14C Respiration/Production Methods

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Overview

The simultaneous measurement of bacterial respiration and production will give the best results for estimating growth efficiency. Here, we have constructed custom bacterial respiration/production vials (Fig 1). We will use these vials with 14C-resources. However, first we must optimize the methods.

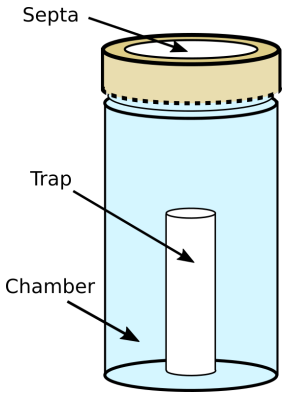

Figure 1: Bacterial Respiration and Production Vial

Optimizations

As we start there are a few things we need to optimize. We are going to start by determining the efficiency of our apparatus. We are doing this using 14C-Bicarbonate. But we need to check a few things first:

1. How much Bicarbonate should we add
2. What is the efficiency of the trap

1) Bicarbonate

I did a simple experiment where I added known volumes of the bicarbonate stock to 1500 μL of scintillation cocktail (1, 5, and 10 μL). Based on this I discovered that the LSC could only measure 1 μL max. The CPM for this voucher was ~2,300,000 cpm.

2) Trap Efficiency

```
# Import Data
trap.data <- read.csv("../notes/20150921_BRBP.csv", header=T)

# Raw Data For Intra Vial Test
```

```
in1  <- trap.data[trap.data$Sample == "Tube1_in" & trap.data$Time == "1", 3]
out1 <- trap.data[trap.data$Sample == "Tube1_out" & trap.data$Time == "1", 3]

# Define Theoretical Value
voucher <- trap.data[trap.data$Sample == "Voucher" & trap.data$Time == "1", 3]

# Correct For Vol
in1.c <- in1 * 5 # used 100ul of 500ul
out1.c <- out1 * 50 # used 100ul of 5000ul

# Percent of Theoretical
in1.p <- in1.c / voucher
out1.p <- out1.c / voucher

# Calculate mean and sem
means <- c("Trap" = mean(in1.p), "Residual" = mean(out1.p))
ses <- c("Trap" = se(in1.p), "Residual" = se(out1.p))

# Plot
png(filename="../figures/Recovery1.png",
    width = 800, height = 800, res = 96*2)

par(mar=c(3,5,0.5,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
bp_plot <- barplot(means, ylab = "Percent Recovery",
                   ylim = c(0, 1), lwd=3, yaxt="n", col="gray",
                   cex.lab=1.5, cex.names = 1.25)
arrows(x0 = bp_plot, y0 = means, y1 = means - ses, angle = 90,
       length=0.1, lwd = 2)
arrows(x0 = bp_plot, y0 = means, y1 = means + ses, angle = 90,
       length=0.1, lwd = 2)
axis(side = 2, labels=T, lwd.ticks=2, las=2, lwd=2)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```

3) Alkaline Solution Experiment

One thing that we need to test is if if matters what alkaline solution we use (NaOH or KOH) and at what concentration. So we are going to do a simple experiment to test this. I filled 21 BP vials with 1500 μL scintillation cocktail. I then added 100 μL of either NaOH or KOH at either 5N, 1N, or 0.1N. I then added 1 μL of 14C-Bicarbonate. I analyzed on the LSC immediately.

```
# Import Data
alk.data <- read.csv("../notes/20150924_AlkalineExp.csv", header=T)
alk.data$Group <- sapply(strsplit(as.character(alk.data$Sample), "OH"), "[[", 1)

pairwise.t.test(alk.data$CPM, alk.data$Group, p.adjust.method="BH")
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
```

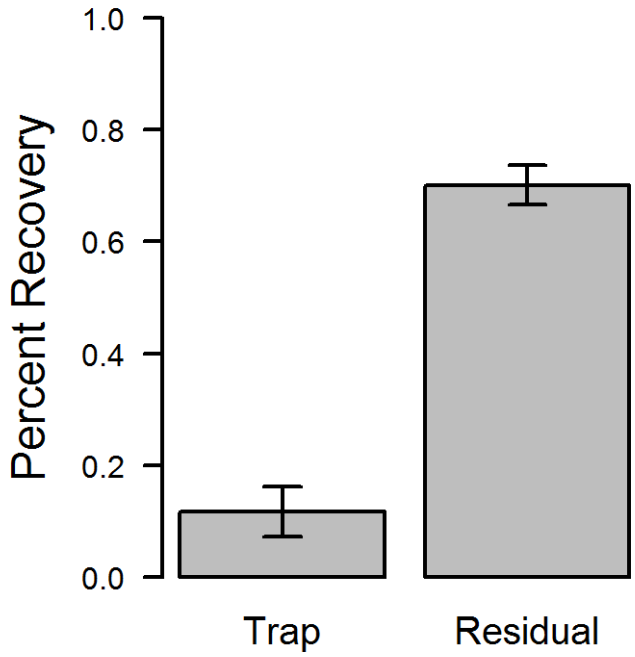

Figure 2: 14C-Bicarbonate Recovery

```
## data:  alk.data$CPM and alk.data$Group
##
##       K     Na
## Na    0.748 -
## Water 0.036 0.036
##
## P value adjustment method: BH
```

```
alk.model <- lm(alk.data$CPM ~ alk.data$Group + alk.data$Sample)

# Group Differences
round(TukeyHSD(aov(alk.model))$'alk.data$Group', 4)
```

```
##                diff        lwr        upr  p adj
## Na-K       28792.78  -23048.94   80634.49 0.3419
## Water-K  -308336.89 -381652.14 -235021.63 0.0000
## Water-Na -337129.67 -410444.92 -263814.41 0.0000
```

```
# Make Dateframe for Plotting
alk.data.plot <- as.data.frame(matrix(NA, length(levels(alk.data$Sample)), 5))
colnames(alk.data.plot) <- c("Sample", "Group", "Normality", "mean", "sem")
alk.data.plot$Sample <- rev(levels(alk.data$Sample))
alk.data.plot$Group <- sapply(strsplit(alk.data.plot$Sample, "OH"), "[[", 1)
alk.data.plot$Group[2:7] <- paste(alk.data.plot$Group[2:7], "OH", sep="")
alk.data.plot$Normality[2:7] <- sapply(strsplit(alk.data.plot$Sample[2:7], "OH"), "[[", 2)
```

```
for (i in alk.data.plot$Sample){
  alk.data.plot$mean[alk.data.plot$Sample == i] <- mean(alk.data$CPM[alk.data$Sample == i])
}

for (i in alk.data.plot$Sample){
  alk.data.plot$sem[alk.data.plot$Sample == i] <- se(alk.data$CPM[alk.data$Sample == i])
}

# Plot
png(filename="../figures/Alkalinity.png",
    width = 1600, height = 1200, res = 96*2)

par(mar=c(4,5,0.5,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
bp_plot <- barplot(alk.data.plot$mean / 1000000, ylab = "Counts Per Minute (millions)",
                   names.arg = alk.data.plot$Normality,
                   space = c(0, 0.5, 0, 0, 0.5, 0, 0),
                   lwd=3, yaxt="n", col="gray", ylim = c(0, 2.5),
                   cex.lab=1.5, cex.names = 1.25)
arrows(x0 = bp_plot, y0 = alk.data.plot$mean / 1000000,
       y1 = alk.data.plot$mean / 1000000 - alk.data.plot$sem / 1000000, angle = 90,
       length=0.1, lwd = 2)
arrows(x0 = bp_plot, y0 = alk.data.plot$mean / 1000000,
       y1 = alk.data.plot$mean / 1000000 + alk.data.plot$sem / 1000000, angle = 90,
       length=0.1, lwd = 2)
axis(side = 2, labels=T, lwd.ticks=2, las=2, lwd=2)
mtext(alk.data.plot$Group[c(1,3,6)], side = 1, at=bp_plot[c(1,3,6)], line = 2.5, cex=1.5)

dev.off() # this writes plot to folder
```

```
## pdf
##   2
```

```
graphics.off() # shuts down open devices
```

Trap, Time and Solution Experiment

I think I finally figured things out. The key is the use just 1N KOH and to let it stand a while.

```
# Import Data
trap.data <- read.csv("../notes/20151002_BRBP.csv", header=T)

# Raw Data For Intra Vial Test
ina  <- trap.data[grep("_in", trap.data$Sample), 2]
outa <- trap.data[grep("_out", trap.data$Sample), 2]
in1  <- trap.data[trap.data$Sample == "Tube1_in", 2]
out1 <- trap.data[trap.data$Sample == "Tube1_out", 2]
in5  <- trap.data[trap.data$Sample == "Tube5_in", 2]
out5 <- trap.data[trap.data$Sample == "Tube5_out", 2]

# Define Theoretical Value
voucher <- mean(trap.data[trap.data$Sample == "Voucher", 2])
```

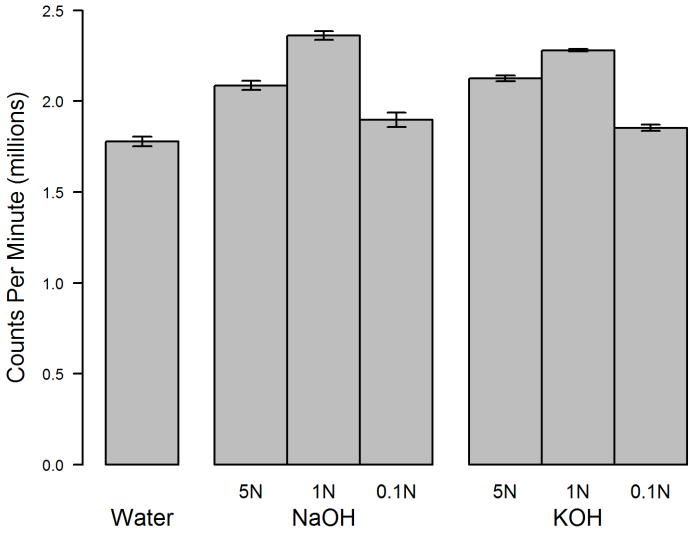

Figure 3: 14C-Bicarbonate Recovery

```
# Correct For Vol
in.c   <- ina  * 5  # used 100ul of 500ul
out.c  <- outa * 50 # used 100ul of 5000ul
in1.c  <- in1  * 5  # used 100ul of 500ul
out1.c <- out1 * 50 # used 100ul of 5000ul
in5.c  <- in5  * 5  # used 100ul of 500ul
out5.c <- out5 * 50 # used 100ul of 5000ul

# Percent of Theoretical
in.p   <- in.c   / voucher
out.p  <- out.c  / voucher
in1.p  <- in1.c  / voucher
out1.p <- out1.c / voucher
in5.p  <- in5.c  / voucher
out5.p <- out5.c / voucher

# Calculate mean and sem
means <- round(c("Trap" = mean(in.p), "Res." = mean(out.p) ,
                 "Trap" = mean(in1.p), "Res." = mean(out1.p),
                 "Trap" = mean(in5.p), "Res." = mean(out5.p)), 3)
ses <- round(c("Trap" = se(in.p), "Residual" = se(out.p) ,
               "Trap" = se(in1.p), "Res." = se(out1.p),
               "Trap" = se(in5.p), "Res." = se(out5.p)), 3)

# Plot
```

```
png(filename="../figures/Recovery2.png",
    width = 1600, height = 1200, res = 96*2)

par(mar=c(4.5,5,0.5,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
bp_plot <- barplot(means, ylab = "Percent Recovery",
                   ylim = c(0, 1), xlim = c(1,9), lwd=3, yaxt="n", col="gray",
                   cex.lab=1.5, cex.names = 1.25,
                   space = c(1, 0, 1, 0, 1, 0))
arrows(x0 = bp_plot, y0 = means, y1 = means - ses, angle = 90,
       length=0.1, lwd = 2)
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means - ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means - ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means - ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
arrows(x0 = bp_plot, y0 = means, y1 = means + ses, angle = 90,
       length=0.1, lwd = 2)
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means + ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means + ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
## Warning in arrows(x0 = bp_plot, y0 = means, y1 = means + ses, angle = 90, :
## zero-length arrow is of indeterminate angle and so skipped
```

```
axis(side = 2, labels=T, lwd.ticks=2, las=2, lwd=2)
mtext(c("Overall", "Tube #1", "Tube #5"), side = 1, at=bp_plot[c(1, 3, 5)],
      line = 2.5, cex=1.5, adj=0)

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```

Bicarbonate Concentration Experiment

So far we have shown that we have approximately 90% efficiency with the BP/BR vials However, we do not yet know if efficiency could be a function of CO2 respired. So, we are going to test the set-up with different starting concentrations of bicarbonate

```
# Import Data
trap.data <- read.csv("../notes/20151007_BRBP.csv", header=T)

# Raw Data For Intra Vial Test
```

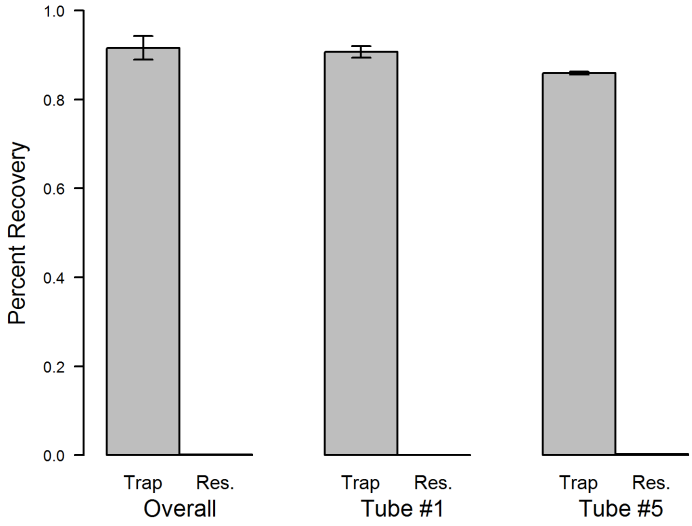

Figure 4: 14C-Bicarbonate Recovery

```
vouchers  <- trap.data[grep("Voucher", trap.data$Sample), ]
dil.test <- summary(lm(vouchers$CPM ~ vouchers$Conc))
dil.test$r.squared

Tube1.T <- mean(vouchers[vouchers$Conc == 1, 2])
Tube2.T <- mean(vouchers[vouchers$Conc == 0.1, 2])
Tube3.T <- mean(vouchers[vouchers$Conc == 0.01, 2])
Tube4.T <- mean(vouchers[vouchers$Conc == 0.001, 2])
Tube5.T <- mean(vouchers[vouchers$Conc == 0, 2])

Tube1 <- trap.data[trap.data$Sample == "Tube1", 2]
Tube2 <- trap.data[trap.data$Sample == "Tube2", 2]
Tube3 <- trap.data[trap.data$Sample == "Tube3", 2]
Tube4 <- trap.data[trap.data$Sample == "Tube4", 2]
Tube5 <- trap.data[trap.data$Sample == "Tube5", 2]

# Correct For Vol (used 100ul of 500ul)
Tube1.c <- Tube1 * 5
Tube2.c <- Tube2 * 5
Tube3.c <- Tube3 * 5
Tube4.c <- Tube4 * 5
Tube5.c <- Tube5 * 5

# Percent of Theoretical
Tube1.p   <- Tube1.c / Tube1.T
Tube2.p   <- Tube2.c / Tube2.T
```

```
Tube3.p   <- Tube3.c / Tube3.T
Tube4.p   <- Tube4.c / Tube4.T
Tube5.p   <- Tube5.c / Tube5.T

obs <- c(Tube1.c, Tube2.c, Tube3.c, Tube4.c, Tube5.c)
exp <- rep(c(Tube1.T, Tube2.T, Tube3.T, Tube4.T, Tube5.T), each=3)

obs.log <- log10(obs)
exp.log <- log10(exp)

model <- lm(obs.log ~ exp.log)

# Plot
png(filename="../figures/Recovery3.png",
    width = 1200, height = 1200, res = 96*2)

par(mar=c(5,5,0.5,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
plot <- plot(obs.log ~ exp.log, cex = 2, las = 1, cex.lab=1.5, pch = 22, type='n',
             xlab = expression(paste("Expected Recovery (log"[10], " CPM)")),
             ylab = expression(paste("Observed Recovery (log"[10], " CPM)")),
             lwd=3, col="black", bg = "gray", xlim = c(2, 7), ylim = c(2, 7))

axis(side = 1, labels=F, lwd.ticks=2, las=2, lwd=2)
axis(side = 2, labels=F, lwd.ticks=2, las=2, lwd=2)
axis(side = 1, labels=F, lwd.ticks=2, tck=0.01, las=2, lwd=2)
axis(side = 2, labels=F, lwd.ticks=2, tck=0.01, las=2, lwd=2)
axis(side = 3, labels=F, lwd.ticks=2, tck=0.01, las=2, lwd=2)
axis(side = 4, labels=F, lwd.ticks=2, tck=0.01, las=2, lwd=2)

abline(a = 0, b = 1, lty=3, col="black", lwd=2)
abline(model, lty = 2, col = "gray20", lwd=3)

points(jitter(obs.log, amount = 0.1) ~ jitter(exp.log, amount=0.1), cex = 2, pch = 22, lwd=3, col="blac

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```

Succinate Test Experiment

```
# Import Data
succ.data <- read.csv("../notes/20151105_BRBP.csv", header=T)

# Raw Data For Intra Vial Test
vouchers  <- succ.data[grep("Voucher", succ.data$Sample), ]

SetA.T <- mean(vouchers[vouchers$Conc == 10, 2])
SetB.T <- mean(vouchers[vouchers$Conc == 0.5, 2])

SetA_supp <- succ.data[succ.data$Sample == "Tube1" |
                         succ.data$Sample == "Tube2", 2] * 50
```

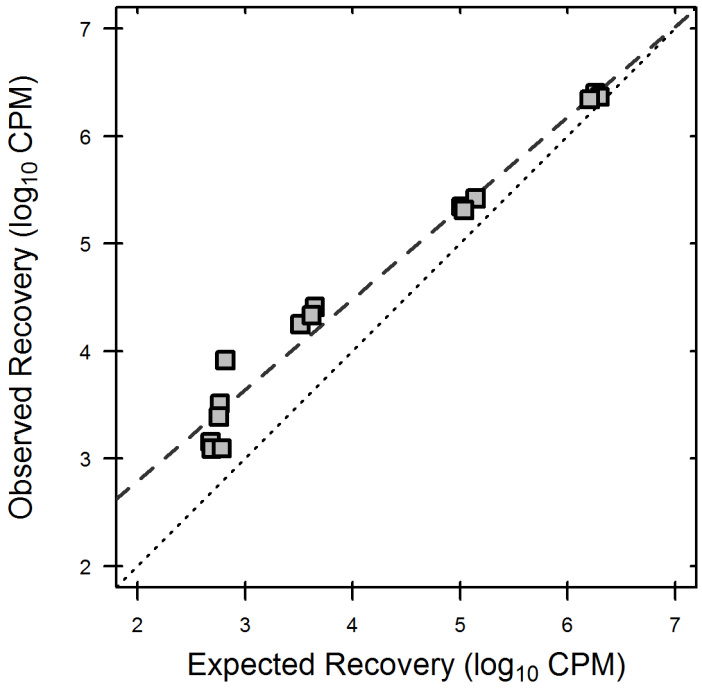

Figure 5: 14C-Bicarbonate Recovery

```
SetA_trap <- succ.data[succ.data$Sample == "Tube1_trap" |
                         succ.data$Sample == "Tube2_trap", 2] * 5
SetA_head <- succ.data[succ.data$Sample == "Tube1_head" |
                         succ.data$Sample == "Tube2_head", 2] * 15

SetB_supp <- succ.data[succ.data$Sample == "Tube3" |
                         succ.data$Sample == "Tube4", 2] * 50
SetB_trap <- succ.data[succ.data$Sample == "Tube3_trap" |
                         succ.data$Sample == "Tube4_trap", 2] * 5
SetB_head <- succ.data[succ.data$Sample == "Tube3_head" |
                         succ.data$Sample == "Tube4_head", 2] * 15

ctr_supp <- succ.data[succ.data$Sample == "Tube5", 2] * 50
ctr_trap <- succ.data[succ.data$Sample == "Tube5_trap", 2] * 5
ctr_head <- succ.data[succ.data$Sample == "Tube5_head", 2] * 15

# Ctrl Subtraction and Percent of Theoretical
SetA_supp.c <- mean((SetA_supp - ctr_supp) / SetA.T)
SetA_trap.c <- mean((SetA_trap - ctr_trap) / SetA.T)
SetA_head.c <- mean((SetA_head - ctr_head) / SetA.T)
SetB_supp.c <- mean((SetB_supp - ctr_supp) / SetB.T)
SetB_trap.c <- mean((SetB_trap - ctr_trap) / SetB.T)
SetB_head.c <- mean((SetB_head - ctr_head) / SetB.T)

SetA <- c(SetA_supp.c, SetA_trap.c, SetA_head.c)
SetB <- c(SetB_supp.c, SetB_trap.c, SetB_head.c)
```

```r
names(SetA) <- c("Supp", "Trap", "Head")
names(SetB) <- c("Supp", "Trap", "Head")

bps <- as.matrix(data.frame(SetA, SetB))

# Plot
png(filename="../figures/Succinate1.png",
    width = 1200, height = 1200, res = 96*2)

par(mar=c(5,5,3,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
barplot(bps, main = "Succinate Metabolism Mass Balance",
        xlab = "Succinate Concentration",
        ylab = "Percent Recovery",
        names.arg = c("10 mM", "0.5 mM"),
        legend = c("Cells", "KOH Trap", "Head Space"),
        las = 1, cex.lab = 1.5,
        args.legend = c(x= "topleft", bty="n"))

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```

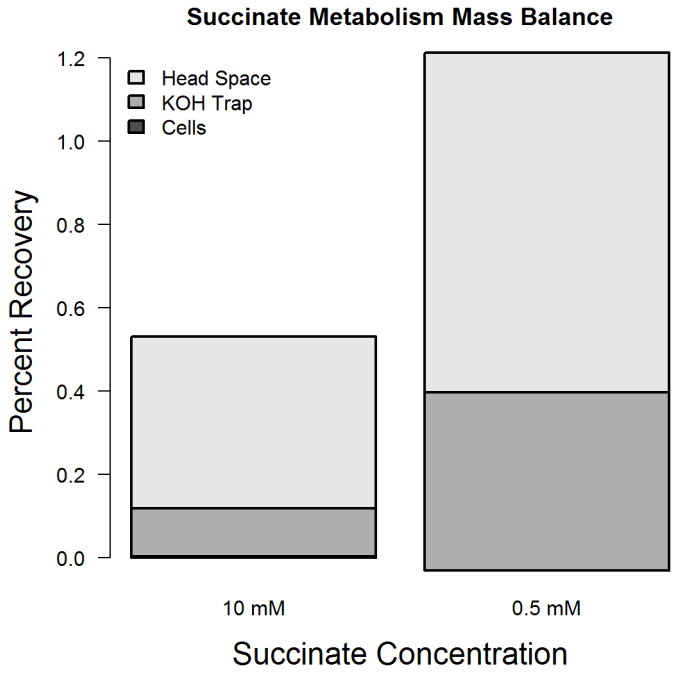

Figure 6: Succinate Mass Balance

Succinate Test Experiment 2

```
# Import Data
succ.data <- read.csv("../notes/20151119_BRBP.csv", header=T)

# Raw Data For Intra Vial Test
vouchers  <- succ.data[grep("Voucher", succ.data$Sample), ]

SetA.T <- mean(vouchers[vouchers$Conc == 10, 2])
SetB.T <- mean(vouchers[vouchers$Conc == 0.5, 2])

SetA_cells <- succ.data[succ.data$Sample == "Tube1_cells" |
                        succ.data$Sample == "Tube2_cells", 2] * 1.5/5
SetA_supp <- succ.data[succ.data$Sample == "Tube1_supp" |
                        succ.data$Sample == "Tube2_supp", 2] * 50
SetA_ac.cells <- succ.data[succ.data$Sample == "Tube1_ac-cells" |
                        succ.data$Sample == "Tube2_ac-cells", 2] * 1.5/5
SetA_ac.supp <- succ.data[succ.data$Sample == "Tube1_ac-supp" |
                        succ.data$Sample == "Tube2_ac-supp", 2] * 50

SetB_cells <- succ.data[succ.data$Sample == "Tube3_cells" |
                        succ.data$Sample == "Tube4_cells", 2] * 1.5/5
SetB_supp <- succ.data[succ.data$Sample == "Tube3_supp" |
                        succ.data$Sample == "Tube4_supp", 2] * 50
SetB_ac.cells <- succ.data[succ.data$Sample == "Tube3_ac-cells" |
                        succ.data$Sample == "Tube4_ac-cells", 2] * 1.5/5
SetB_ac.supp <- succ.data[succ.data$Sample == "Tube3_ac-supp" |
                        succ.data$Sample == "Tube4_ac-supp", 2] * 50

ctr_cells <- succ.data[succ.data$Sample == "Tube5_cells", 2] * 1.5/5
ctr_supp <- succ.data[succ.data$Sample == "Tube5_supp", 2] * 50
ctr_ac.cells <- succ.data[succ.data$Sample == "Tube5_ac-cells", 2] * 1.5/5
ctr_ac.supp <- succ.data[succ.data$Sample == "Tube5_ac-supp", 2] * 50

# Ctrl Subtraction and Percent of Theoretical
SetA_cells.c <- mean((SetA_cells - ctr_cells) / SetA.T)
SetA_supp.c <- mean((SetA_supp - ctr_supp) / SetA.T)
SetA_ac.cells.c <- mean((SetA_ac.cells - ctr_ac.cells) / SetA.T)
SetA_ac.supp.c <- mean((SetA_ac.supp - ctr_ac.supp) / SetA.T)
SetB_cells.c <- mean((SetB_cells - ctr_cells) / SetB.T)
SetB_supp.c <- mean((SetB_supp - ctr_supp) / SetB.T)
SetB_ac.cells.c <- mean((SetB_ac.cells - ctr_ac.cells) / SetB.T)
SetB_ac.supp.c <- mean((SetB_ac.supp - ctr_ac.supp) / SetB.T)


SetA <- c(SetA_cells.c, SetA_supp.c, SetA_ac.cells.c, SetA_ac.supp.c)
SetB <- c(SetB_cells.c, SetB_supp.c, SetB_ac.cells.c, SetB_ac.supp.c)

names(SetA) <- c("Cells", "Supp.", "Acidified Cells", "Acidfied Supp.")
names(SetB) <- c("Cells", "Supp.", "Acidified Cells", "Acidfied Supp.")

bps <- as.matrix(data.frame(SetA, SetB))
```

```r
# Plot
png(filename="../figures/Succinate2.png",
    width = 1200, height = 1200, res = 96*2)

par(mar=c(5,5,3,0.5), oma=c(1,1,1,1)+0.1, lwd=2)
barplot(bps, main = "Succinate Metabolism Mass Balance with Acidification",
        xlab = "Succinate Concentration",
        ylab = "Percent Recovery",
        names.arg = c("10 mM", "0.5 mM"),
        legend = c("Cells", "Supp.", "Acidified Cells", "Acidfied Supp."),
        las = 1, cex.lab = 1.5,
        args.legend = c(x= "topright", bty="n"))

dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```

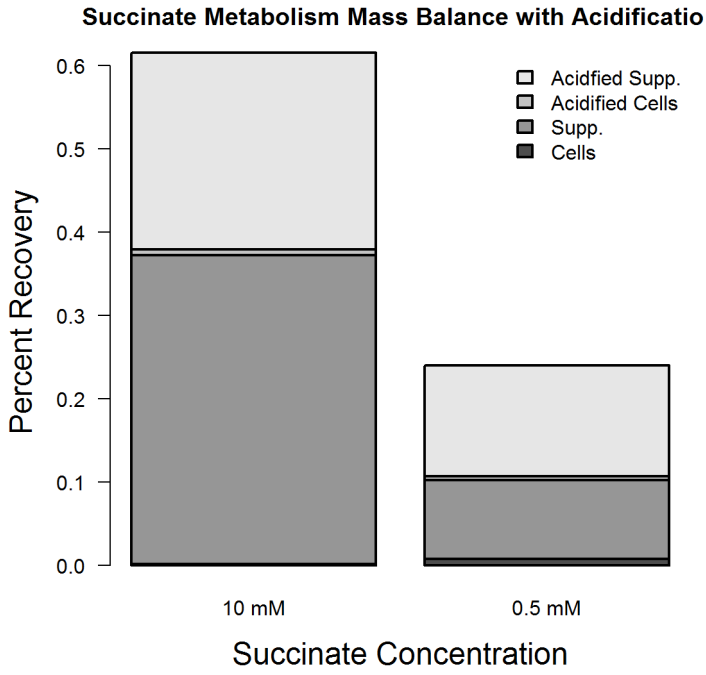

Figure 7: Succinate Mass Balance

Conclusions: Acidifiction does not lyse cells. It is clear, however, that we need to increase the 14C succinate in the succinate solution.