -1°C$

$x = .996$

or $x - 1 = -.004$

$1 - x = .004$

$= -1°C$

NO

It is $0°C$ not $-1°C$

This is true. $x^{-173} = 2.0$ also.

or $.996^{-173} = 2.00$

$n = -174$

$\frac{1}{.996^{173}} = 2.00$

This is correct. $x > 1$

Digital years

Page 198

We seem to have a 3% error @ 40 k startup. Why?

Nov 24 2014

Ok, it is time to move on to aerosols.

Ok, we have figured out something very important today.

Any HTML code can be performed with Javascript. This is huge.

This means if you have figured out something in HTML like it HTML 5. or otherwise you can set it into Javascript.

This means, for example, any button in HTML that does anything. It may need to be on a single line, not sure yet.

This is very powerful.

You have also learned how to transfer values from HTML tags into the Javascript code.

~~You~~ have also learned how to make a text link w/ a java script but that looks sloppy. A button is much better.

This was all painful as no one told me how to do it, but understandable

Page 200

Just use separate arrays

relatively lecture HTML & JS was crucial.

Also, using Web Editor to simulate simple & functional HTML was an absolute necessity.
You generated button code & pasted it over.
You are on to write that.

I now have a selected menu item and a corresponding value.
Coming into the javascript routine
Very good.

Very cool

Need specific heats & molar masses

	Cp	molar mass
Carbon (Graphite)	0.71	12.02
Sulfate	-3.051 kJ	96.06
Barium	0.29	137.33
Aluminum	~~0.897~~ 0.91	26.98
H_2SO_4	.853	98.08
Sulphur is 0.11	0.71	32.06
$Ba(OH)_2$	.823	171.342
Aluminum Oxide	.776	101.96

x1 a character phenomenon observable in compounds

x3

x4

x2

```
var Items = [[1,2], [3,4], [5,6]];
```

Cp of $Ba(OH)_2$ is $\frac{141\ J}{mol \cdot K}$.

1 mol = 171.342 gms so

$\frac{141\ Joules}{171.342\ gms} = \frac{.141\ KJ}{.171342\ kg \cdot K^0} = \frac{X}{1.0\ kg\ K}$ .823

$X = .823$ so Cp of $Ba(OH)_2 = \frac{.823\ KJ}{kg \cdot ^0C}$

Pure Barium = $\frac{0.29\ KJ}{kg \cdot C^0}$

units are moles. NO

Sulfate Ion SO_4^{2-} has a Cp of $-.\cancel{293.1}\ \frac{KJ}{kg\ C^0}$ (negative!?)

Aluminum Oxide is Al_2O_3 = 101.96 gms/mol $\frac{79.08\ J}{mol \cdot C^0}$

$\frac{79.08\ J}{101.96\ gms \cdot C^0} = \frac{X}{1000\ gms \cdot C^0}$ $X = 775.6\ J$ gms C^0

R. = $\frac{.776\ KJ}{kg \cdot C^0}$ vs $\frac{0.87\ KJ}{kg \cdot C^0}$ for elemental aluminum

Aluminum Elemental

$\frac{24.5\ J}{mol \cdot C^0} = \frac{24.5\ J}{26.98\ gms} = \frac{X}{1000\ gms\ C^0}$ 910 J

$X = .91\ \frac{KJ}{KC^0}$

Cp of sulfate:

$$\frac{-293.1\ J}{9606\ gms\ C^\circ} = \frac{X}{1000\ gms} - 3051.2$$

$X = -3.051\ \frac{kJ}{kg \cdot C^\circ}$ this is remarkable

most common sulfate is
$H_2SO_4 \cdot H_2O$ has a cooling effect

Sulfur Dioxide
SO_2

H_2SO_4 Cp = $\frac{83.7\ J}{mol\ C^\circ} = \frac{83.7\ J}{98.08\ gms\ C^\circ} = \frac{X}{853.4\ gms\ C^\circ}$

$X = \frac{853\ J}{kg^\circ C^\circ} = \frac{.853\ kJ}{kg \cdot C^\circ}$

Start your list with

	Cp	Molar mass
Carbon (Graphite)	.71	12.02
H_2SO_4	.85	98.08
Barium (OH_2)	~~.99~~ .82	~~137.33~~ 171.34
Al Oxide	.78	101.96

Ok, I have the index number
the name
the amount
the specific heat
the molar mass.

Nov 25

Lets go to work compute an aerosol!

We have

$$C_{p_{mix}} = \sum_{i=1}^{n} C_{p_i} \cdot mf_i$$

Now our atmosphere has 6 Components

Nitrogen, oxygen, argon, CO_2, CH_4 & aerosol

So.

$C_{p_i} m f_i$

$C_{p_{mix}}$ = Constant + CO_2 + CH_4 + aerosol

$\Delta C_{p_{mix}} = \emptyset + C_{p_{CO_2}} \Delta mf_{CO_2} + C_pCH_4 \cdot \Delta mf_{CH_4} + A_{cj} \Delta mf_A$

Our program already handles CO_2 & CH_4.

We only need to add in the aerosol effect.

So we need $\Delta C_{p_{mix}}$ to be modified w/ the addition of the term.

$C_{p_{aerosol}} \cdot mf_{aerosol}$.

Lets look at mass fraction of the aerosol

mass fraction = $\frac{\text{mass of a component}}{\text{mass of the total}}$

Mass fraction = Mole fraction of total * molar mass

Lets say we have 50 ugms of $BaOH_2$

The mass of this is 50 ugms / CV m^3

What is the mass of 1 CV mole of air?

@ Std temp & pressure

mass of CV m^3 of air = 1.2041 kg/m^3

So mass fraction = $\frac{50E-6 \text{ gm}}{1.2041 * E3 \text{ gms}}$ = .04152 E-6

Therefore our term of addition is:

$C_{p(aerosol)}$ * Concentration in ug/m³ = (mag ~ .04E-6)
1204.1 gms/m³

Now the density @ altitude is much less.

Approximation of density = $1.24 \frac{kg}{m^3} \cdot e^{\left(-\frac{\text{height in meters}}{8000m}\right)}$

So @ 30,000 ft = 9144 meters

$\therefore$ density $\approx 1.24 e^{-\frac{9144}{8000}} \approx .386 \frac{kg}{m^3}$

So this leads to

$\frac{2E-6}{.386E3}$ gms = .130E-6 mass fraction. A factor of roughly 3.

We can use 15,000 feet as an example = 4572 meters

so density $= 1.21 e^{-\frac{4572}{8000}} \approx 0.68 kg/m^3$ reasonable.

We may have it already!

At this point we are having no effect. Why?
We are not using the molecular weight yet.

Does the molar mass have anything to do with this?
You have not been using a m^3
You have been using the mass fraction defined by:

Mole fraction * Molar Mass = mass fraction
(which is volume)

So how does this number compare w/ m^3?

5E-6 gms

How many moles are in a m^3 of air?

Approx 1 m^3 air = 40.8 moles of air

1 m^3 of air has a mass of 1.2041 kg/m3

Your results indicate that you have to put GRAMS into a m^3 to have an effect

like 10-20 gms per m^3 This is huge

If you put 20 gms into the air m³
this is a mass fraction of - $\frac{20 \text{ gms}}{600 \text{ gms (@ 15c)}}$ = = 2.9%

$\frac{10 \text{ gms}}{600 \text{ gms @ 15c}} \simeq 1.5\%$ mass fraction

This low is on the level of Argon so
this is not entirely out of range.
Remember it accumulates!

10 gms once = 10 flights w 1 gm
100 " @ 0.1 gm = 100 mg.
1000 " @ .01 gms = 10 mg m³
So number of application is a factor.

It is looky very intesting

2 problem has come up of duration of influence.

With C = 1 ng & n=10 heat energy rate = +.1E-5
so it is positive.

So whether you compute it:
high conc
C= [redacted] u/(high number) n= 26 years

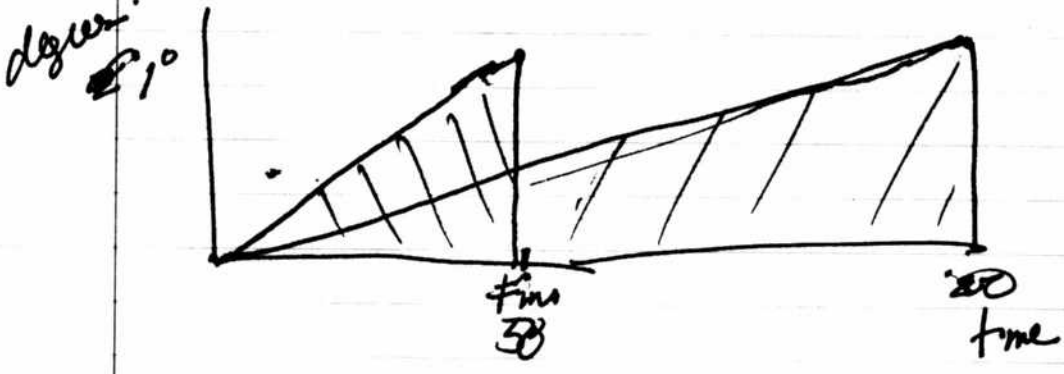
So how do you want to explain this?

It is current rate ± one degree year

one degree 800 years
current 50 years.

Will leakage by 2° in current rate

The rate will
still leaks but
>50 slower than the current rate.



How about an instead water point of opposed to an additive water?

The atmosphere will heat up by 2° in

The atmosphere will heat up by an additional degree in about 26 years.

The current condition about lead to a heating of 1°C in about 50 years.

Your answer will also heat the earth by an additional degree in ___ years.

Situation:
Your particulates only increase the heat of the earth. Is that true?

Water = 4.187 KJ/kgK

You have a problem. You know that water Cools down the air by a factor of.
$\frac{4.00}{1.00} = 4$ You know that
< air is heated it
by a rating of $\frac{1.00}{4}$

Specific heat ratio

cools $\frac{K_i}{1.005}$ Water = 4.00 4/1.005 = 3.98

heats Barrie Ø.3 .299

1 if specific heat is less than air it is additive prevents the air it is subtractive

What are the physics of heating & Cooling of particulates in the atmosphere.

Particulates block sunlight Particulates can heat up.

So we have some questions. Do we want

specific heat ... or heat capacity.

$\frac{KJ}{KS\ C^\circ}$ $\frac{KJ}{C^\circ} = \frac{KJ}{kg \cdot C} \cdot kg$

relates to a ~~physical~~ unit mass.

relates to a physical property body.

Working out heat capacity of one aerosol.

Planetary ave albedo is 30 - 35%
0 mean total absorption
100 all reflection

So like say we have the specific heat of an aerosol and a mass. What would we have to do with it?

$$C_{p_{mix}} = C_{pi} \cdot m_{fi}$$

$$\frac{KJ}{kg \cdot C^\circ} \quad \Delta C_{p_{mix}} = C_{pi}\, \Delta m_{fi}$$

We could certainly find the heat capacity of the atmosphere as it is modified.

We know the mass fraction.
We know the C_{pi}
Use 100 mg.

ΔC_p Heat capacity for Carbon for m^3 = $\frac{.71\ KJ}{kg \cdot C^\circ}\left(\frac{\text{mfi}}{\frac{5\text{mg E-3}/m^3}{.68\ kg/m^3}}\right) = \frac{.00010\ KJ}{m^3 C^\circ}$ of air.

Mass of atmosphere = 5.1E18 kg.

$C_p \cdot m_{fi}$ mfi m = 100 mg

$m_f = \frac{(100\text{E-3 gms})/1000\text{gms}}{.68\ kg/m^3} = .00015$ this is a fraction. It is unitless

$C_p \cdot m_f = \frac{.71\ KJ}{kg \cdot C^\circ}(.00015) = \frac{.00010\ KJ}{kg \cdot C^\circ}$

Now to atmosphere $\frac{.00010\ KJ}{kg \cdot C}(5.1E18\ kg) = 5.325E14\ \frac{KJ}{C^\circ}$

Heat capacity of an aerosol

Now heat capacity of atmosphere

$\cong 1.005\,(5.1E18\,kg) = 5.1255\,E18\ \frac{kJ}{C^\circ}$

So our ratio is

$\frac{5.325\,E14\ kJ/C^\circ}{5.1255\,E18\ kJ/C^\circ} = .00010$

or .0104 % of whatever the atmosphere does.

We have a calculation that gives us the total energy accumulated within the atmosphere for carbon aerosolized per deg C° change.

of $5.325\,E14\ \frac{kJ}{C^\circ}$ (per degree C°) !!

Now what source of energy do we want to apply?
The sun?
The global warming budget?
CO_2, methane relationship

We have an observed energy correlation to give us a temperature rise but that does not apply here.

We may want albedo estimates.

Sola Radiation vs Temperature Rise of an aerosol.

Earth albedo is about 31-35%

We anticipate 240 W/m² actually absorbed by earth

is how much is actually absorbed = $\frac{240 J}{s \cdot m^2}$

Earth surface area = 510 E6 km²

= 5.101E14 m²

So we have $\frac{240 J}{s \cdot m^2}$ (5.101E14 m²) = $\frac{1.224E17 J}{sec}$

In a year we have:

365 · 24 · 60 · 60 sec = 31536000 sec per year

So $\frac{1.224E17 J}{sec}$ · 31536000 sec = 3.86E24 J =

= 3.86E21 KJ

We have a heat capacity of $\frac{5.325E14 KJ}{C^\circ}$ / 3.86E21 KJ

= .138E-6 °Centigrade per year

this is a very small number.

= 1E-5 of actual change taking place now

Note this

Lets Consider different strategies.

You can also consider albedo.

Revised:

$\frac{5.325E14 J}{9.75E19 J}$ per year = .0001 °C

≈ 3.E-4 of what is happening now

≈ .03%

This is too high

See next page

We have at mapleton

annual heat budget @

9.75 E19 Joules/year

Nov 26 2014

We had an interesting result that has developed w.r.t. aerosols. What we learn, under our current thinking, is that everything is additive. That is not realistic.

In the case of CO_2 & methane we had

$$C_{p_{mix}} = \sum C_{p_{CO_2}} mf_{CO_2} + C_{p_{CH_4}} mf_{CH_4}$$

Now the answer to this problem is that the mass fractions can be both positive and negative. That is all fine & the law worked well.

But particulates are a difficult matter. You really are only additive w.r.t. mass fraction because you start out w/ zero in all cases.

Now one way of approaching the problem is to attach a sign w.r.t. a comparison of C_p air & C_p aerosol. You know a C_p < air increases heat & a C_p > air decreases heat. So you can attach sign on that basis.
I am not sure that this is physically correct.

What you really need are 3-4 variable effects particulates:

1. specific heat relative to air
2. Albedo
3. Damage level
4. # of applications
5. Light obscuration
6. Cloud formation

Therefore your modelly is certainly to be more complex.

We started out with

$C_{p_{mix}} = \sum C_{p_i} \cdot m_{f_i}$

In our world with CO_2 + methane
We assume C_{p_i} to be a constant. This is reasonable. But w/ already a major factor on the C_p w.r.t. to air so this difference is important!
Is your model?

$$C_{p_{mix}} = (N + O_2 + A) + C_{p_1} m_{f_1} + C_{p_2} m_{f_2} + (C_{p_3} - air)\, m_{f_3}$$

and when $C_{p_3} >$ air it cools it down
" " " $<$ air it heats it up so it would be

$$C_{p_{mix}} = (N + O_2 + A) + C_{p_1} m_{f_1} + C_{p_2} m_{f_2} + (air - C_{p_3})\, m_{f_3}$$

$$\Delta C_{p_{mix}} = C_{p_1} \Delta m_{f_1} + C_{p_2} \Delta m_{f_2} + (air - C_{p_3})\, \Delta m_{f_3}$$

We have air. We have C_{p_3}.
What exactly is Δm_{f_3}?

$$m_{f_3} = \frac{\text{mass per unit volume}}{\text{mass of unit volume}}$$

and mass = dosage × no. of applications

SO

Aerosol Term: (Cms · 1E-3)

$\Delta = \frac{(Air_{Cp} - Aerosol_{Cp}) \cdot dosage \cdot no\ of\ applications}{mass\ of\ unit\ volume}$ gms · n

gas per m^3 @ 15k feet

Note: C_p is in $\frac{KJ}{Kg \cdot C^\circ}$

Now, this looks good to attack sign of change.

But the annual temperature change is a next question. Do not aerosols affect this? The only way that we can come up with a temperature change is how!???

Heat Capacity * ΔT = Δ Heat $\quad C_p = \frac{KJ}{Kg \cdot C^\circ}$

$\frac{Joules}{\cdot C^\circ}$ (not a unit of temperature!)

units

So Heat Capacity is $\frac{J}{C^\circ}$

Heat Capacity = $\frac{KJ \cdot Kg}{Kg \cdot C^\circ}$

= Joules / $Kg\,C^\circ$

= Joules per C° (real unit)

We multiply specific heat by a mass to get to heat capacity of that mass. There is nothing unit about it.

So water really is cooling it down well. Good. Carbon is a modest increase. Good.

Page 217

Blue Griffon has wonderful
[illegible] the cost
but no right click
Keyboard Controls should work

We need a net [illegible] of

Cooly number –
[illegible]
This ~~[illegible]~~ will cancel the current
heating of the atmosphere in
approximately (Cooly number – 50 years)

→ 50 years

← 46 years

if no degreeyears > 50 then
this will cancel (subtract)

else if ~~[illegible]~~
[illegible]
else

this will cancel the current.
within about no degreeyears

1 degree in 249 years. 244.9 years

50 yrs →

← 245 years

What if you set up all the aerosols and slides and you carried them all over, even with gravity.

		Cp
0	Carbon black	.71
1	Al_2O_3 Al Oxides	.78
2	Sulfuric Acid	.85
3	Water Vapor	4.19
4	Barium Hydroxide	.82
5	Ice	2.03

Something wrong w/ slides

Nov 27 2014

The model is looking much cleaner

1. Graphics?

2. Albedo?

3. Concentration & block out sun?

4. Volcanic Ash C_p = Ø.84
   Aluminum Ø.87
   Barium Ø.29
   Graphite-Carbon Ø.71
   Ice 2.Ø ~~1.8 @ -40°C~~
   Magnesium 1.05
   Air 1.005

One table has
   ~~Carbon~~ 17 Innovation City
   Atom.
   Lithium 3.58.
   Plastics 1.67
   Strontium Ø.3Ø 550 kJ/mol

Albedo		Cp	Albedo?
.10	01	Carbon	.71
~~.15~~	02	Aluminum Oxide	.78
.30	03	Sulfuric Acid	.05
.75	04	Water Vapor	4.19
	05	Barium	0.29
.37	06	Ice	2.03
	07	Volcanic Ash	.84
.25	08	Magnesium	1.05
.25	09	Strontium	0.30

Looks to me like we have a reasonable model shaping up.

"max = 50.0" → max = 100.0

High numbers Reflect
Low Numbers Absorb.

Thick Clouds	60-90
Thin Clouds	30-50
Ice	30-40
Water	10

Example: Thick Clouds Reflect: Use 75
Thin Clouds Absorb. Use 37

So Reduce ~~has~~ Specific Albedo by 75% for thick clouds (water) and Ice by 37%

So Water: ~~4.04 - .75~~

(4.04 − 1.005) = 3.035

(3.035) − .75(3.035) =

ΔCp = 0.76

High albedo reflects
Low albedo absorbs
Albedo measures reflection

Ice Clouds:

$(2.03 - 1.005) = 1.025$

$1.025 - .37(1.025) = \underline{\underline{0.65}}$

This says high clouds heat up a little more than low clouds. Which is true. But I bet the difference is greater.

$(1 - \text{albedo})(\Delta Cp)$

This says clouds heat but still up.

Thick ~~Water~~ clouds: $(0.25)(3.035) = .76$

Ice $(.37)(1.025) = .30$ looks more better.

But this is not realistic but 2X is.

Suggestion

$2(1 - \text{albedo})(\Delta Cp)$

Low $2(1 - .75)(3.035) = 1.52$

High $2(1 - .37)(1.025) = 1.29$ should be LI? Still Still cool down but heat more than high clouds

What do we want to accomplish?

~~reflect~~ ~~reflect~~ ~~less~~ reflect less high clouds reflect more

High Clouds ~~absorb less, more, low cloud absorb more~~

Page 222

Thick (low, water) ~~coto~~ clouds
reflect more (i.e. absorbs more)

~~High~~:
Thin (high, ice) reflect less (absorbs less)

Hhmmm: is more or less the albedo.

Thick (water) ⇒ $\Delta C_p = 4.04 - 1.005 = +3.035$

ΔC_p.
≈ 75% albedo. means they reflect 75%,
so 25% come in
$.25(3.035) = .76$ this says it
still heats up, not freeze.
∴ Needs to be reduced by ~~ice~~.
2X clear seen about right

Low Clouds $2(1-\text{albedo})\Delta C_p = 2(1-.75)3.035 = 1.52$
$2(1-.37)\Delta 1.025 = 1.29$ and this is too high

$2(1-\text{albedo}^{1/2})\Delta C_p$

$2(1-.75^{2})3.035 = 2.65$

$2(1-.37^{2})(1.025) = 1.77$ NO

3D Albedo - ΔCp model

$f(\text{Albedo}, \Delta cp) = Cp^*$

$Cp^* = f(\text{Albedo}$

Cp is + or -

A	ΔCp	
.75	3.055	→ 1.5
.31	1.025	→ .85
.3	1.005	→ 1.005
.25	.715	→ 0.7
X	4	

3D plot

xy plane solution

$3.9 \cdot \text{Albedo} - .42\,\Delta cp - .16 = Cp^* \quad = 1.5$

$3.9\,(.31) - .42(1.025) - .16 =$

$\Delta cp = (\text{air}_{cp} - \text{aerosol}_{cp}) - \text{Reversed Sign}$

$Cp^* = 1.84\,A - .06\,\Delta cp + 0.29$ $\quad r = .94$ good

Page 224

Observations
local

1. @ ~~maximum~~ minimum of methane
by CO_2 = +8%
CH_4 ≅ −12.4%
Rate of cooling = −4.2%

2. Now introduce Ba: aerosols

I now have a balanced situation between:

CO_2 +8%
CH_4 −26%
Ba +15 @ 400
Thin High Clouds @ 400
Thick Low Clouds @ 45

Your model is looking rather realistic.

Now if Core of particulate gets high enough it starts to cool things down because the sun is blocked.

Why are we off expected value by a factor of 1000?

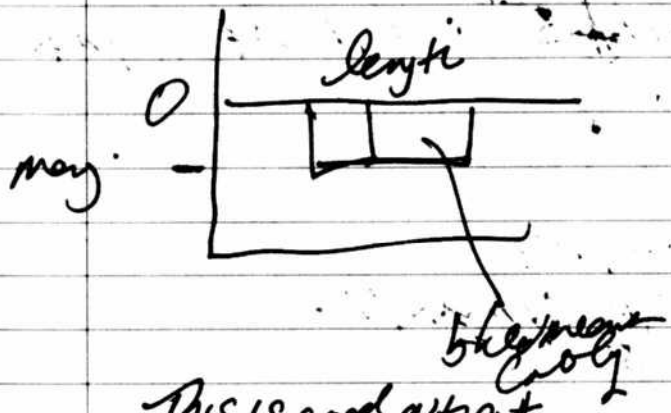
Nov 28, 2014

It is now time to see if we can draw a line.
But before we do this, ask
what would we actually like to draw?

Our output is

1. a Cooley rate . .
2. a reference value of 1° +/- at time . .
3. of course our inputs are intensity alone .

We could start out of a simple diagonal slope
and a ~~green~~ blue.

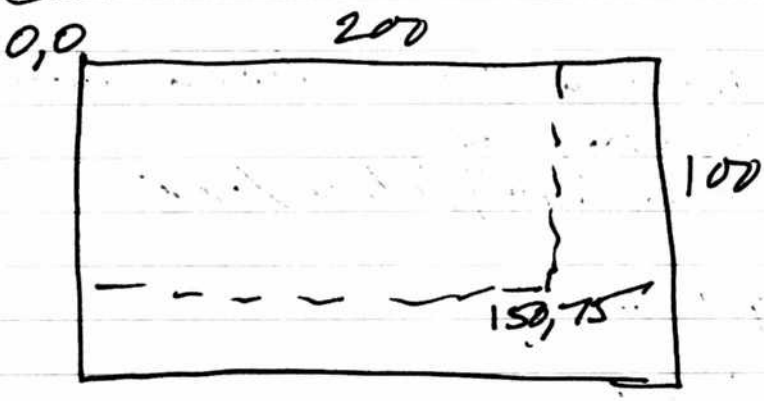


The width of the bar. Could be how long it takes.

This is good output.

OK We have a Colored box!

Canvas element initiated



Ok, we have a box and a line
Very good.

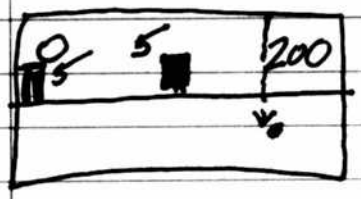
Assume the box scales to 10 max on y.
If sonarky is 10 it scales by a ratio of $\frac{200}{10}$
but since it is +10 to -10

lets imagine a point at 5. (It is 1/4 of ymax
150, we have to

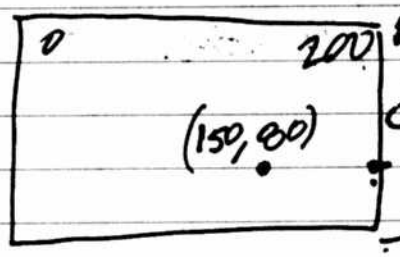
Steps:
1. Move to y axis, ie
   Move to Xorigin, ymax/2
2. Take the number 5

Page 227

bouquet



pt = (newy − yorigin) / newx − xorigin



My coords are 750 years

$\frac{150}{200}(1000) = 750$

1000 years

$y = \frac{80}{100}(20) - 6$

me 60x

x 750 goes to 150

−6 goes to 80

$\frac{750 \cdot 200}{1000} = 150$ easy enough $X_{box} = \frac{myx \cdot xmax}{myxmax}$

my x

xmax = 200

myymax = 20

myxmax = 100

ymax = 100

−6 $-6 + \left(-\frac{myymax}{\cdot}\right)$

$\left(y_{box}\right) \frac{-6 + -10}{-2 \cdot myymax} = \frac{-16}{-20}(100) = 80$

So $y_{box} = \left(\frac{myy - myymax}{-2 \cdot myymax}\right) ymax$

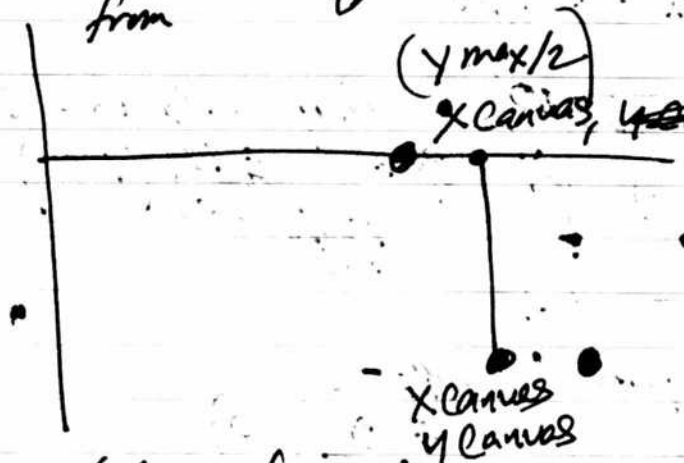
y is ok

x is not

We have a line now.
Now draw a box

box (x1, y1, x2, y2) & it fills it.

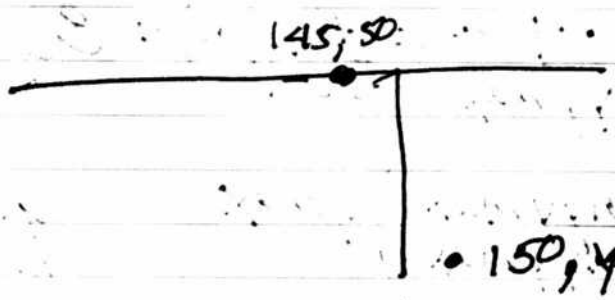
from to



so lets so from
xCanvas − 5 to xCanvas + 5
~~yCanvas~~ to yCanvas
ymax/2.

xCanvas = 150.
yCanvas = 80 & it still's
150, 80

145, 50, 150,

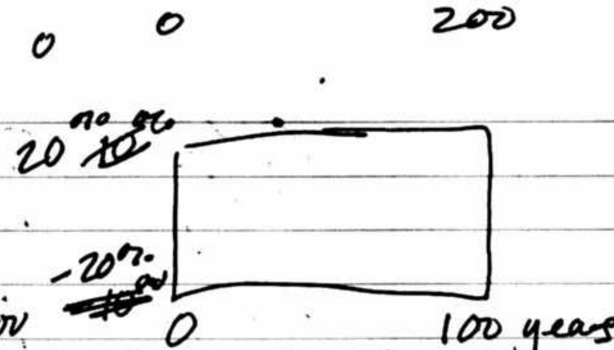


OH!
IT IS WIDTH!
Not x2, y2

Page 229

It is width & height!
NOT x_2, y_2!

This was tricky!!!!



It is:
fill rect (x_1, y_1, width, height!)!!
note

Ok you are getting close.

10.6% per year this is y! $CO_2 = 7.3$
5.9 years this is x! $CH_4 = 593$

percent fixed that every median years

Our coords are
21.2 & the box is the wrong direction.
35.2 ~~122.51~~
Work on the line first. Then the box.

my x = 10.6% good...
my y = 5.9 years. good!

Canvas x = 21.2°? matches.
Canvas y = ~~[illegible]~~ 35.2. NO! This is wrong.

$\frac{10.6}{20}$ = our y coordinate should be about 23.5 no!

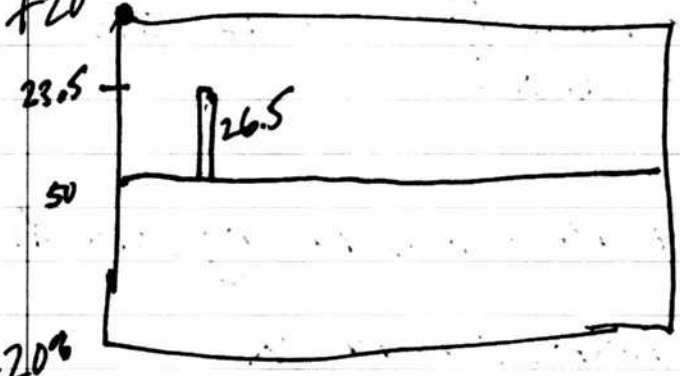
Ok, you had coordinates swapped.
The line looks good now.
Now get the box!

Page 230

Old x	5.9 years	~~the~~ one degree year
Old y	10.6 m.	heat energy percent
New x	11.8	out at 200
New y	23.5	out at 100

So for the rectangle
~~how high is the box~~
how wide is the box in pixels? 10 always.
how high is the box. in pixels?

y 0 +20 m. 200 x



100 -20 m.

So the height is $-(y_{max}/2) - y_{canvas}$

You want the smallest bar furthest away.

Draw a line @ x no matter what

So we think ΔM_4 should be:

$$\Delta M_4 = \frac{\left(\frac{\text{Relative Change}}{100}\right) \cdot \text{Current Growth Rate in \%}}{100}$$

Example: 3% Change:

$$\frac{\left(\frac{.03}{100}\right)(.4\%)}{100} = .00012$$

But we believe that we are getting .012. This means it is scaled up by a factor of 10!

Yes, we are getting .012

!!! but it should be reduced to ΔCP to .00012

Error

We got 1 degree in 52 year

We got ±1.3% per year
It should be .013% per year.

Because of this error I believe
we got to close to 1% per m³ !!!

So we are dealing with
hundreths of a percent per
year & 1% of aerosols

m

We need to close to 1000 µg
not 100

This will be still be in range of 1 mg per m³

lets leave aerosols in mg and take
from 0 to 1 mg step .01

Change graph to 0.3 not 3%

Dec 01 2014

I believe that I have uncovered a really problem of percent.

We also have a question w.r.t. relative change

We also have a question as to what is happening w/ methane.

Back to scaly problem:
We are after $C_p = C_4 M_4 + C_5 M_5$
We ended up with:

$$\Delta M_4 = \frac{(\%\ CO_2 \text{ growth rate})(\%\ \text{relative change})}{100}$$

~~2~~ 0.4% 100 3%

$$\Delta M_4 = \frac{(\%\ CO_2 \text{ growth rate})(\%\ \text{relative change})}{100^2}$$ Assume

Check it. Yes this is right. It should be

$\Delta M_4 = (.004)(.03) = .00012$

Now, is this a volume change, or a mass change?
I believe it is volume, lets check.
It is ppmV notice. yes it is by volume.

We must convert change in volume change in % to a mass change in %.

We have

$.03(.4) = .012$ this is percent per year.
decimal we divide by 100
$CO_2 + 3\%$ = we get .00012 this is right
now methane
$CH_4 - 3\% = -.00027$ (factor of 2)

add annual temperature range
CO_2 +3% = .0006

Should n't this be 10% if it is leaving us @ .006?

The temperature of .006 is the closest of to atmosphere - forget the 2% business

Methane is Cooling down @ -.003 deg C
and then the progma locks up w/ 3.

CO_2 +3% - Total annual temp change is now in .0066

This is right.
Our percentage should be $\frac{.0066 - .006}{.006}$

= 10%. this is not right
what we have

We are using the empirical approach right now to determine Δ°C.

Heat Capacity Units $\frac{J}{C^o}$

Heat Capacity • ΔT = Joules

Right now we are not using the Δspecific heat a heat capacity. Why?

Heat Change in Atmosphere

Use +3% CO_2 4.11998466 Joules Now it is by also
−3% CH_4 Methane is off the Chart - why?

Heat energy ratio
+3% CO_2 = 4.22E-5
−3% CH_4 4.54E-5 and it locks up the program.

Something is wrong here?

Use the specific heat of the atmosphere to determine the global reference!
Not to 2% rate.

We formed a new number.

$$\frac{\Delta \text{ Heat of atmosphere}}{\text{Heat of atmosphere}}$$

Rather than the 2% rule. This make a lot of sense.

A negative number is locky up.

OK, I found that problem.
RAGE vs RATE in the variable.

Now the problem is
that methane sensor is back leaky?

Why?

Temperature change is signed!
Estimate

Graphics take my x, my y

Methane is leaky -.005%
Compare:

+3% CO_2 5113 .013% +15% CO_2 758 +.09
+3% CH_4 4809 -.014 -15% CH_4 401 +.17 ?

	CH_4
+3%	-.014
-6%	-.006

So it is decreasy - why?

Why is the CO_2 change so apparently the methane

I believe we have a problem with the total annual heating change.

−.004 + .006 = +.002 net rise.

So a decrease of about 7% almost balances the current heating rate.

If it is negative we have a problem.

I don't like the 0% term?

You are putting out + Total ΔT
additional ΔT

+.006 anything

.0037 / .006 = .61%

−.01% =

So the heating ratio = $1 + \frac{X}{100}$

or $\frac{X}{100}$ So the slope has to be very small now

First of all temp is changing .0061 per year
Not 0.02

We have some problems now / the code.
Methane does not decrease.
Methane incomplete @ low levels.
Heats at very low levels
Thick low clouds do not work also.

One dozen years:

1 ~~#~~ heat energy ratio:

1-.77 1-.77= .23

1-

~~You have cooled to earth~~

You have decreased the earths heats rate
by 30%

You have increased the earth heats rate by

You are now cooling the earth at a rate
of

Page 241

What is the CO_2 percent increase here?

Really?

~~We know that~~ 1°

The relationship is

$\Delta 5°C = 100\%$ increase in CO_2

$\frac{5°C}{100\%\text{ increase}} \quad \frac{1°C}{x} \quad x = 20\%$ increase

So $1° = 20\%$ increase in CO_2
So CO_2 increase $= .05 \cdot$ temperature rise
So example if temp rises by ~~.006~~ .006 deg.
CO_2 rises by $.00030\%$

% increase = ~~$\Delta 20 \cdot \Delta deg C$~~ $20 \cdot \Delta deg C$ this is percent!!!

$.006 = .12\%$ ∴ $\Delta deg C = \frac{\%\text{ increase } CO_2}{20}$
$5 = 100\%$
$1 = 20\%$

What exactly are you doing here?

How time is one of your last indicators

The earth is heating up about 20°. faster

You have thrown away your model - why?

You are now

1. Setting a CO_2 amount
2. Computing an empirical temperature change
3. ~~Computing it~~ Adding a subtracting it to the current rate
4. Calling it good.

Then you would change methane by a GWP & not adjusted rate & call it good.

This might be sufficient but it is hardly based upon specific heats.

How do they compare & why?

It all about because if you found a potential error & recalculate.

Lets go back

$$C_p = C_4 \Delta m_4 + C_5 \Delta m_5$$

Work w/ CO_2 only right now.

$$\Delta m_4 = \left(\frac{1 \pm CO_2 \text{ slider which is in percent}}{100}\right) \cdot \text{Current } CO_2 \text{ m}\Delta - \text{Current } CO_2 \text{ m}\Delta$$

example

20% increase: $\frac{1+20}{100}(5.7E-4) - 5.7E-4$

$$\Delta m_4 = .00011$$

Your number IS the mass fraction change.

You don't need to change anything.

I set $\Delta C_p = .02346$ (sounds too high)

$(.023)(1.02) = .02346$ OK ✓

$(1.02)(.025) + .03_{m_4}0(2.22) =$ OK

$$\frac{kJ}{kg \cdot C^\circ} \cdot kg \cdot 1000 = \frac{J}{\Delta C^\circ}$$

Methane & CO_2 look very scary.
You seem to have lost your aerosols.

Ok, there is some kind of contribution from the aerosol.

Ok, the problem is there is no temperature change.

So how do you relate aerosol temperature change to CO_2?

The problem is, what if you put aerosols in by themselves with no CO_2 change or methane change

Use the carbon cycle model

use what is a

Minimum Established @ + Ratio of Specific Heats

How about a filter for aerosols based upon existing heat charge .006 per year

We have an already contribution to the specific heat we even have it through the heat capacity term

If additional annual temperature change = ∅ then addition = current heating

[redacted]

OK I believe we have a very good model.

One of the solutions is that ΔM_4 & ΔM_5 are the direct inputs from the slides.

$$\Delta c_p = C_4 \Delta m_4 + C_5 \Delta m_5 \stackrel{?}{=} \emptyset$$

$$-C_4 \Delta m_4 = C_5 \Delta m_5$$

$$1.02 - \frac{C_4}{C_5} = \frac{\Delta m_5}{\Delta m_4} \qquad = 2.17 \qquad \text{Yes this works}$$
$$2.22$$

Set CO_2 @ +5.0

adjust CH_4 to $+5.0 = -2.17$ $\quad t = 80,439$ years
-2.3

or other way around. You can set either one

Komposer creates very clean code.
Komposer is a daily used program.

Composer starts out of a div tag
Makes some blank lines
Insert a table
Rt Click, Properties, Center

(It is using CSS style tag to center the table)
The bar @ the top can resize

Blue Griffon can be used to generate HTML5 elements

This means BG program needed to generate
the HTML5 element code.

Komposer also has a very easy to use
Style editor.

3. Programs make up your suite
Composer is your new html editor of choice
Blue griffon is your html5 element
generator program
Netbeans is your project manager
and javascript editor

Page 248

[redacted]

We continue to work through the model

Heat Capacity = $\Delta C_p \cdot Mass \cdot 1E3$

$\frac{Joules}{C^\circ}$ = ——

[redacted]

Next step.

1. We have estimate for $\Delta T = f(\Delta CO_2) = .05 CO_2^{n_0}$

2. Next we need methane estimate for ΔT
   It is stated that GWP of methane is 25 · CO_2
   So start w/ $\Delta T = 25 \cdot f(\Delta CO_2)$
   or $\Delta T = 25(.02) \cdot CO_2$ However this assumes equivalent mass, which is hardly the case.

So

$$\Delta T_{CH_4} = GWP_{methane} \cdot a_1 \cdot \left(\frac{\Delta m_5}{\Delta m_4}\right) \cdot CO_2^{n_0}$$

Thus

$$\Delta T_{mix} = a_1 CO_2^{n_0} + GWP_{CH_4} \cdot a_1 \cdot \frac{\Delta m_5}{\Delta m_4} \cdot CO_2^{n_0}$$

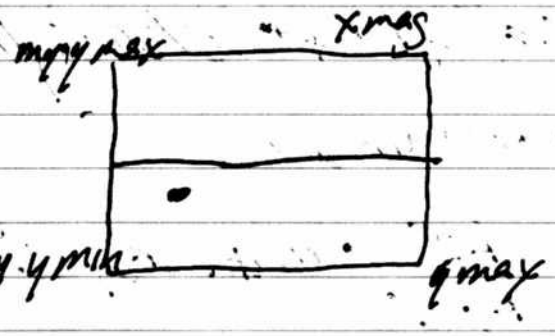
① Grey CL bag [illegible] Grey- has books
② Blue lunch box bag- has soda in cans
③ Blue [illegible] bag with CDs.

$$\Delta T_{mix} = a_1 CO_2^{n_0} \left(1 + GWP \frac{\Delta m_5}{\Delta m_4}\right)$$

Page 249

I think that our y coordinate is messed up

my ymax = 20



or my y =

$$y = \frac{(myy - myymax)}{2 \cdot myymax} \cdot ymax \qquad -.5$$

$= -.5 - 2 = -2.5$

This is better.

abs

This is currently wrong w/ the graphic

(area definitions must be strings.

I have had problems w/ the Firefox cache clearing at.

Dec 05 2014

It is dimensional analysis time. We have

$$\Delta E = \left(C_4 \Delta M_4 + C_5 \Delta M_5\right) M_{atm} \Delta CO_2^{no} \left(1 + 25\left(\frac{M_5}{M_4}\right)\right)$$

So our units are

$$\frac{KJ}{Kg \cdot C^\circ} \cdot M_{atm}(Kg)\, C^\circ = = KJ$$

~~So mass of atmos~~ So KJ. have to be multiplied by 1000 to get Joules.

And our constant term .05 is in $\frac{deg\ C}{per\ no\ CO_2}$

So now we have

$$\frac{KJ}{Kg \cdot C^\circ} \cdot mass_{atm}(Kg) \cdot \frac{deg\ C^\circ}{per\ percent\ Co2} \cdot \Delta CO_2^{no}$$

leads to KJ

So somewhere it is multiplied by 1E3 to get Joules,

So we note that $\Delta CO_2\% = \frac{\Delta m_4 \cdot 100}{\text{Total}}$

Dec 06 2014 For CO_2 alone

Repeat Dimensional Analysis (ΔT)

$$\Delta E_{atm} = C_4 \Delta m \cdot M_{atm} \cdot 1E3 \cdot .05^{\circ} \Delta CO_2^{2} \left(1 + .25 \frac{m_5}{m_4}\right)$$

$$= \frac{KJ}{kg \cdot C^{\circ}} \cdot Ratio \cdot kg \cdot 1E3 \cdot deg \cdot (Constant + Ratio)$$

$$= KJ \cdot 1E3 = Joules \quad \text{good.}$$

But you said that $\Delta CO_2\% = \Delta m_4 \cdot 100$

So what the rocky says is that

$$\Delta E_{atm} = C_4 \Delta m \cdot M_{atm} \cdot 1E3 \cdot .05^{\circ} \Delta m_4^{2} \left(1 + .25 \frac{m_5}{m_4}\right)$$

$$= \Delta m C_4 \cdot M_{atm} \cdot 1E3 \,(.05^{\circ}) \left(1 + 25 \frac{m_5}{m_4}\right) \cdot \Delta_4 m \cdot 100$$

$$= \Delta m C_4 \cdot M_{atm} \cdot 1E(.05) \left(1 + 25 \frac{m_5}{m_4}\right) \Delta m_4$$

? This would be a constant?

$$= 5000 \cdot C_4 \cdot M_{atm} \cdot \Delta m_4^2 \left(1 + 25 \frac{m_5}{m_4}\right)$$

$$= 2.55E22 \, \Delta m_4^2 \left(1 + 25 \left(\frac{m_5}{m_4}\right)\right)$$

$\frac{m_5}{m_4} = .17544$ check these

$$= 2.55E22 (5.386) \Delta m_4^2 = 1.3734 \Delta m_4^2 \; E23$$

Isot $0.65E18$ methane

$m_5 = 1.0E-4$

$m_4 = 5.7E-4$

CO_2 Eg $\Delta m = .5\%$

$\Delta E = 3.493 E18$ Joules

So you do seem to be on to right track but you do have a discrepancy.

For $\Delta M_4 = 0.5\%$ (= .005 decimal)

I calculate $\Delta E = 3.433 E18$
but code gives
$\Delta E = .65 E18$
Factor of error = 5.28 why?

One thing we see is that $\%\Delta CO_2 = \Delta M_4 \cdot 100$
or that
$\Delta M_4 = \%\Delta CO_2 / 100$

So try again.

$$\Delta E = C_4 \Delta M_4 \cdot M_{atm} \cdot 1E3 \cdot .05 \Delta CO_2^{\%} \left(1 + 25\left(\frac{m_5}{m_4}\right)\right)$$

recall that $\%\Delta CO_2 = \Delta M_4 \cdot 100$

so

$$\Delta E = C_4 \Delta M_4 \cdot M_{atm} \cdot 1E3 \cdot .05 \Delta M_4 \cdot 100 \left(1 + 25\left(\frac{m_5}{m_4}\right)\right)$$

$$\Delta E = C_4 \Delta M_4^2 \cdot M_{atm} \cdot 1E5 \cdot .05 \left(1 + 25\left(\frac{m_5}{m_4}\right)\right)$$

$$\Delta E = C_4 \Delta M_4^2 \cdot M_{atm} \cdot 5E3 \left(1 + 25\left(\frac{m_5}{m_4}\right)\right)$$

$$\Delta E = (1.02)(5.1E18)(5E3)\Delta M_4^2 \left(1 + 25\left(\frac{m_5}{m_4}\right)\right)$$

$$\Delta E = 2.60E22 \Delta M_4^2 \left(1 + 25(.17544)\right)$$

$$\Delta E = 1.4E23 \Delta M_4^2 \quad \Rightarrow \Delta E = 3.5E18$$

$\Delta M_4 = .005 = 0.5\%$ vs $0.65 E18$

So we do have a difference. Lets learn why.
Break into parts
$C_4 \cdot M_{atm} \cdot$

$\Delta C_p = .0057$ Check.

OK $\Delta C_p = C_4 \Delta M_4 = 1.02(.005) = .0051$

atm $\Delta C = \cancel{2.6}\ 26.01\ EIB = 2.601 E19$

$\Delta C = (.0051)\ 5.1 E18 \left(\frac{1}{1000}\right) =$

~~Step of this term~~

$\Delta E = \left(C_4 \Delta M_4 \cdot M_{atm} \cdot 1E3 \cdot \frac{.05}{276}\right) \cdot \Delta M_4 \cdot 100 \left(1 + 25 \left(\frac{MS}{M_4}\right)\right.$

$.0051 ? .0051 \cdot 5 \cdot 1 E18 \cdot 1000$

✓OK $= 2.601 E19$ OK ✓

This takes care of $C_4 \Delta M_4 \cdot M_{atm} \cdot 1E3$

$.05 \cdot \frac{\text{slider}}{100}$ should be $\Delta M_4 = .05(.005) = .00025$

We get .025 this is $.05 \cdot \Delta M_4 \cdot 100$

$.025(2.601 E19) = 6.52 E17 = .\cancel{62}\ .65 E18$

This is a match.

So the problem is the term $1 + 25 \frac{MS}{M_4}$

There could be no such term when methane = 0.

Why are you including it?

You know you learn fastest when incorrectly

Page 254

We have
$\Delta T_{CO_2} = .05\,\Delta CO_2 = .05\,\Delta M_4 \cdot 100$

You have an error in your paper here. and we fixed that.

Now
$\Delta CH_4 = 25 \cdot .05\,\Delta CO_2 \cdot \frac{M_5}{M_4}$ Notice this gives a contribution to methane when there are no methane changes in units. This is wrong.

So
~~$\Delta T = .05$~~
You have an error. It requires a conditional statement.
if $CO_2 > 0$ ~~then~~ and $CH_4 = \emptyset$
$\Delta T CO_2 = .05\,CO_2$

~~if $CH_4 > 0$ then~~ if $CO_2 > \emptyset$ and $CH_4 > \emptyset$ then
$\Delta T CH_4 = 25(.05)\,\Delta CO_2 \cdot \frac{M_5}{M_4}$

What if $CO_2 + CH_4 = \emptyset$?
$CO_2 = \emptyset$ + $CH_4 > \emptyset$? This is a tricky one.

Dec 01 2014.

Let's remove CO_2-CH_4 dependency

$\Delta T_{CO_2} = 0.05\ \%CO_2$

$\Delta T_{CH_4} = 25\left(\frac{m_5/m_4}{m_4}\right) 0.05\ \%CO_2$

%

$$\text{Methane Impact} = 25(0.05)\left(\frac{m_5}{m_4}\right) \%CO_2 \text{ impact}$$

$$CO_2 \text{ Impact} = \frac{\text{Methane Impact}}{25(.05)\left(\frac{m_5}{m_4}\right)}$$

So this goes both ways

Notice one of them will be zero.

Now in terms of %: $\%\Delta CO_2 = \Delta m_4 \cdot 100$

$\Delta m_4 = \%CO_2 / 100$

$\Delta m_5 = \%CH_4 / 100$

$$\Delta T_{CO_2} = 25\left(\frac{m_5}{m_4}\right)(0.05)\,\Delta m_4 \cdot 100$$

slider-CH_4 slider-CO_2

$$\Delta T_{CH_4} = \frac{\Delta m_5 \cdot 100}{25(.05)\left(\frac{m_5}{m_4}\right)}$$

Then add

Check again:

$\Delta T_{CO_2} = .05\,\%\,\Delta CO_2 = .05\,\Delta m_4 \cdot 100$

$\Delta T_{CH_4} = .05\,(25)\left(\frac{m_5}{m_4}\right)\,\%\,\Delta CO_2$

$\Delta T_{CH_4} = .05\,(25)\left(\frac{m_5}{m_4}\right)\Delta m_4 \cdot 100$

~~$\%\,\Delta CO_2 =$~~

$\Delta T_{CO_2} = .05\,(\%\,\Delta CO_2)$

$\Delta T_{CH_4} = (.05)(25)\left(\frac{m_5}{m_4}\right)(\%\,\Delta CO_2)$

$\frac{\Delta T_{CO_2}}{\Delta CH_4} = \frac{1}{25\left(\frac{m_5}{m_4}\right)} = \text{a number} = \frac{1}{25\left(\frac{1.0E-4}{5.7E-4}\right)} = .228$

So $\Delta T_{CO_2} = \underline{\Delta CH_4}$

$\therefore \Delta T_{CO_2} = .228\,\Delta T_{CH_4}$

$\Delta T_{CH_4} = 4.39\,\Delta T_{CO_2}$

Therefore

$\Delta T_{methane} = \frac{.05}{.228}\,\Delta CH_4\,\% = \frac{.05}{.228}\Delta m_5 \cdot 100$

$\Delta T_{CO_2} = .05\,\%\,\Delta CO_2\,\% = .05\,\Delta m_4 \cdot 100$

The reset button is not working correctly.
OK, the temperature looks good
w/out a reset.
Now test it

CO_2 ~~CH_4~~ $+ \emptyset.20\%$ $\Rightarrow$ 0.11%
$- \emptyset.20\%$ $\Rightarrow$ $+1.06\%$?

Without Reset

$CO_2 + .20\%$ $\Delta T = .01$
$\Delta heat = +\emptyset.11\%$

$-.20\%$ $\Delta T = -.01\%$
$\Delta heat = +\emptyset.11\%$???

CH_4 $+.20\%$ $\Delta T = .045^\circ$ (OK)
$\Delta heat$ $+1.06\%$

$-.20\%$ $\Delta T = -.045$ (OK)
$\Delta heat = +1.06\%$ (???

OK, you have the math model much better & more elegant.
It allows for both $\emptyset$ Co_2 & CH_4 now
You also found a sign error in the data & corrected.

The next button is not working correctly.
OK, the temperature looks good
w/out a reset.
Now test it

CO_2 ~~CH4~~ + Ø.20% ⇒ 0.11%
− Ø.20% ⇒ +1.06% ?
without Reset

CO_2 +.20% ΔT = .01
Δheat = +Ø.11%

−.20% ΔT = −.01%
Δheat = +Ø.11% ???

CH_4 +.20% ΔT = .045° (OK)
Δheat +1.06%

−.20% ΔT = −.045 (OK)
Δheat = +1.06% (???)

OK, you have the math model much better & more elegant.
It allows for both Ø CO_2 & CH_4 now
You also found a sign error in the theory & code.

Your next problem is the graphing. Something screwy @ high values, as already

my	y'
80	20
85 ~~80~~	42 (this should be less)
80	21
81	25 NO
82	
83	70
84	
85	
79 %	16
77	8
78	12
79	16
76	4
75	0

	y'	100 − y
74	4	96
75	0	100
76	4 (should be −4)	

The problem occurs @ 1.5%
it should be adding it

Model is looking very good. Problem w/ Aerosols
$CH_4 = +.25\%$
High Clouds +50y n = 160 .15 near glo pt *

	% heating	y Canvas	300 − y
0	.166	0	100
25	.82 %	68	232
50	+.01	149	151
75	.84 (should be −.84)		

difficulty breathing — Upper & Lower
deep in
shallow breathing
deep breathing
absence of breathing

Emphysema
bronchitis, Chronic
Asthma

Bad breath

1. mucus sputum
2. persistent
3. eating
4. Chest pain

Something is happening w/ aerosols when
m_y is < 0.
Aerosol contribution is < 0 as it should be
but m_y stays > 0.

When the heat capacity flips negative
it stabilizes at a heating

So heat capacity is negative from lots of
cloud cooling.

The ΔT from methane is positive

(Abs) Heat capacity $\cdot \Delta T$ is positive
leads to a positive which is incorrect

Ok, I found the problem. What do I want to do about it?

1. Clouds low → heat capacity eventually of h2os negative
2. High methane w/ no aerosol dependence means that ΔT stays positive.
3. Using abs(heat capacity) to positive ΔT leads to a false positive.

We could flip to a variance point of view?

If you You have the ΔT totally dependent upon CO_2 & methane.

We should be able to get an estimate of ΔT from aerosols alone.

OK, the model is looking better.

Now assume you put volcanic aer in by itself. What happens? Aerosols by themselves w/ no gases You have it back this up.

Next we will go after solar heating of aerosols w/ no gas Contribution.
This will give ΔT

$\Delta C_{pmix} = C_i^* \Delta m_f$ * for aerosols = effective C_p.

$\Delta C = \Delta C_{pmix} \cdot kg$

$\Delta E = \Delta C \cdot \Delta T$ ← use ΔT from Solar Energy in Joules.

Look at long wave greenhouse gases also. $\Delta E = C_i^* \Delta m_f \cdot kg_{atm} \cdot \Delta T$ → Solar

Two approaches to aerosol influence
1. Change in C_{pmix} relative to air (numbers look small e.g. .01% etc
2. Solar radiation.

The product of these & mass of atm could be a validity estimate.

Col @ aerosol contribution · Matm · 1000 · Solar Energy in Joules

$\frac{kJ}{kg \cdot C^\circ} \cdot kg \cdot C^\circ = kJ * 1000 = \underline{\text{Joules}}$

Just use the average annual atmospheric budget.

Dec 08 2014

[redacted]

Lets go after default aerosol contribution:

$\Delta C_{pmax} = C_e^* \cdot \Delta M_{faerosol}$

this is the aerosol contribution term
it is also delta specific heat of air
we also have the heat capacity from it.
Just multiply it by annual heat change
We have the heat capacity of aerosol influence ok.
Now what.

$\Delta C = \frac{\text{Joules}}{C^\circ}$ we need it by C° to get Joules

Now we do have the annual earth heating in Joules.

$\frac{\text{Joules}}{C^\circ} = \frac{\text{Joules}}{C^\circ} \cdot \frac{1}{\text{Joules}} = \frac{1}{C^\circ}$ invert?

$\frac{\text{Joules}}{1} = \frac{C^\circ}{1}$

If the earth's atmosphere absorbs so many Joules per year, how much did the temperature rise?

$\Delta E = \Delta C \cdot \Delta T$ so $\Delta T = \frac{\Delta E}{\Delta C}$ we have this / we have this

for ΔC we get $\approx$ 5E18 J. to .76 E18 J of atmosphere per 1 degree C

Annual Earth heating $\approx$ 4.87 E21 J
so we expect a ratio of .004

The situation is that

ΔT_{annual} is now determined from CO_2 & methane alone.

so aerosols have no temperature reference to work with.

But we do have the means to determine a heat capacity change from the aerosols alone. Then look to units of J/C° for the atmosphere. You also have an annual heating OF THE ENTIRE EARTH

Right now you are forming a rate that you cannot quickly find out seem to be working? & without the 2nd atmosphere vs earth adjustments. Why?

$\frac{J}{C°} = \frac{J}{C} \cdot \frac{1}{J} = \frac{1}{C°}$ $\frac{20E18\ J/C°}{4.87E21\ J}$

$\frac{J}{1} =$

Think about it. Something takes 10 Joules per 1°C. $= \Delta C$

So if you put 10 Joules into it, it raises one degree C°.

$\frac{10\ J/C}{10\ J} = 1°C$ OK!! So it works but I am not sure why on units

What if you only put in 5 Joules?

$\frac{10}{5} = 2°$ but indeed it is only 1/2°

So indeed it is $\frac{1}{C°}$ units.

So we have: $\Delta C = n 20E18 J$

We have (atm)

$\frac{KJ}{Kg \cdot C} \cdot Kg \cdot 1000 = \frac{Joules}{C^\circ}$ the # of atmosphere mg.

Indeed this is interesting. Our aerosol contribution may be way too high.

Why do we always get 100^{ppm}?

$n: \frac{20E18 \; J/C^\circ}{(.02) \times 4.87E21} = \Delta .2 = 5^\circ$ This is huge.

The only one no. is so large.

$= \frac{20E18 \; J/C^\circ K}{9.74E20 \; J}$ ← the one very large

You would need this no. very small

Something is not making sense here.

Your aerosol contribution is heat capacity change, not heat capacity.

Page 266

What does it mean to form a term.

(Jules per C°) Heat Capacity Change of the atmosphere due to Aerosols

annual
Actual Heat energy of the entire earth in Jules

or

actual annual heat energy of the atmosphere in Jules.

Is this nonsensical?
This would be like Miles per hour / Miles

This would be like someone has taken a trip of 100 miles but their speed is now changing like 0.2 mph
How do you interpret that? How do you translate that. How do you translate that to miles?

---

diagnosed

IRB. 1. Anonymous
2. Conduct
3.

Known to Know

What, how long
Severity
Treated
Results

---

additional

Do you think that... If so,

Scaling of aerosol impact on
Consequences of greenhouse gas $\Delta \pm 1.5^{\circ}$

With respect to aerosols, we seem to have a physical solution, but we have no idea why it works.

$$\Delta C_{atm} = \Delta C_{p\,mix} \cdot M_{atm} \cdot 1E3 = \frac{J}{C^{\circ}}$$

Why, when we scale this by the annual heating of the entire earth, does it perform so well at the sfc level? Makes no sense to me.

If we assumed there was a 1° change in temperature of the atm then,

$$\Delta C_{atm} = \Delta C_{p\,mix} \cdot M_{atm} \cdot 1E3 \cdot (1^{\circ}C) = J$$

and then it make sense to have the ratio:

$$\frac{\text{Heat Change of } 1^{\circ}C \text{ for the aerosol atmosphere}}{\text{Annual heating of the earth}} \quad \frac{\text{Joules}}{\text{Joules}}$$

is at least a more relative term. But what makes you think you know that it is a 1 degree change.

This is sort of saying the temperature we change here or not so important. 1°, ½ deg, .2° deg. Given same order of magnitude.

$$\text{Example } \frac{\Delta E_{Atm}}{\Delta E_{earth}} = \frac{5E18}{4.87E24} = .10^{\%} \qquad \frac{76E18}{4.87E24} = 16^{\%}$$

Then in exactly in our range.

So we have eventually found a scaly of aerosol energy change that is commensurate w greenhouse gas change. I don't know if there was a lucky break or not.

Page 267

The next factor you would like to add

1. When Concentration of aerosols gets thick enough, it blocks sunlight & the annual earth temp declines

2. The more method declines, the less influence it has.

3. Changing a picture depending upon season..

Hot Earth
Cool Earth
Neutral Earth..

There is a problem w/ sign of temperature change

Aerosols by themselves are OK
Greenhouse by itself is OK

but greenhouse w/ aerosols is messed up w/ aerosols going in wrong direction

When my γ is
When you have greenhouse gases and add aerosols. then my γ always stays positive

The aerosols are backwards??

Low Clouds causes el-problem reversed

Dec 09 2014

1. You still have a problem w/ image centralizing on the canvas.

2. You have a problem w/ the alignment.

!! Conditional expressions cannot have an assignment at the end of them!

There is a problem w/ loading the first image. I do not know why. The other faces were superior.

[redacted]

Ok, we have an initial loading problem. I do not know why.

Next we have a problem w/ aerosols.

$42^{?}$ CO_2
$\sim 10^{?}$ CH_4

Now introduce Ba. It cools it.?
High Clouds cool it ?
Thick Clouds also cool it. They should!
Carbon black cools ?

With CO_2 alone C 40°
~~High clouds heat~~
Ba Heats
~~High Clouds heat until~~ ?
High Clouds cool until 13°, then they heat. ???
Al cools to 40°, then it starts heating ??
Ba & high clouds heat all the time
Carbon black heats all the time
Volcanic ash heats all the time
Magnesium cools it slightly forever
Strontium heats it forever
H_2SO_4 heats continuously

Observations: w/ CO_2 alone, Thick Clouds & Al_2O_3
each local minimums ?

Test case is with CO_2 alone @ 0.40%

	Cp	Albedo
High Clouds	4.19	0.75
Aluminum Oxide	0.78	.45 ~~.29~~

1.84 −.06 .29

$Cp^* = C_1 \cdot albedo + C_2 \Delta Cp_{air} + C_3$

		Cp	~~dt albedo~~ Δair	albedo	Fx
	0 Carbon	.71	−.295	.20	.67 ~~.58~~
Cools	1 Al_2O_3	.78	−.225	.45	1.13
	2 H_2SO_4	.85	−.155	.30	.85
Cools	3 Thick Low Clouds	4.19	3.185	.75	1.45
	4 Barium	0.29	−.715	.30	.88
	5 Thin High Clouds	2.03	1.025	.37	.91
	6 Volcanic Ash	0.84	−.165	.30	.85
	7 Magnesium	1.05	.045	.40	1.02
	8 Strontium	0.30	−.705	.30	.88

A question to use ΔCp for methane and CO_2?

This makes great sense

The is very revealing.

If $Cp^* <$ air @ 1.005 it heats more to no end.

If $Cp^* \cong$ Air @ 1.005 it has less effect

If $Cp^* >$ Air it Cools the air but it reaches a minimum and then goes the other way. Why ???

Page 273

Carbon black is ind directional

Now methane seems to be one way only but you know that this is not true.

The mass fraction is changing.

	C_p	ΔC_p
CO_2	1.02	
methane	2.22	
Air	1.005	

What if you were to look @ ΔC_p vs C_p?

$C_p = C_i M_i$ what if you allow C_p to change?

$\Delta C_p = C_i \cdot \Delta M_i + M_i \Delta C_i$ take a look at this.

or $(\Delta C_p) \cdot \Delta M_i$ What does this term actually mean?

this no. is a constant / ~~if negative~~ → this goes negative and positive

a constant term which expresses the relative difference from air.

f(x) So you have $\Delta C_{p_1} \Delta M_1 + \Delta C_{p_2} \Delta M_2 \quad < 0, > 0$

$0 = \Delta C_{p_1} \Delta M_1 + \Delta C_{p_2} \Delta M_2$

$-\Delta C_{p_1} \Delta M_1 = \Delta C_{p_2} \Delta M_2$ This is the relationship

$\frac{-\Delta C_{p_1}}{\Delta C_{p_2}} = \frac{\Delta M_2}{\Delta M_1}$

Page 274

$-\frac{\Delta C_{p1}}{\Delta C_{p2}} = \frac{\Delta m_2}{\Delta m_1}$

C_p CO_2 1.02
C_p CH_4 2.22
C_p Air 1.005

$C_{p1} = CO_2$ $\Delta C_{p1} = +.015$

$C_{p2} = CH_4$ $\Delta C_{p2} = +1.215$

$-\frac{\Delta C_1}{\Delta C_{p2}} = -\frac{.015}{1.215} = -.012$

$-\frac{\Delta C_{p2}}{\Delta C_{p1}} = 81$

% $\Delta CO_2 = 4\%$
% $\Delta CH_4 = -.049$

Eg $CO_2 = +4\%$
$CH_4 =$

$\frac{4}{-.049} = -\underline{\underline{81.6}}$

Reject.

This says that there is a maximum cooling influence @ these ratios.

Now the magnitude is affected by GWP and mass fraction sat [illegible]

Page 275

CO_2 6 ppm
CH_4 - .013 ppm — Neutral
High Clouds 30 @ 100. — .65 ppm
Low Clouds 6 @ 100 — Neutral
18 Carbon — 1.32 ppm
48 Al_2O_3 — Neutral

Next problem
Save your values
Load your values.
We are going to try and save a value.

I have local Storage working!
It lasts as long as the browser stays open.

LocalStorage is fantastic!

Now I have graphics & data Storage capability. These are huge advances

Use global variables if you need to call the function from within html!

Anytime that you:
1. Load values
2. Run the program (No value)
3. Back up browser

the values are transfered to the sliders

The cache is a problem.

It must be cleared when you retain history

Run it locally first.
Then test a duplicate on server.

I have it!!!
Am loading stored values!!!

Great work.

Local Minimum Explanation

We have $-\frac{\Delta Cp_1}{\Delta Cp_2} = \frac{\Delta m_2}{\Delta m_1}$

Then express the balance pt of cooling:

$$\Delta m_1^{CO_2} = \frac{\Delta Cp_2^{CH_4}\ \Delta m_2^{CH_4}}{-\Delta Cp_1\ CO_2}$$

It would be of interest to see this function

We need to look at this ratio.
Try a calculator first.
Assume CO_2 & methane

1.02 2.22

$\Delta Cp = .015 \quad 1.215$ so $\frac{\Delta Cp_2}{\Delta Cp_1} = 81.0$

so $\Delta m_1^{CO_2} = (81.0)\,\Delta m_2^{CH_4}$ or $81.0 = -\frac{\Delta m_1}{\Delta m_2}$

So when the mass ratio $= -1 \cdot Cp$ ratios the heating - cooling effects are balanced.

$$\frac{\Delta Cp_1}{\Delta Cp_2} = \frac{(Cp_1 - a)}{(Cp_2 - a)}$$

eg Aluminum & CO_2 $CO_2 = 1.02$ ΔCp +.015

Al = .78 −.225

Ratio $= (-1) \cdot \frac{-.225}{.015} = (15)$

4000 $\frac{\mu gms\ Al_2O_3}{m3}$ ⇔ 5% increase in CO_2

[redacted]

Carbon Black + Water: Use c_p^* !!!!

	.67	1.45
Δc_p	−.335	−.445

Ratio = 1.33

Carbon Black SD@300 = 15000 dynes/in3 = +,29"
Water | 91@300 = 9300 " "

$\frac{15k}{1.33}$ = 11278 Close but not exact.

Any other variables? Yes, albedo!!!

The closer to the c_p of air the less influence the component has.

This means the water has a greater effect than Al_2O_3

Methane - CO_2 GWP

years	GWP
20	72
100	25
500	7.6

It follows a power rule

$y = 596.7t^{-.6985}$ $r^2 = .999$

for $t=1$ GWP = 596 but this is for unit masses.

Surprising how close I am. So temperature required really.

~~Substitution of the specific heats~~

So the ratio of the mass fractions = the ~~opposite~~ ratio of the ~~specific heats~~ (or modified specific heats) determines the equilibrium point. Inversely relatively. Like -

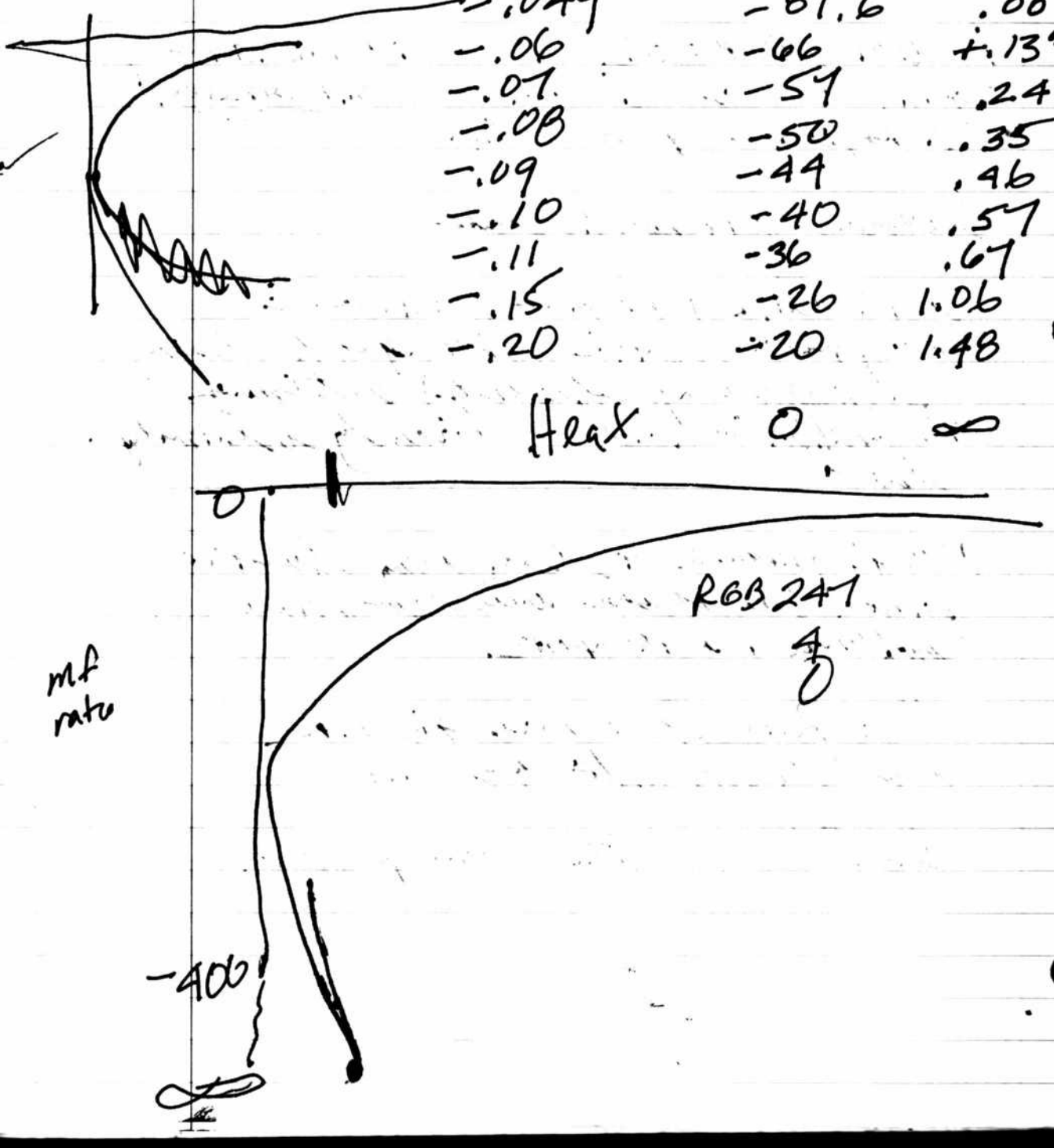
Now the quanta about heating away from the point. One of them will have more influence than the other.

A Ratio of the mass fractions would be very ~~interesting~~ to see.

Lin536 This works perfectly.

Page 280

CO_2	CH_4	mf ratio	Heats
4	0	∞	.63 a.
	-.01	-400	.49 a.
	-.02	-200	.36
	-.03	~~-80~~ -133	.24
	-.04	-100	.11
	-.049	-81.6	.00
	-.06	-66	+.13 a.
	-.07	-57	.24
	-.08	-50	.35
	-.09	-44	.46
	-.10	-40	.57
	-.11	-36	.67
	-.15	-26	1.06
	-.20	-20	1.48



What would it take to develop a graph of the mass ratios?
It is duplicated in duplicate.
You can only right flow to the cache not a file.

Cp CO_2 + CH_4

propose

$$C_p = (CO_2 - C_{air})\Delta M_1 + (CH_4 - C_{air})\Delta M_2$$

In this case only $CO_2 - C_{air}$ is + + $CH_4 - C_{air}$ is pos
So changes in mass will reflect signs accurately.
But what if something cools?

$(-)(-)$
$= +$

$C_i > C_{air}$ cools.
$C_i < C_{air}$ heats.

(Cool)(+Δm) = Cooling
(Cool)(−Δm) = heating influence } This is valid.

(Heating)(Δm) = Heating
(Heating)(−Δm) = Cooling } valid

Dec 13 2014 Page 283

What if we were to save graph circle plots?

I do not think the global variable is doing anything!

You are having a problem w/ global variables

We did indeed access a global number. Yes.

I have done it!

I have incremented a number to storage. It is indeed tricky!

Saving a Counter to Local Storage:

One time

1. Establish a global variable & initialize it
   eg `var global_number`
   `global_number = 1`

2. Go to a function (eg storeACounter())

3. Initialize a temporary variable
   eg `var temp_global_number`

4. Echo the global variable
5. Local storage the global number + 1
   now read the number right back from local storage
   but place it into the temp variable

6. echo the temp variable

7. Make the global variable the temp variable

8. Store the incremented global variable

When you go back it is reinitializing the program.

Ok, instead of going back

Hitting back is the problem

What instead of going back, can you want to the top of the menu

No, that wont work

I seem to have a problem here w/ go back.
What instead of go back we went
to ~~last values~~?
Save & then load???

You have a class problem.

1. You have succeeded in using Create to date.
   The may or may not help you.

2. You would like to loop on the main program & store values as you go along, but you do not know yet.

So you should work up a simple program template.

1. Open a Window

2. Store a counter
3. Do something with the Create
4. Return to the window with the mouseProcedure

2. Is this what you want?

Inner HTML allows you to change the Content of a selected HTML page.

Page 287

You have the
1. HTML have a button
2. Hit the button, do something w/ that number.
3. return that number & display it w/ an alert box.

Part of your problem might be using a canvas
It draws on a page.

CO_2 Methane Time Ratio Adjusted to ΔC_p Determination

We have determined a ΔC_p ratio of approximately 81 based upon annual change. This agrees very well with GWP stats.
Since our temperature determination is based upon a 25 to 30 year span of GWP data we bring the ΔC_p in accord by this.
We know that the GWP is a power function.

$$GWP = 596.7^{t^{-.6985}} \qquad r = .999$$

We are interested in the ratio.

For GWP = 81 $\quad t^{-.6985} = .6985$

$$81 = 596.7 t$$

$$t^{-.6985} = 81/596.7 = .13575$$

$$-.6985 \ln(t) = \ln(.13575)$$

$$\ln(t) = \frac{\ln(.13575)}{-.6985} = 2.8406$$

$$t = 17.126 \text{ years.}$$

for $t = 100$ years GWP = ~~29.35~~ 23.92

17.13 years 81
20 years 72
100 years 25
500 years 7.6

Our GWP ratio is $\frac{81}{23.92} = 3.39$

So we would like to decrease ~~increase~~ the impact by a factor of 3.4 We currently have $+0.2$ to -0.2

We would therefore increase the allow combustion by 3.4

$= +0.2(3.4) = 0.68$
$-0.2(3.4) = 0.68$

So we can go from
a.
$+1.0$ to -1.0 ~~%~~.

~~but we also increase the~~
So we have

$$\Delta C_{p_{max}} = \Delta C_4 \Delta M_4 + \frac{1}{3.04} \Delta C_5 \Delta M_5$$

This adjustment
to a 100 year time span GWP in theory
vs. 17 years GWP

It is
indeed entirely 25 times
now. So this is scaled on a 10 year
period now.

444.100
KTMT
//

Page 291

$\frac{2.2-1.005}{1.02-1.005} \approx 80$ $\frac{80}{3.4} = 23.[illegible]$

We are seeing a curious behavior. Double Max/Min

Start $CO_2 = 5\%$ $CH_4 = 0\%$ $\Delta H = 0.98\%$

CO_2	CH_4	mf Ratio (CO_2/CH_4)	
5%	0	∞	0.98%
+5	-.02	-250	.07%
	-.04	-125	.76%
	-.06	-83	.66
	-.08	-62.5	.56
	-.1	-50	.47
	-.12	-42	.37
	-.14	-36	.28
	-.16	-31	.20
	-.18	-28	.12
	-.20	-25	.04
	-.21	-23.8	Note → .0%
	-.25	-20	.14
	-.30	-17	.31%
	-.35	-14	.45%
	-.40	-12.5	.56%
	-.45	-11.1	.66%
-.52 .76%	-.5	-10	.74%
-.55 .79%	-.6	-8	Note! .83%
max -.58 .81	-.7	-7	.83%
-.65 .84%	-.8	-6	.75%
	-.9	-6	.58%
	-1.0	-5	.32%
	-1.05	-5	.17

This is incredible. Introducing the constant temperature model has disclosed additional & more complex behavior.

wrong!

This was wrong.

Seems to be heading toward another minimum....

	CH_4	Ratio	
Min	-1.096	-5	(Note!!) 0%
	-1.115	-4	-.22%
	-1.2	-4	-.44%
	-1.3	-4	-.95%
	-1.4	-4	-1.54
	-1.5	-3	-2.22

What is our function here?

$C_{p_1} \Delta C_{p_1} = \Delta C_1 \Delta m_{f_1}$

$C_{p_2} \Delta C_{p_2} = \frac{\Delta C_2 \Delta m_{f_2}}{3.4}$

When they offset each other

$-C_{p_1} = C_{p_2}$ or $\frac{\Delta C_1 \Delta m_{f_1}}{\Delta C_1 \Delta} = -\frac{\Delta C_2 \Delta m_{f_2}}{3.4}$

or $3.4 \Delta C_1 \Delta m_{f_1} = -\Delta C_2 \Delta m_{f_2}$

or $\frac{\Delta C_1}{\Delta C_2} = \frac{-\Delta m_{f_2}}{(3.4)\Delta m_{f_1}}$

$\Delta C_1 = 1.02 - 1.005 = .015$ so

$\Delta C_2 = 2.22 - 1.005 = 1.215$

$3.4(.015)\Delta m_{f_1} = -1.215 \Delta m_{f_2}$

or $\frac{\Delta m_{f_1}}{\Delta m_{f_2}} = \frac{-1.215}{(3.4)(.015)} = 23.8235$ and 0

This is first minimum. $\frac{5^{th}}{23.8235} = .20988$

They are also $\emptyset$ when?

$\emptyset.21 = 1486$ MY years

at what other occasion in the future $\emptyset$?

$\emptyset = -C_{p_1} + \Delta C_1 \Delta m_{f_1}$

$\emptyset = -C_{p_2} + \frac{\Delta C_2 \Delta m_{f_2}}{3.4}$ when does this $= \emptyset$?

$\Delta C_1 \Delta m_{f_1} + \frac{\Delta C_2 \Delta m_{f_2}}{3.4} - (C_{p_1} + C_{p_2}) = \emptyset$

or $\Delta C_1 \Delta m_{f_1} + \frac{\Delta C_2 \Delta m_{f_2}}{3.4} = C_{p_1} + C_{p_2}$

~~$C_{p_1} + C_{p_2} = .015 + 1.215 = 1.230$~~

$\Delta C_1 = .015$ $\Delta C_2 = 1.215$

Page 293

$y = 5x$
$y' = 5$

bus ch

$y_1 = a x_1$

$\Delta m_1 = 5$

$\Delta m_2 = -.65$

$y_2 = \frac{b x_2}{d} = b^* x_2$

$.015(.05) = .00075$ .232

$\frac{1.215}{3.4}(-.65) = -.232$

$b^* = \frac{\Delta C_2}{3.4}$

$.015 = \Delta C_1$ CO_2

$\Delta m_2 = CH_4$

If $y_1 = -y_2 = a x_1 = -b x_2$ or $\frac{a}{b^*} = \frac{-x_2}{x_1}$ $\Delta m_1 = CO_2$

23.82 or $\frac{b^*}{a} = \frac{-x_1}{x_2}$ OK

1.215 ΔC_2 CH_4

a 2nd solution

$.015(.05) = .00075$ local max

$\left(\frac{1.215}{3.4}\right)(-.065) = -.00232$ Ratio = 3.09

$\Sigma = -.00154$

$f(x) = 0$

$\Delta C_1 \Delta m_1 + \frac{\Delta C_2 \Delta m_2}{3.4} - C_{p1} - C_{p2} = 0$

$C_{p1} + C_{p2}$ $f(x,y) = \Delta C_1 \Delta m_1 + \frac{\Delta C_2 \Delta m_2}{3.4}$

So this is the first one

$\frac{f'(x,y)}{\partial m_1} = \frac{\partial f_1}{\partial \Delta m_1} + \frac{\partial f_2}{\partial \Delta m_2} = 0$

or $\Delta C_1 + \frac{\Delta C_2}{3.4} = 0$ or $\Delta C_1 = \frac{-\Delta C_2}{3.4}$

$\Delta C_1 = .015$

$\Delta C_2 = \frac{.65}{3.4} = \frac{1.25}{3.4} = .357$ Ratio = 23.82

OK

Page 294

$ax^2+bx+c=\phi$

~~$(C_{p_1} \pm C_{p_2})^2$~~

$C_{p_1}^2 = \Delta C_1^2 \Delta m_{f_1}^2$

$C_{p_2}^2 = \left(\frac{\Delta C_2}{3.4}\right)^2 \Delta m_{f_2}^2$

$.5625E\text{-}6 = (.015)^2(.05)^2 = C_{p_1}^2$

$+5.395E\text{-}6 = \left(\frac{1.215}{3.4}\right)^2 (-.0065)^2 = C_{p_2}^2$

$\frac{C_{p_2}^2}{C_{p_1}^2} \sim +9.6$ ~~$\frac{C_{p_2}}{C_{p_1}} = 3.09$~~ $\frac{C_{p_2}}{C_{p_1}} = 3.10$ No. 1+15~28.

$\Delta m_{f_1}^2 = \frac{C_{p_1}^2}{\Delta C_1^2}$

$\Delta m_{f_2}^2 = \frac{C_{p_2}^2}{\left(\frac{\Delta C_2}{3.4}\right)^2}$

$\frac{\Delta m_{f_1}^2}{\Delta m_{f_2}^2} = \frac{\frac{C_{p_1}^2}{\Delta C_1^2}}{\frac{C_{p_2}^2}{1}} = \frac{\frac{C_{p_1}^2}{\Delta C_1^2}}{\frac{C_{p_2}^2 \cdot 3.4^2}{\Delta C_2^2}}$

$\frac{\Delta C_2^2}{3.4^2}$

Page 295

$$\frac{\Delta m_{f_1}^2}{\Delta m_{f_2}^2} = \frac{C_{p_1}^2}{\Delta C_1^2} \cdot \frac{\Delta C_2^2}{C_{p_2}^2 \cdot 3.4} = \frac{C_{p_1}^2 \cdot \Delta C_2^2}{3.4\, C_{p_2}^2 \cdot \Delta C_1^2}$$

$$\frac{\Delta m_{f_1}}{m_{f_2}} = \frac{[(.015)(.05)]^2 (.0065)^2}{(3.4)(1.215)^2 (.05)^2} = .02377E-9 \quad \text{(.00002E-6)} \quad 106.65E-6$$

Note: .05

$$\frac{\Delta m_{f_1}^2}{\Delta m_{f_2}^2} \cong \frac{.05^2}{.0065^2} = 59.17$$ This looks like a target

$$C_{p_1}^2 = \Delta C_1^2 \Delta m_{f_1}^2 = (.015)^2 (.05)^2 = .5625E-6$$

$$C_{p_2}^2 = \frac{\Delta C_2^2 \Delta m_{f_2}^2}{3.4^2} = \frac{(1.215)^2 (.0065)^2}{(3.4)^2} = 5.395E-6$$

$$\frac{C_{p_1}^2}{C_{p_2}^2} = .104 \qquad \frac{\frac{C_{p_1}^2}{\Delta C_1^2}}{\frac{C_{p_2}^2 \cdot 3.4^2}{\Delta C_2^2}} = \frac{C_{p_1}^2 \Delta C_2^2}{\Delta C_1^2 C_{p_2}^2 3.4^2}$$ This is the acting term!

$$= \frac{[(.015)(.05)]^2 (1.05)^2}{(.015)^2 [(1.215)(.0065)]^2 (3.4)^2} = \frac{.0304E-6}{.1622E-6}$$ MISSED a 2 somewhere

$= .512$ Ratio more reasonable

Ratio of $(3.4)^2$ off