We used Euler's Method to solve the above differential equations, and our formulas are documented below (Figure 22). As figure 22 shows, by plugging in values for [Ethanol] and [ACH] at t_0, a fixed value for V_{max} and K_m (depending on what ALDH2 variant is used), and the time interval Δt, dE/dt = R_1 and dA/dt = Y_1 at t = 0 can be calculated. In other words, we can find the activity of ADH and ALDH2 enzymes at t = 0. As for values of the next time point, t_1, we assume that the concentrations of the substrates are the net gain or loss of concentration added to the previous concentrations. Hence, $E_1 = E_0 - (\Delta t)R_1$ and $A_1 = A_0 - (\Delta t)Y_1$. Because the instantaneous reaction rates are dependent on the substrates' concentrations at the particular time point, the ethanol concentration for dE/dt at t_1 is equal to E_1, while the acetaldehyde concentration for dA/dt at t_1 is equal to A_1. Using the new values, new substrate concentrations and reaction rates can be calculated at the next time step. The same method is repeated for subsequent calculations.

Time (sec)	[Ethanol]	[ACH]	V_{max}	K_m	Δt (interval)	dE/dt	dA/dt
t_0	E_0	A_0	V_{max} of ALDH2	K_m of ALDH2	Δt	$(0.82*[E_0])/(0.9 + [E_0]) = R_1$	$(V_{max} * [A_0])/(K_m + [A_0]) - R_1 = Y_1$
t_1	$E_1 = E_0 - (\Delta t)R_1$	$A_1 = A_0 - (\Delta t)Y_1$	"	"	"	$(0.82*[E_1])/(0.9 + [E_1]) = R_2$	$(V_{max} * [A_1])/(K_m + [A_1]) - R_2 = Y_2$
t_2	$E_2 = E_1 - (\Delta t)R_2$	$A_2 = A_1 - (\Delta t)Y_2$	"	"	"	$(0.82*[E_2])/(0.9 + [E_2]) = R_3$	$(V_{max} * [A_2])/(K_m + [A_2]) - R_3 = Y_3$
t_3	$E_3 = E_2 - (\Delta t)R_3$	$A_3 = A_2 - (\Delta t)Y_3$	"	"	"	$(0.82*[E_3])/(0.9 + [E_3]) = R_4$	$(V_{max} * [A_3])/(K_m + [A_3]) - R_4 = Y_4$

Figure 4-22. This table is a visual representation of the formulas we input into excel to solve the two differential equations. The value of V_{max} and K_m can be easily altered, depending on the genotype of the ALDH2 enzymes.

Time (sec)	[Ethanol]	[ACH]	V_{max}	K_m	Δt (increment)	dE/dt	dA/dt
0	100	300	2.1	0.2	2	0.81268583	1.28591511
2	98.3746283	297.42817	2.1	0.2	2	0.81256608	1.28602277
4	96.7494962	294.856124	2.1	0.2	2	0.81244236	1.28613418
6	95.1246115	292.283856	2.1	0.2	2	0.81231447	1.28624955
8	93.4999825	289.711357	2.1	0.2	2	0.8121822	1.28636908
10	91.8756181	287.138619	2.1	0.2	2	0.81204532	1.28649299
12	90.2515275	284.565633	2.1	0.2	2	0.81190359	1.28662151
14	88.6277203	281.99239	2.1	0.2	2	0.81175674	1.28675491
16	87.0042068	279.41888	2.1	0.2	2	0.8116045	1.28689346
18	85.3809978	276.845093	2.1	0.2	2	0.81144655	1.28703745
20	83.7581047	274.271018	2.1	0.2	2	0.81128258	1.2871872

Figure 4-23. This is a sample calculation using our excel formulas. This sample assumes that ALDH2*1 enzymes are catalyzing the reaction. [Ethanol] and [ACH] have a unit of μM; V_{max} and K_m are constants specific to the ALDH2 variants; Δt has a unit of seconds; dE/dt and dA/dt have a unit of μM/sec.

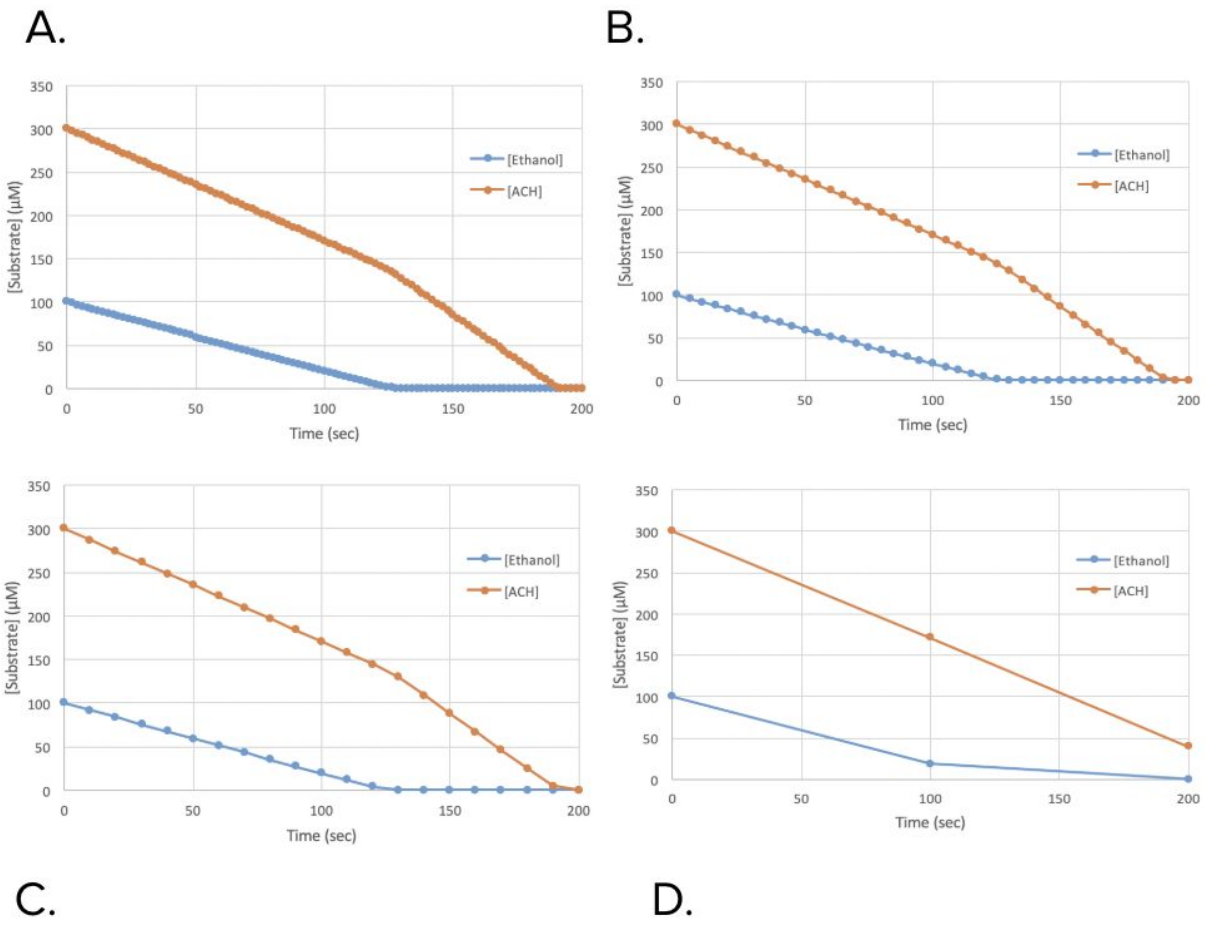
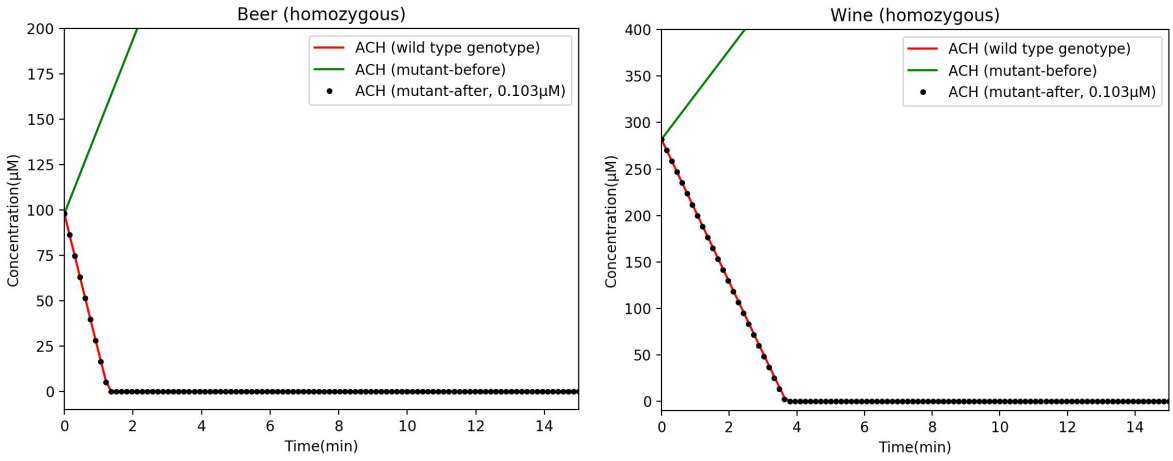


Figure_____. Graphs of [Ethanol] & [ACH] versus time (seconds) with different Δt. The four graphs were plotted with the same numbers except for the Δt values. Graph A to D had Δt values of 2, 5, 20, and 100.

Referenced in Figure 4-8 on Modeling page:

Acetaldehyde concentration over time after supplying various concentrations of ALDH2*1 enzymes for Beer and Wine:

The same concentration (the same as in spirits) of 0.103 μM ALDH2*1 also adjusts acetaldehyde levels in homozygous mutants to match those found in wild type people.



COMPARING OUR ALDH2*1 ENZYMATIC ACTIVITY TO LITERATURE VALUES

Literature Values:

We obtained the Specific Activity of purified ALDH2*1 enzymes from Rashkovestky, 1994. This represents V_{max}, the maximum rate of acetaldehyde elimination or acetate production, for a given amount of ALDH2*1 enzymes. We convert the literature values to relate a molar concentration of ALDH2*1 with its rate of acetate production.

The following known values were from experiments run at 25°C by (Rashkovestky, 1994). The "ALDH2 enzymes" referred to in this section are wild type ALDH2*1 enzymes.

Specific Activity: 5.5 μmol/min/mg of ALDH2
Protein ALDH2: 8.5mg
Volume: 47ml
k_{cat} at pH 9.5: $1180/min$

Calculation 1. Calculating initial ALDH2*1 concentration [E_0]. Using the molecular mass of an ALDH2*1 tetramer, we calculated the [E_0], or initial [ALDH2*1] used, to be *0.8 μM.*

$$\frac{8.5mg\ ALDH2}{225000g/mol}.\frac{1}{0.047L} = 0.80\mu M$$

Calculation 2. Calculating maximum rate of acetaldehyde elimination (V_{max}) of ALDH2*1 enzymes. Equation 1 converts the given Specific Activity of ALDH2*1 to concentration over time through dimensional analysis, and the V_{max} was calculated to be *1.0 mM/min*. **Equation 2** converts V_{max} from *1.0 mM/min* to *17 µM/sec*.

Equation 1

$$\frac{5.5\mu mol\ acetate}{min} \cdot \frac{1}{mg\ ALDH2} \cdot \frac{1}{0.047L} \cdot \frac{8.5mg\ ALDH2}{1} = \frac{1.0\ mM}{min}$$

Equation 2

$$\frac{1.0\ mM}{min} \cdot \frac{1\ min}{60\ sec} = \frac{17\mu M}{sec}$$

Based on these literature values, we concluded that, given excess acetaldehyde, the maximum reaction rate of **0.80µM** of ALDH2 enzymes at 25°C is about **1.0mM/min**, or **17µM/sec**.

*Our Experimental ALDH2*1 Values:*

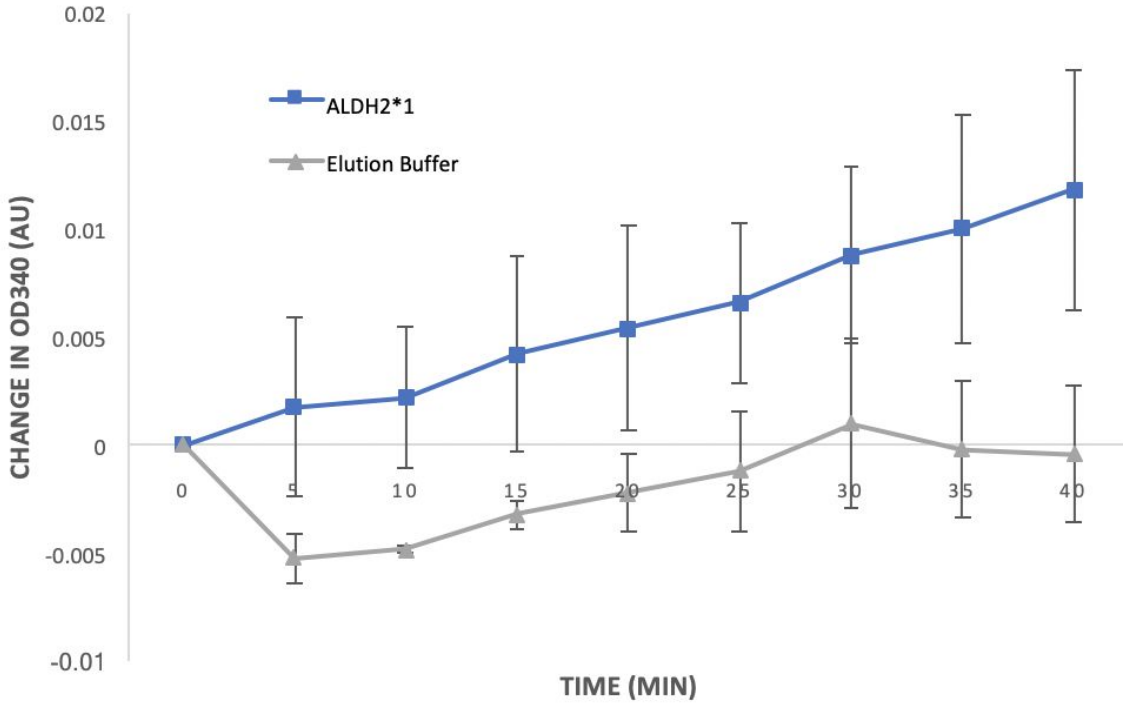


Figure 4-2. Experimentally Determined Purified Enzyme Activity of ALDH2*1 at 25°C. The OD340 values of NADH are recorded and the change in OD340 values over time is plotted in order to measure the enzymatic activity of purified ALDH2*1. The experiment was run in water at 25°C. A negative control containing only elution buffer (from the protein purification process) was included (gray). The error bars on the graph represent standard error. *For more details regarding the experiment, click here*. (Experiment: Justin W; Figure: Justin L & Justin W)

Next, we used the Beer-Lambert Law to convert changes in NADH absorbance values to changes in concentration of NADH (Figure 11).

$$A = \varepsilon b C \qquad \frac{dA}{dt} = \varepsilon b \frac{dC}{dt}$$

Figure 4-3. Conversion of absorbance values to changes in NADH concentration. (Left) The variable ***A*** denotes a NADH absorbance at 340nm; the variable ***ε*** is a constant that denotes the NADH molar extinction factor at the same wavelength of light; the variable ***b*** is a constant that denotes the pathlength of the light; and ***C*** denotes the NADH concentration. **(Right)** The Beer-Lambert Law in the form of a differential equation, with changes in ***A*** relating to changes in ***C*** over time. We used this equation to determine the corresponding change in NADH concentration using a change in ΔA_{NADH} from our functional test. (Figure: Justin L)

From the graph in Figure 4-4, we calculated the change in OD340 of NADH over a time period of 40 minutes. We first corrected the change in ΔA_{exp} at t = 40 min by subtracting it with the value of the control at t = 40 min. We then used the difference as the overall change in OD340 and calculated $\mathbf{dA_{NADH}/dt}$ to be

0.0122/40min. By dividing dA/dt with the extinction coefficient ε_{NADH} (6220/$M^{-1}Cm^{-1}$, as indicated on the Megazyme kit manual) and the pathlength ***b*** (1 cm, which is the length of the cuvette), we calculated the rate of change in [NADH] over time, or **dC_{NADH}/dt**, to be **0.817nM/sec.** Because acetaldehyde, acetate, and NADH all react in a one-to-one ratio in the oxidation of acetaldehyde (Figure 4-1), we assumed that a change in NADH concentration was equivalent to changes in acetaldehyde and acetate concentrations. In other words, we assumed that **dC_{NADH}/dt = dP/dt (**or **$dC_{acetate}/dt$)** in the reaction (FIgure 4-1). For specific calculation processes, please see the Modeling Lab notebook.

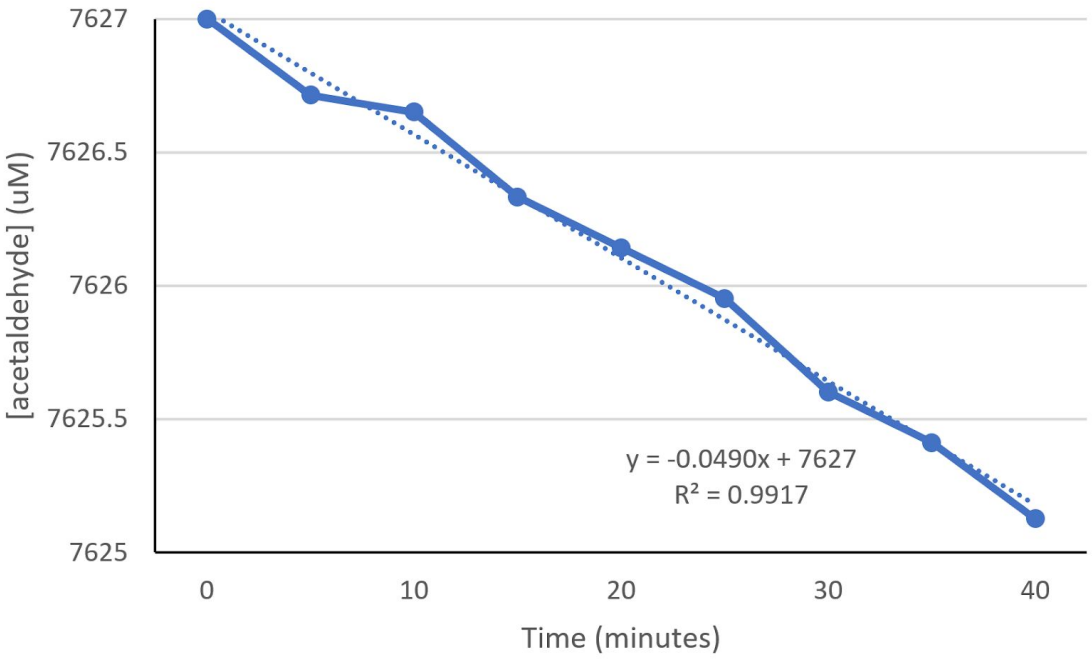


Figure 4-4. Decrease in acetaldehyde concentration as a result of purified ALDH2*1 at 25°C. The acetaldehyde concentrations were calculated from the raw absorbance values and the Beer-Lambert Law. The calculated acetaldehyde metabolism rate is 0.0490 uM/min or 0.817 nM/sec. (Figure: Justin W)

We also measured the ALDH2 concentration with a nanodrop spectrophotometer and found it to be 0.1 mg/ml. In our test, we used 500µL of protein solution. We then converted the value's unit to be in terms of M (with the molecular weight of an ALDH2*1 enzyme given us 225000g). Hence, we concluded that 0.222 nM of ALDH2*1 enzymes yielded 0.817nM/sec enzymatic activity.

PROTEIN EXPRESSION OF ALDH2*1-*Ec*N

In Figure 4-9, we calculated that **4.79*10^8 *Ec*N cells/ml produced about 0.0536nM of ALDH2 over 16 hours at 37°C**. With these values, we could determine the protein expression rate of our engineered ALDH2-carrying *Ec*N.

To calculate how much ALDH2*1-*Ec*N should be cultured to convert a target acetaldehyde level, we also determined the growth rate of our ALDH2*1-*Ec*N. We grew ALDH2*1-*Ec*N bacteria and recorded OD600 values to keep track of the culture's turbidity (for specific experiment procedures, please see Modeling Lab Notebook). A total of two trials under the same conditions (37°C) were performed, and the data recorded were averaged. The averaged plotted data are presented below in Figure 4-10. The averaged data are plotted and fit with a logistic equation specifically for ecological population growth called the Verhulst Equation (Figure 4-11). The graph is represented by Figure 4-12.

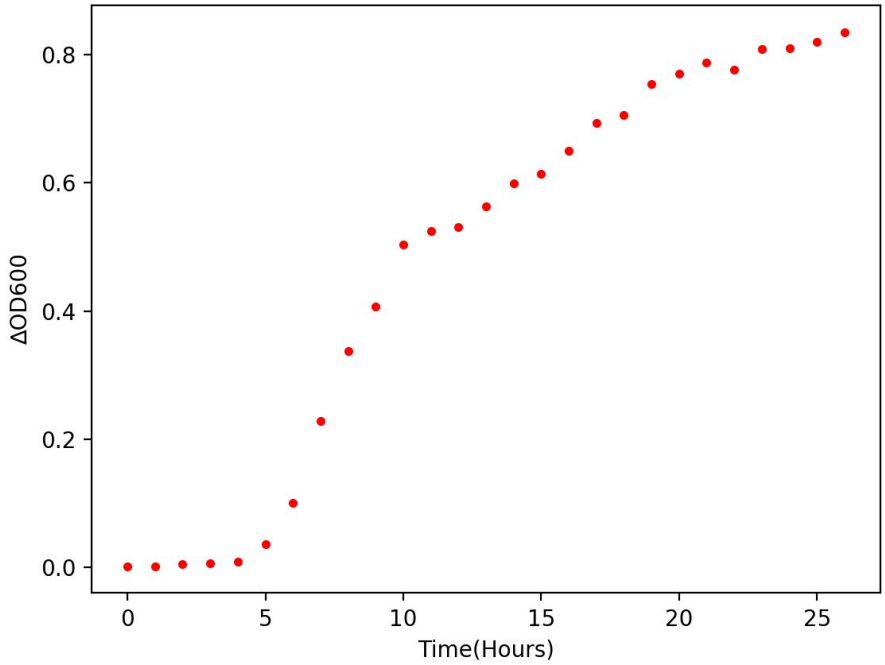


Figure 4-10. Growth curve of *Ec*N bacteria based on OD600 values at 37°C. We plotted each experimental absorbance value of our bacteria over time. (Figure: Justin L)

We then converted the absorbance values to concentrations of bacteria, based on the conversion ratio that 8*10^8 *Ec*N cells/ml is equivalent to 1.000 OD600 value.

$$P(t) = \frac{KP_0e^{rt}}{K + P_0(e^{rt} - 1)}$$

Figure 4-11. The Verhulst Equation was used to model *Ec*N bacteria population over time. P(t) represents the bacterial concentration at any given time. K represents the maximum bacteria

concentration, while P_0 represents the initial bacteria population. The r constant is the bacteria growth rate determined by our Python software. (Figure: Iris H)

Based on the experimental values, we used the Verhulst Equation (Figure 4-11) to model our bacteria growth over time, with the initial and final experimental *Ec*N populations (calculated from OD600 values) used as the model's P_0 and K constants, respectively. The optimal value for the constant r was automatically calculated by our Python software and represents the bacteria growth rate. The best fit equation of bacteria population over time is plotted below in blue (Figure 4-12). According to our program, the ***r*** value is about **0.5890** for our experimental data.

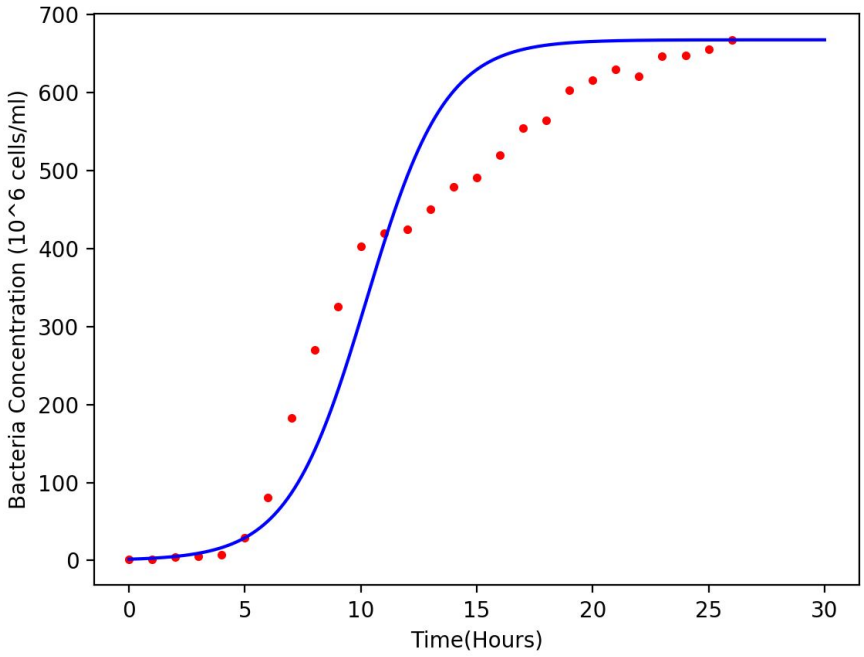


Figure 4-12. Theoretical best-fit curve based off our experimental absorbance values and conversions to bacteria concentration over time. The red dots represent the experimental bacteria population, while the blue represents the theoretical best-fit bacteria growth curve. (Figure Justin L)

Because we used a constitutive promoter (BBa_J23100) in our engineered ALDH2*1-*Ec*N bacteria, the proteins should be expressed at a constant rate. Hence, we created a **constant ß value** to model the expression rate of our bacteria (figure 18).

$$\frac{dE}{dt} = \beta P(t) \qquad E(t) = \int_{t=a}^{t=b} \beta P(t)dt$$

Figure 4-13. Equations that model ALDH2*1 level expressed by our engineered *Ec*N bacteria. The symbol ***E*** represents the ALDH2*1 concentration. The equation on the left represents the protein expression rate by the constitutive promoter (BBa_J23100); the equation on the right represents the ALDH2*1 concentration at any given time (t = b, in hours) since the initial time of bacteria culture (t = a, usually equal to 0h).

Knowing that $4.79*10^8$ *Ec*N cell/ml produced 0.0536 nM ALDH2 in 16 hours, we were able to calculate the numerical value of **ß**, which represents constant rate of production of ALDH2*1 by one bacterium cell in an hour (**$1.38*10^{-20}$ mol ALDH2/cell/hour)**. With this value, we constructed **three** calculators for manufacturers to simplify the production of ALDH2 enzymes from our engineered EcN bacteria and make it more efficient to create a final product.

DIFFERENT GENOTYPES

We obtained the K_m constant values for the homozygous wild type and mutant type from literature. With the available values, we plotted the Michaelis-Menten graphs for all three genotypes of ALDH2 enzyme variants at 37°C, where the initial enzyme concentration [E_0] was 0.80µM. We did this to get a better sense of ALDH2 enzymes' reaction rate at various concentrations of acetaldehyde, the substrate. We also assumed that enzymatic degradation did not occur in our Michaelis-Menten graphs, so the enzyme concentrations would remain constant throughout the course of the reactions.

ALDH2 *1/*1 wild type: $\frac{(17\mu M/sec)\ .\ [ACH]}{0.2 + [ACH]}$

ALDH2 *1/*2 mutant type: $\frac{(3.4\mu M/sec)\ .\ [ACH]}{1.4 + [ACH]}$

ALDH2 *2/*2 mutant type: $\frac{(0.17\mu M/sec)\ .\ [ACH]}{1.4 + [ACH]}$

Figure 4-15. Equations of the three alleles types. Acetaldehyde [ACH] has a unit of µM. We assumed the worst case scenario when building our equations. The heterozygous *1/*2 type's has about a 20 to 40% of wild *1/*1 type's efficiency. We thus graphed our *1/*2 type curve as 20% efficiency of the *1/*1 type. Same logic applies to the homozygous *2/*2 type's equation, where

the *2/*2 type was assumed to have about 1% efficiency of the *1/*1 type. We also assumed the heterozygous *1/*2 type to have the same Km value as the *2/*2 type due to the lack of literature data. (Figure: Justin L)

Comparison of Michaelis-Menten Curves

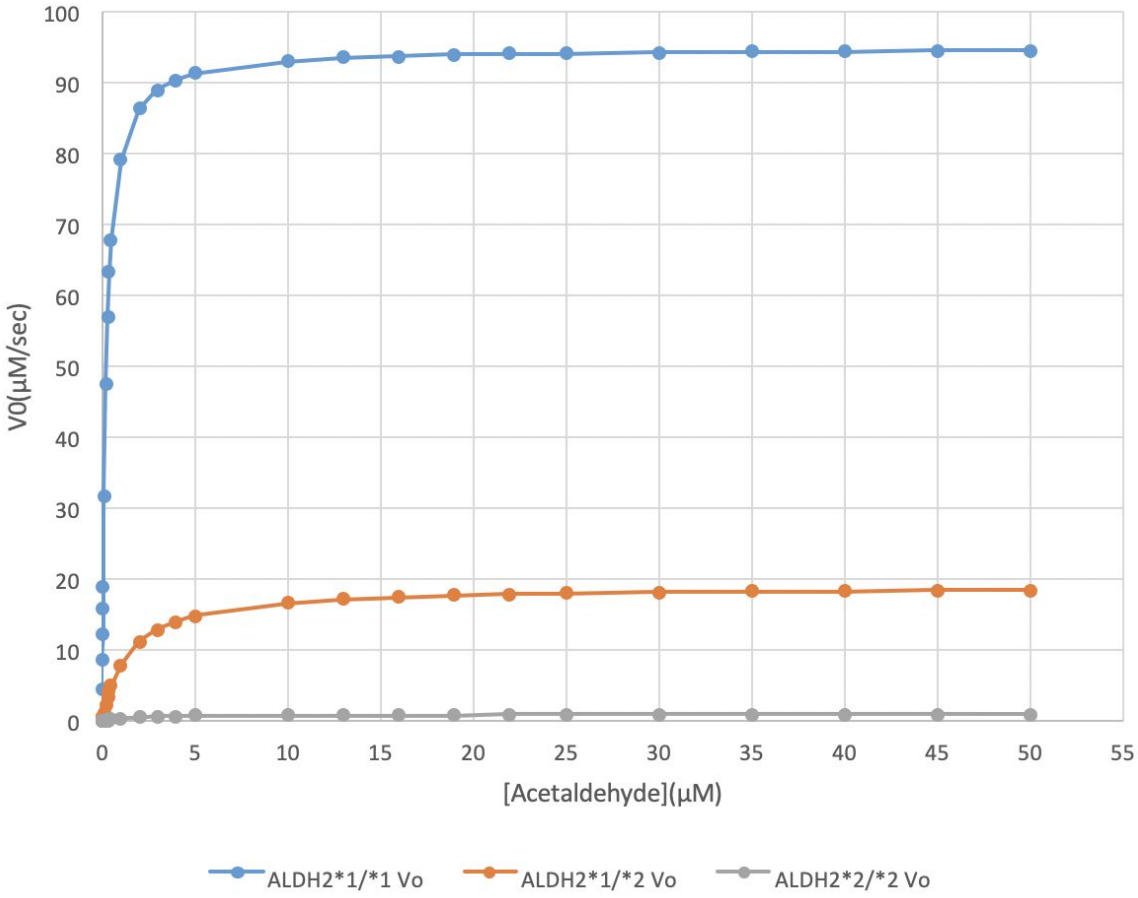
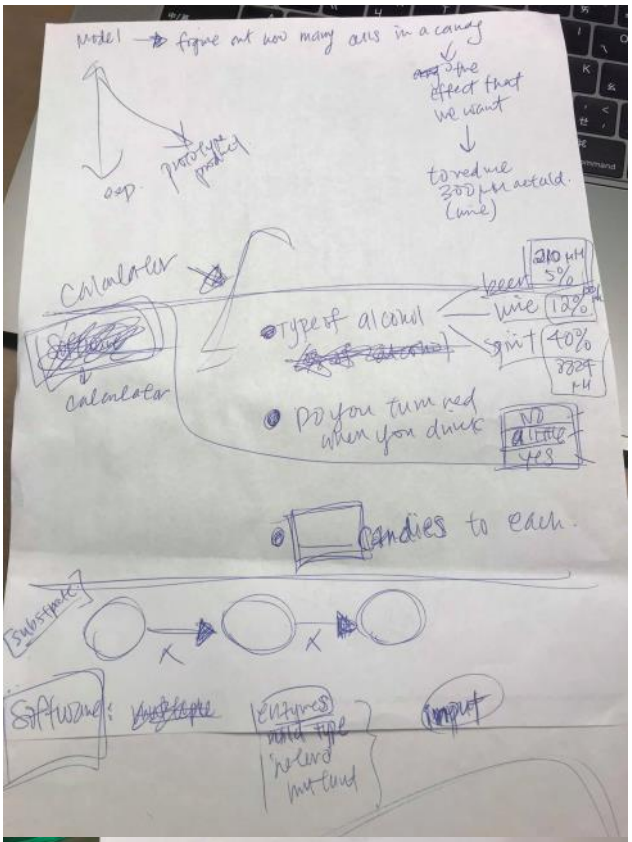
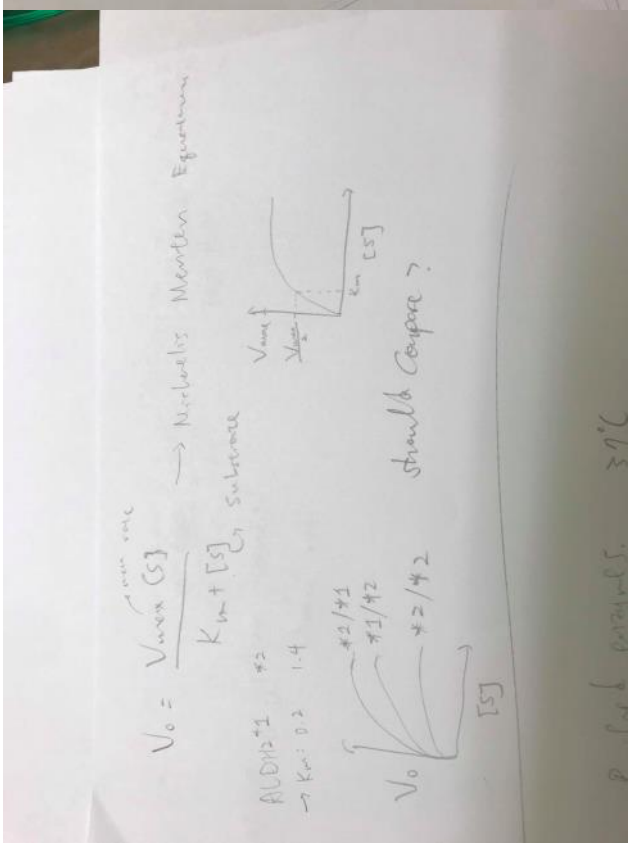


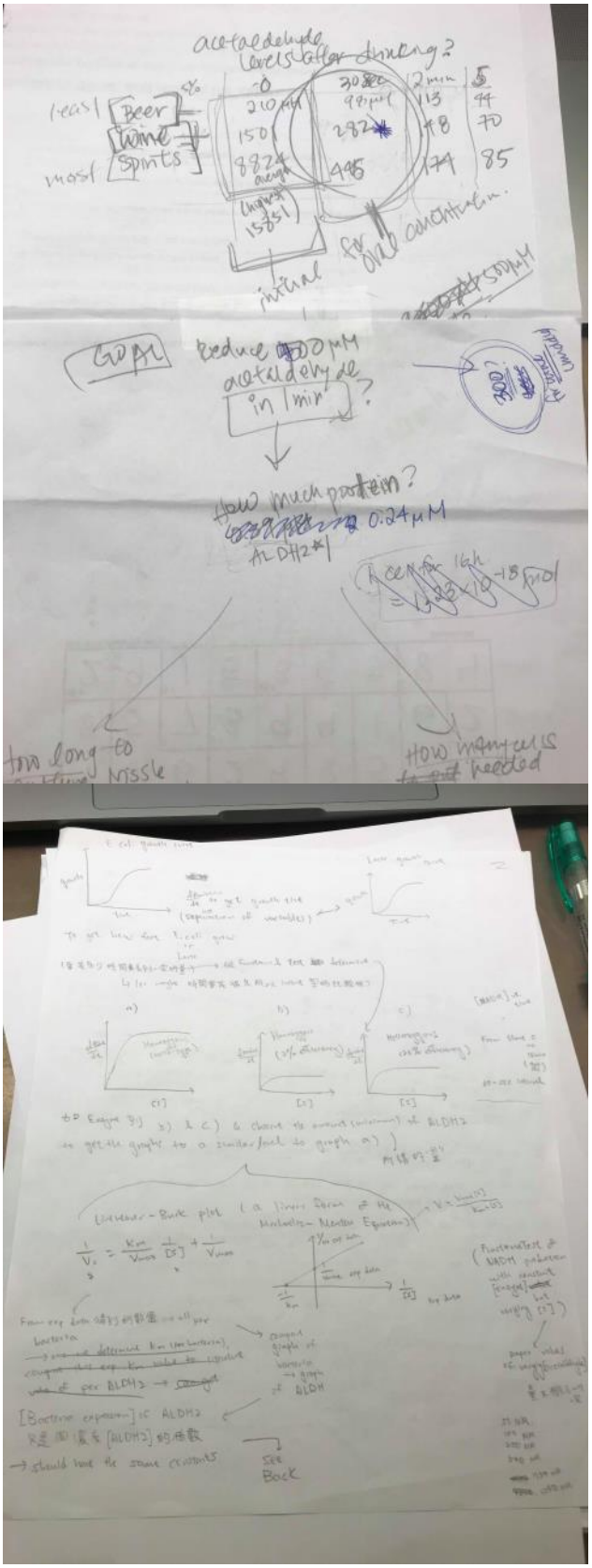
Figure 4-17. Graph of Michaelis-Menten Graphs of 0.80μM ALDH2 Enzymes at 37 °C. The curves reflect the reaction rate V_0 of the three ALDH2 variants in various concentrations of acetaldehyde. (Figure: Justin L)

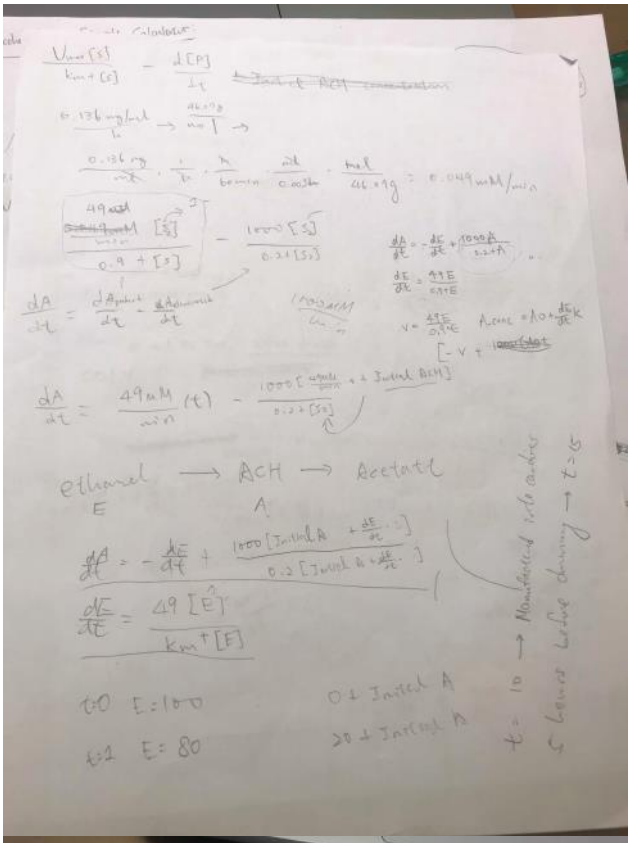
By looking at the graphs in figure 16 and figure 17, we were able to better visualize the differences in reaction rates of ALDH2 enzymes at different temperatures, and we determined two things. First, we concluded that 37°C is a more optimal temperature for ALDH2 enzymes to function in. Second, we decided that the fastest, most ideal solution to treating ALDH2 deficient patients would be to directly deliver ALDH2*1 enzymes to compensate for the differences in reaction rates between wild type and mutant types.

Modeling


model → figure out how many cals in a candy
soap
prototype posted
the effect that we want
to reduce 300 μM acetald. (me)
Calculator
calculator
type of alcohol
beer
wine 10%
spirit 40%
210 μM 5%
do you turn red when you drink
No
a little
Yes
candies to each.
Substrate
Software:
enzymes
input


Michaelis Menten Equation
substrate
should Compare ?
[S]


acetaldehyde levels after drinking?
Beer
Wine
Spirits
GOAL
Reduce μM acetaldehyde in 1 min?
How much protein?
0.24μM
ALDH2*1
too long to
How many cells is needed

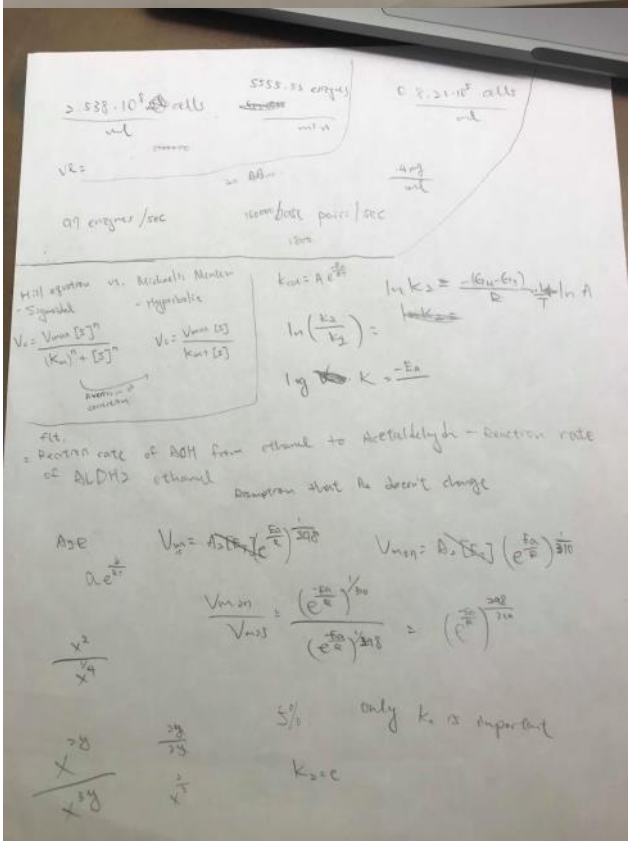


ethanol → ACH → Acetate

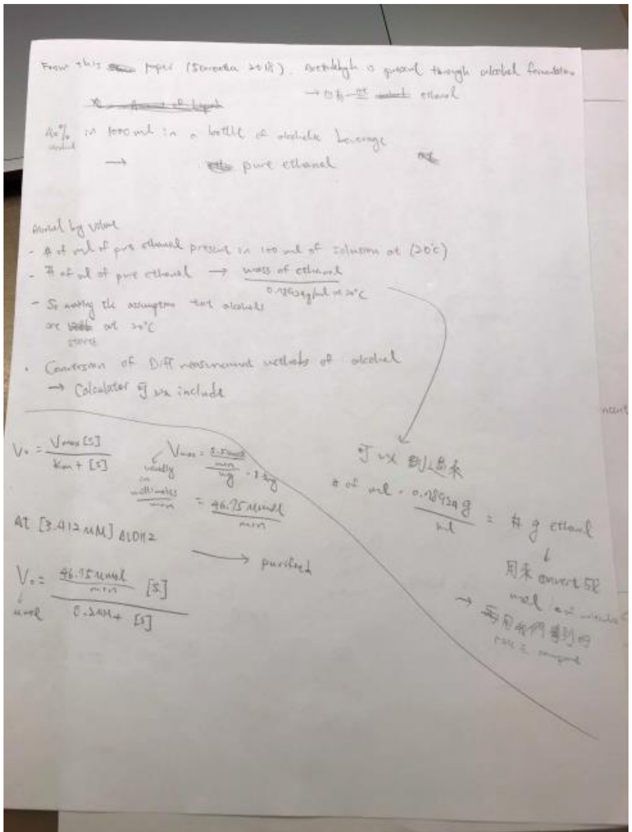
E A

t=0 E=100

t=1 E=80



Exp Log Page 3



From this paper (Sumoda 2018) Acetaldehyde is present through alcohol fermentation
→ ethanol

40% in 1000 ml in a bottle of alcoholic beverage
→ pure ethanol

Alcohol by volume
- # of ml of pure ethanol present in 100 ml of solution at (20°C)
- # of ml of pure ethanol → mass of ethanol / 0.78924 g/ml at 20°C
- So making the assumption that alcohols are stored at 20°C
- Conversion of Diff measurement methods of alcohol
→ Calculator include

$V_0 = \frac{V_{max}[S]}{K_m + [S]}$

$V_{max} = \frac{5.5 \mu mol/min}{mg} \cdot 1 mg$ usually in millimoles/min $= \frac{46.75 \mu mol}{min}$

At [3.412 uM] ALDH2

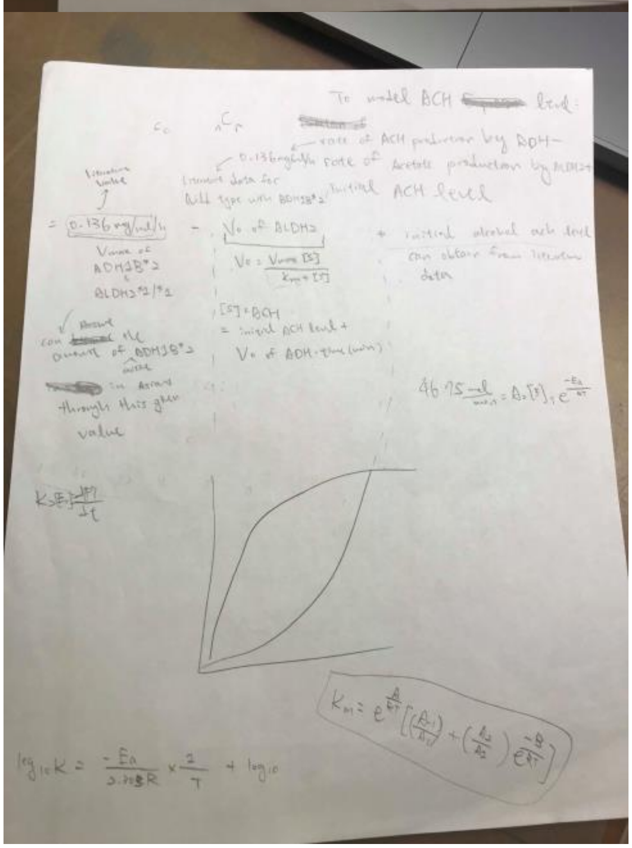
→ purified

$V_0 = \frac{\frac{46.75 \mu mol}{min}[S]}{0.2 mM + [S]}$

umol

# of ml · 0.78924 g/ml = # g ethanol

用来 convert 成 mol



To model ACH level:

rate of ACH production by ADH –
0.136 mg/ml/h rate of Acetate production by ALDH2 +
Initial ACH level

literature value

$= 0.136 mg/ml/h$

Vmax of ADH1B*2 & ALDH2*2/*1

literature data for wild type with ADH1B*2

$-$ V_0 of ALDH2

$V_0 = \frac{V_{max}[S]}{K_m + [S]}$

+ initial alcohol and level can obtain from literature data

[S] = ACH
= initial ACH level + V_0 of ADH · time (min)

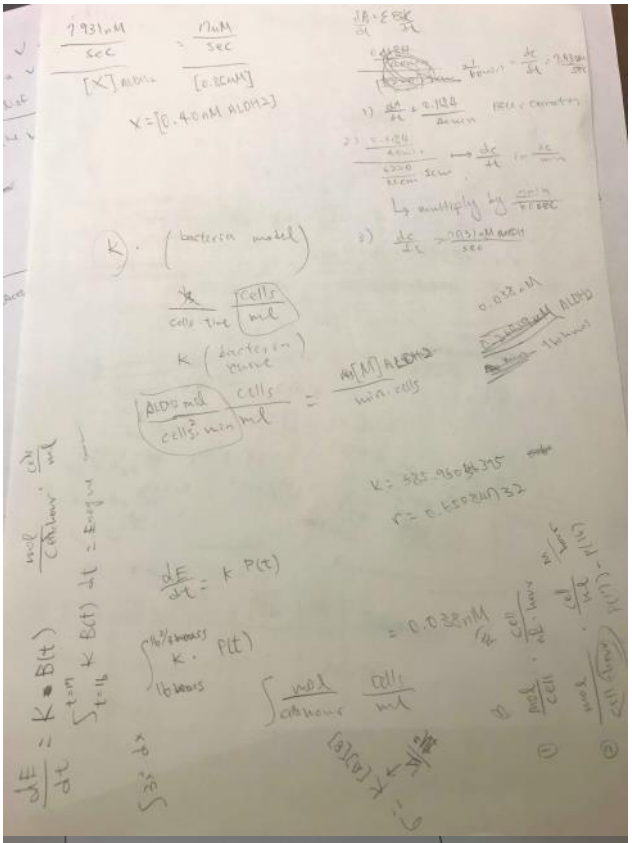
can assume the amount of ADH1B*2 in Asians through this given value

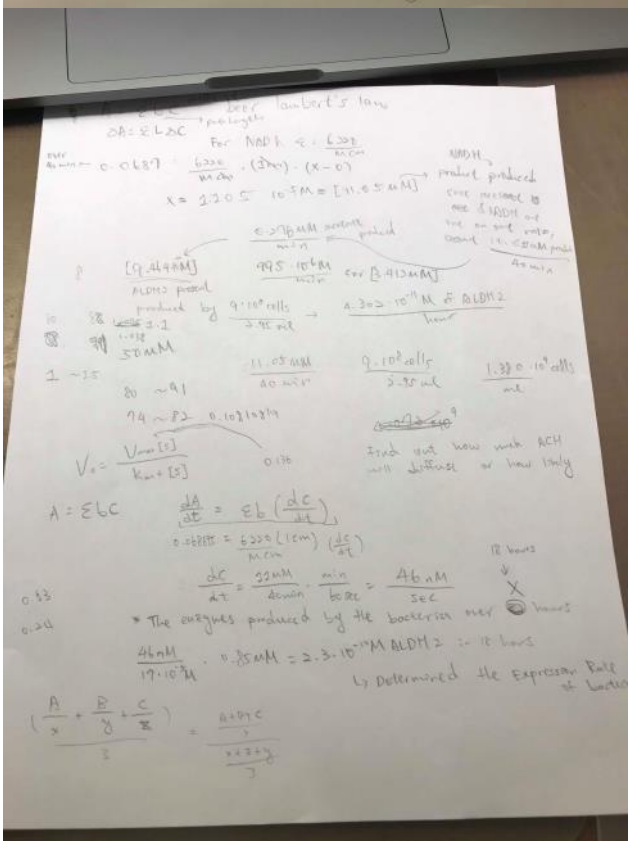
$46.75 \frac{\mu mol}{min} = A_0 [S]_t e^{\frac{-E_a}{RT}}$

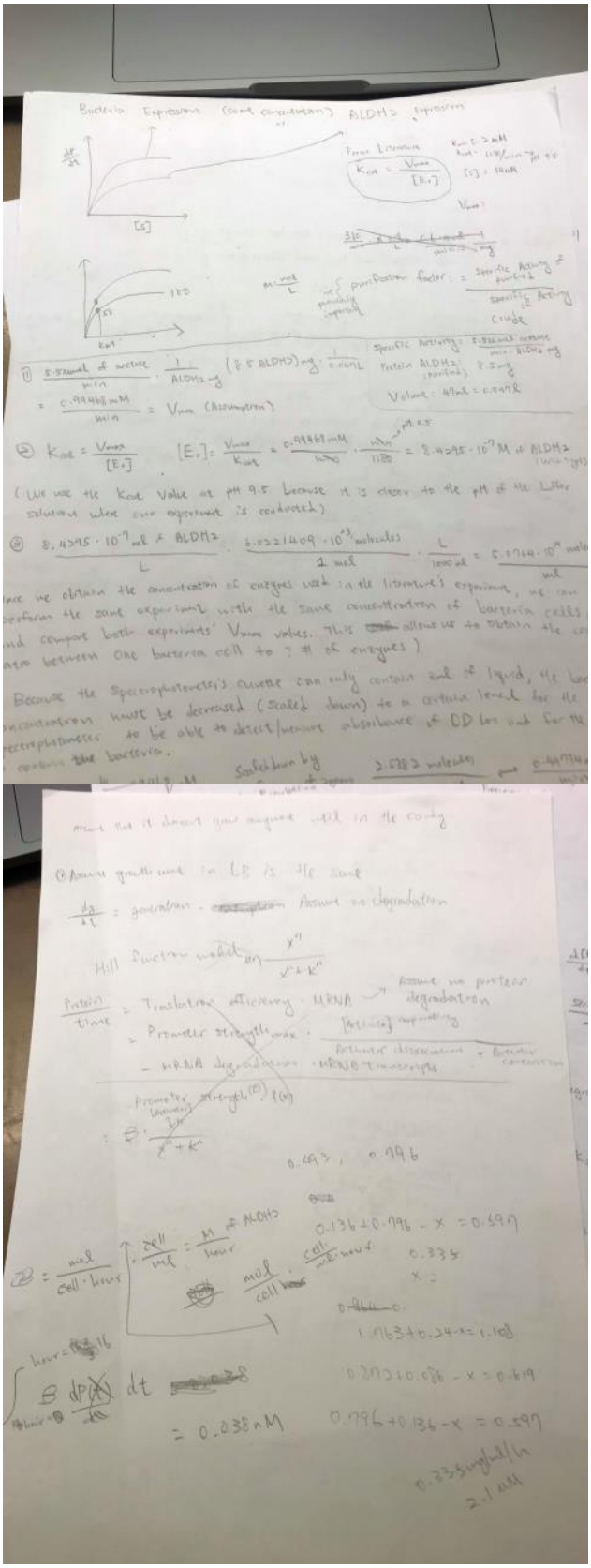
$K_2[E_0] = \frac{d[P]}{dt}$

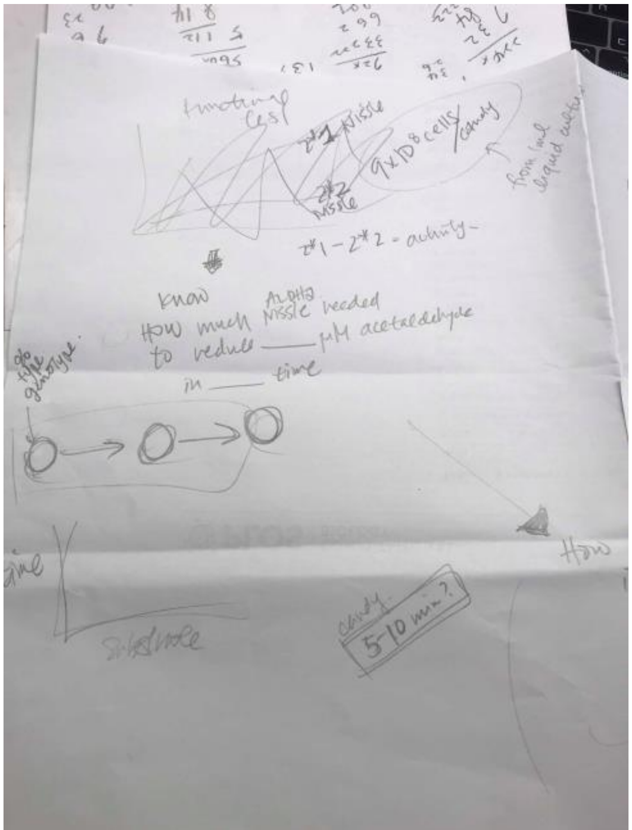
$K_m = e^{\frac{\Delta}{RT}}\left[\left(\frac{A_{-1}}{A_1}\right) + \left(\frac{A_2}{A_1}\right)e^{\frac{-\Delta}{RT}}\right]$

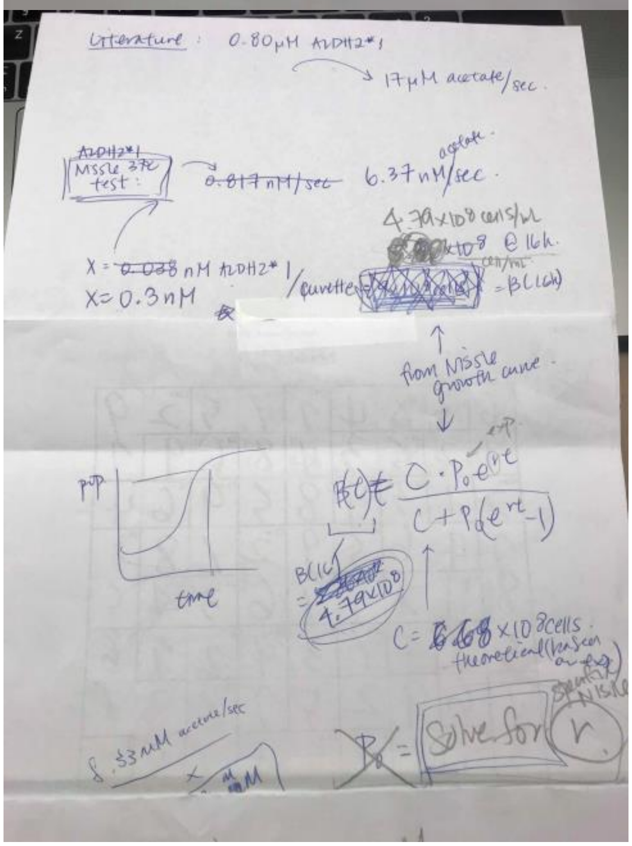
$\log_{10} K = \frac{-E_a}{2.303R} \times \frac{1}{T} + \log_{10}$

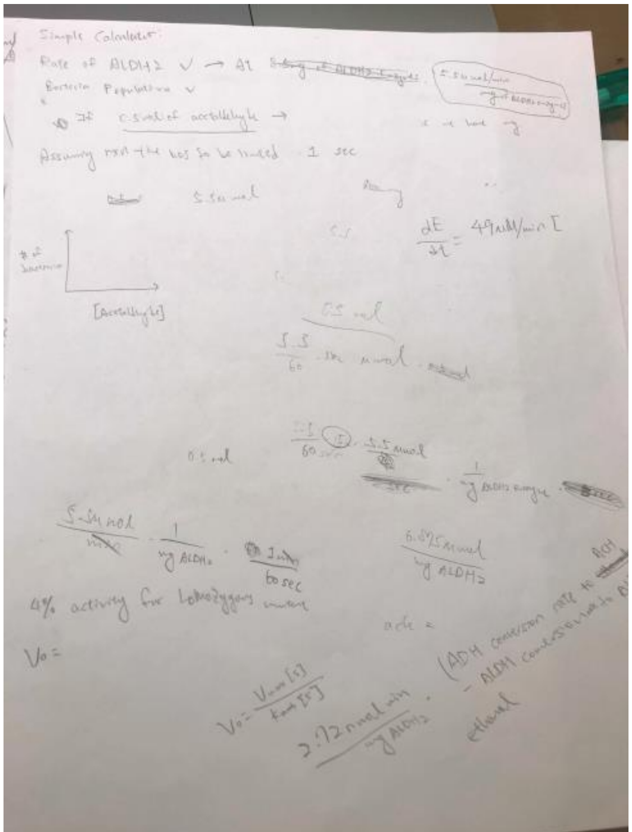


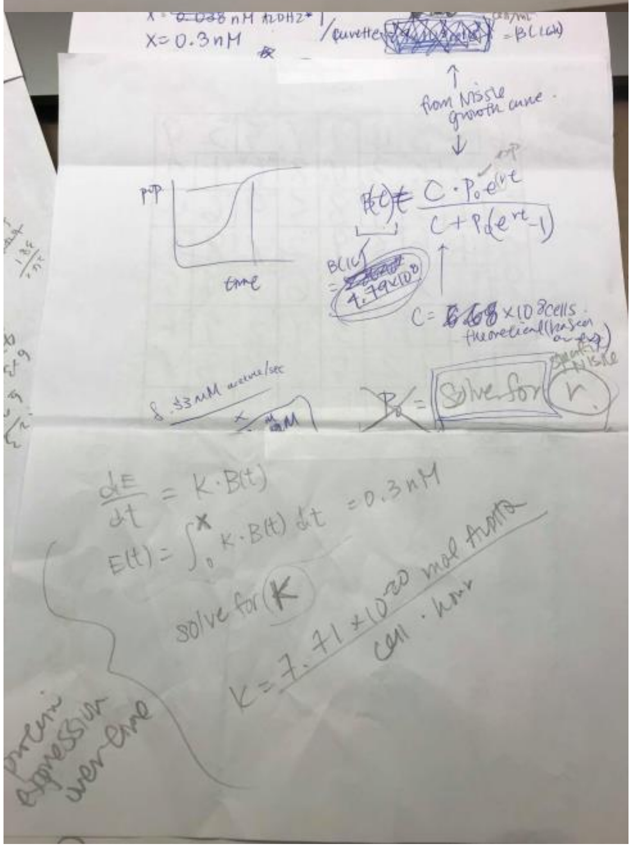


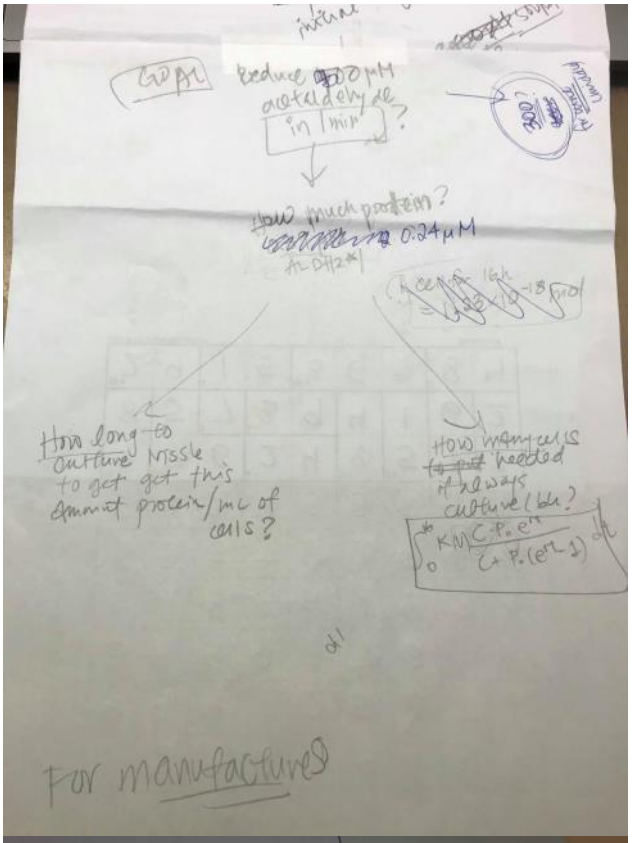


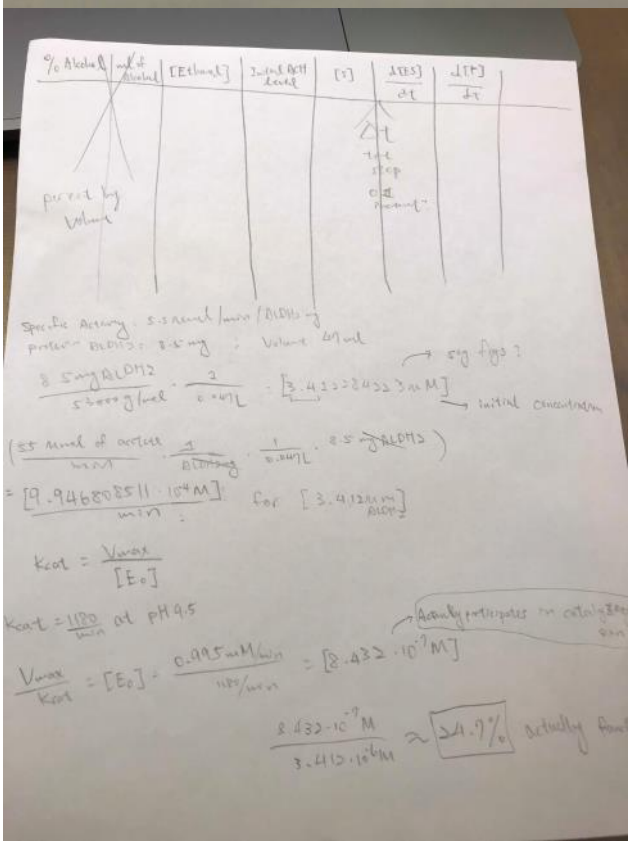

functional test
#1 Nissle
9x10^8 cells/candy
from 1mL liquid culture
#2 Nissle
#1 - #2 = activity
know how much ALDH2 Nissle needed
to reduce ___ µM acetaldehyde
in ___ time
time
substrate
candy 5-10 min?
How


Literature: 0.80µM ALDH2*1
17µM acetate/sec
ALDH2*1 Nissle 37C test:
0.817 nM/sec 6.37 nM/sec acetate
4.79x10^8 cells/mL
@ 16h
X = 0.038 nM ALDH2*1 /cuvette
X = 0.3nM
= B(16h)
from Nissle growth curve
pop
time
exp
P(t) = C·P0 e^rt / C + P0(e^rt - 1)
B(16) = 4.79x10^8
C = 6.68 x10^8 cells theoretical (based on exp)
specific Nissle
Solve for r
8.53nM acetate/sec
x nM


Simple Calculator:
Rate of ALDH2 ✓ → At
Bacteria Population ✓
If 0.5 mol of acetaldehyde →
Assuming rxn the has to be limited 1 sec
5.5 mol
dE/dt = 49 nM/min
[Acetaldehyde]
0.5 mol
5.5/60
0.5 mol
5.5 mmol
1 / mg ALDH2 enzyme
5.5 mol / min · 1 / mg ALDH2 · 1 min / 60 sec
0.0925 mmol / mg ALDH2
4% activity for homozygous mutant
V0 =
V0 = Vmax[S] / Km + [S]
0.092 mmol min / mg ALDH2
(ADH conversion rate to ACH − ALDH conversion to … ethanol


X = 0.3 nM
/cuvette = B(1cm)
from Nissle growth curve
time
B(t) = C · P0 e^rt / C + P0(e^rt − 1)
B(1cm) = 4.79×10^8
C = ×10^8 cells
theoretical (based on graph)
Solve for r
8.33 nM acetate/sec
dE/dt = K · B(t)
E(t) = ∫0^x K · B(t) dt = 0.3 nM
solve for K
K = 7.71 × 10^-20 mol ALDH2 / cell · hour
protein expression over time


reduce 200 μM
acetaldehyde
in 1 min
How much protein?
0.24 μM
How long to culture Nissle to get get this amount protein/mL of cells?
How many cells is needed if always culture 1 L?
For manufactured


% Alcohol
[Ethanol]
[I]
Specific Activity: 5.5 mmol/min/ALDH2-g
protein ALDH2: 8.5 mg
Volume: 0.1 mL
sig figs?
initial concentration
Kcat = Vmax / [E0]
Kcat = 1190 /min at pH 9.5
Actually corresponds to catalase
Vmax / Kcat = [E0] = 0.995 mM/min / 1190/min = [8.432 · 10^-7 M]
8.432 · 10^-7 M / 3.413 · 10^-6 M ≈ 24.7%
actually found

Arrhenius Equation: $k = Ae^{\frac{-E_a}{RT}}$ → Activation Energy; → Temp in Celsius

Rate constant; Frequency Factor; The gas constant

or $\ln\frac{k_2}{k_1} = -\frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$

OD [illegible] 1 → 8 · 10^8

0.0[illegible] → [illegible]

[S] ⇌ V_{ADH} V_{ALDH_2}

+1 → 5 → 4

t → 0.0017

Zeroth Order kinetics

$S(t) = [S]_0 - kt$ $k = \frac{d[P]}{dt}$

k = how rapid the rxn occurs at any concentration of [S]

First order

$v = k[S]$

$S(t) = S_0 e^{kt}$

Second order $S + R \rightleftharpoons P + Q$

$k = [S][R]$

Michaelis Menten → Assume no degradation of enzymes

$V_{max} = K_{cat}[E_0]$

$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$

$k_1[E_0][S]$, $k_2[ES]$, $k_3[ES]$

$k_1 = A_1 e^{\frac{-E_{a1}}{RT}}$

$K_M = K$

$k_1 = A_1 e^{\frac{-E_{a1}}{RT}}$

$k_2 = A_2 e^{\frac{-E_{a2}}{RT}}$

$K_M = \frac{k_2 + k_3}{k_1}$

25°C

37°C

[Ethanol]	[S]	initial mM	$\frac{d[ES]}{dt}$	$\frac{d[P]}{dt}$	Δt

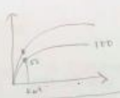
Bacteria Expression (Cont. experiment) ALDH2 Expression

From Literature K_M = 0.2 mM
K_{cat} = 1170/min; pH 9.5

$K_{cat} = \frac{V_{max}}{[E_0]}$

[S] = 14 mM

V_{max} =



purification factor = $\frac{\text{Specific Activity of purified}}{\text{Specific Activity (crude)}}$

Specific Activity = $\frac{\text{2.5 [illegible] acetate}}{\text{min} \cdot \text{ALDH2 mg}}$

Protein ALDH2 (purified) 8.5 mg

Volume: 4 mL = 0.004 L

① $\frac{\text{2.5 } \mu\text{mol of acetate}}{\text{min}} \cdot \frac{1}{\text{ALDH2 mg}}$ (8.5 ALDH2) mg $\cdot \frac{1}{0.004 L}$

$= \frac{0.99468 \text{ mM}}{\text{min}} = V_{max}$ (Assumption)

② $K_{cat} = \frac{V_{max}}{[E_0]}$ $[E_0] = \frac{V_{max}}{K_{cat}} = \frac{0.99468 \text{ mM}}{\text{min}} \cdot \frac{\text{min}}{1170}$ = 8.4395 · 10^{-7} M of ALDH2

(We use the Kcat value at pH 9.5 because it is closer to the pH of the buffer solution where our experiment is conducted)

③ $\frac{8.4395 \cdot 10^{-7} \text{ mol ALDH2}}{L} \cdot \frac{6.0221409 \cdot 10^{23} \text{ molecules}}{1 \text{ mol}} \cdot \frac{L}{1000 \text{ mL}} = \frac{5.0824 \cdot 10^{14} \text{ molecules}}{\text{mL}}$

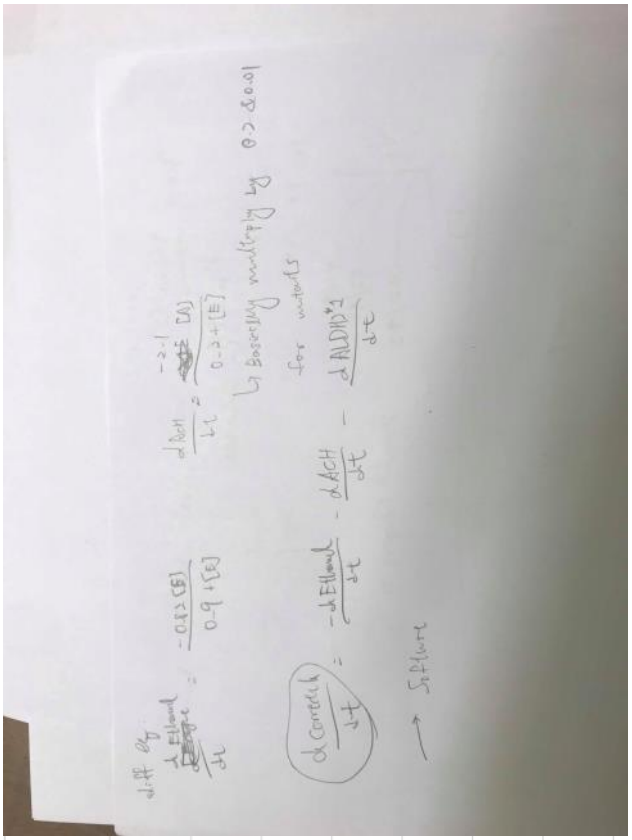
(Once we obtain the concentration of enzymes used in the literature's experiment, we can perform the same experiment with the same concentration of bacteria cells/mL and compare both experiments' V_{max} values. This allows us to obtain the conversion ratio between one bacteria cell to ? # of enzymes)

④ Because the spectrophotometer's cuvette can only contain 3 mL of liquid, the bacteria concentration must be decreased (scaled down) to a certain level for the spectrophotometer to be able to detect/measure absorbance of OD [illegible] for the cuvette to contain the bacteria.

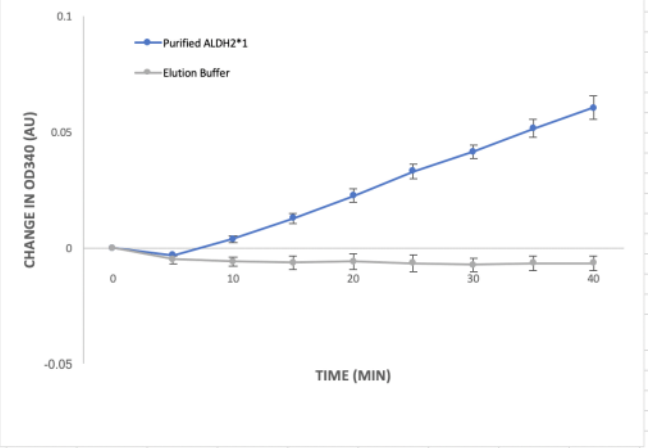
$\frac{5.0824 \cdot 10^{14} \text{ molecules}}{\text{mL}}$ ↔ $\frac{0.99468 \text{ mM}}{\text{min}}$ V_{max}

Scaled down by a factor of [illegible] $\frac{2.5382 \text{ molecules}}{\text{mL}}$ ↔ $\frac{0.49734 \text{ mM}}{\text{min}}$

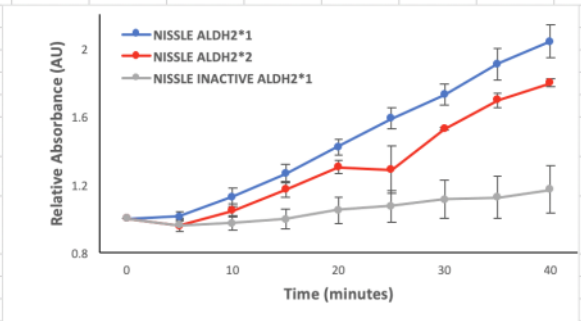
Cont.



Change in ABS									
BOB	0	-0.003	0.006	0.016	0.027	0.038	0.045	0.057	0.066
MUT	1	0.970588	0.980392	0.990196	1.004902	1.019608	1.034314	1.058824	1.073529
E	0	-0.008	-0.009	-0.011	-0.011	-0.012	-0.012	-0.012	-0.012
B	0	-0.004	0.004	0.013	0.023	0.034	0.043	0.053	0.065
M	1	0.979487	0.984615	1	1.015385	1.025641	1.041026	1.061538	1.071795
E	0	-0.005	-0.007	-0.007	-0.007	-0.008	-0.008	-0.007	-0.007
B	0	-0.003	0.001	0.009	0.017	0.027	0.036	0.044	0.05
M	1	0.981818	0.990909	1	1.009091	1.022727	1.036364	1.05	1.063636
E	0	-0.002	-0.002	-0.001	0	0	-0.002	-0.001	-0.001
AVG									
Time (min)	0		10		20		30		40
Purified ALDH2*1	0	-0.00333	0.003667	0.012667	0.022333	0.033	0.041333	0.051333	0.060333
Elution Buffer	0	-0.005	-0.006	-0.00633	-0.006	-0.00667	-0.00733	-0.00667	-0.00667
SE									
B	0	0.000333	0.001453	0.002028	0.002906	0.003215	0.002728	0.003844	0.005175
E	0	0.001732	0.002082	0.002906	0.003215	0.003528	0.002906	0.00318	0.00318



Relative Abs									
ALDH2*1	1	1.0646388	1.2319392	1.3688213	1.5019011	1.6730038	1.7756654	2.0152091	2.1292776
MUT	1	1.0206897	1.1206897	1.2586207	1.3793103	1.4482759	1.5172414	1.7724138	1.837931
BB	1	0.9762712	0.9661017	0.9830508	1.0677966	1.0677966	1.1186441	1.0949153	1.1627119
ALDH2*1	1	0.9785408	1.0515021	1.1759657	1.3476395	1.4678112	1.6008584	1.7253219	1.8454936
MUT	1	0.942623	1.0122951	1.1434426	1.2909836	1.4057377	1.5368852	1.647541	1.7745902
bb	1	1.014218	1.0473934	1.1042654	1.1706161	1.2369668	1.2985782	1.3554502	1.4170616
ALDH2*1	1	0.9843137	1.0941176	1.2431373	1.4117647	1.6196078	1.8	1.9882353	2.145098
MUT	1	0.9066148	1.0038911	1.1089494	1.2373541	1.0038911	1.5252918	1.6614786	1.7782101
bb	1	0.8927039	0.8969957	0.8969957	0.9055794	0.9098712	0.9184549	0.9227468	0.9313305
AVERAGE									
	0		10		20		30		40
Nissle ALDH2*1	1	1.0091644	1.125853	1.2626414	1.4204351	1.5868076	1.7255079	1.9095888	2.0399564
Nissle ALDH2*2	1	0.9566425	1.0456253	1.1703376	1.3025493	1.2859682	1.5264728	1.6938111	1.7969104
Nissle Inactive ALDH2*1	1	0.9610644	0.9701636	0.9947707	1.0479974	1.0715449	1.1118924	1.1243708	1.170368
SE									
	0	0.0277872	0.054451	0.0565203	0.044742	0.0614624	0.0627194	0.0924619	0.0973386
	0	0.0336684	0.0376105	0.0452507	0.0413853	0.1415721	0.0057014	0.0395067	0.0205369
	0	0.0358927	0.0434635	0.0601199	0.0771473	0.094443	0.109784	0.125776	0.1402707



```
# -*- coding: utf-8 -*-
"""
@author: Justin Lin

"""
import numpy as np
import matplotlib.pyplot as plt
from matplotlib import animation
from scipy.integrate import solve_ivp

Alcohol = input("Alcohol type (beer, wine, or spirits): ")

#Codes from line 19 to 27 are questions specific to our project and were included simply out of convenice
#The codes can be taken out if the user wishes to remove them
get_Red = input("Do yo flush red when you drink? (yes or no) ")
ALDH2_type = 'wild'

if get_Red == 'yes':
    question = input("Do you get mildly red or super red? (mild or super)")
    if question == 'mild':
            ALDH2_type = 'heterozygous'
    if question == 'super':
            ALDH2_type = 'mutant'

# Defining the variable ACH_conc
ACH_conc = 0

# Defining constants and enzymatic activity for enzymes. Can be altered
v_max_ADH = 0.82
v_max_ALDH2 = 0.0
v_max_ALDH2_wild = 2.1
km_ALDH2_wild = 0.2
km_ALDH2 = 0.0
km_ADH = 0.9

# Setting ethanol concentration depending on the alcohol beverage
if Alcohol == 'beer':
    ACH_conc = 98
if Alcohol == 'wine':
    ACH_conc = 282
if Alcohol == 'spirits':
    ACH_conc = 446
ethanol_conc = 399*10**6

# calculation for enzyme characteristics
if ALDH2_type == 'wild':
    v_max_ALDH2 = 2.1
    km_ALDH2 = 0.2
if ALDH2_type == 'heterozygous':
    v_max_ALDH2 = 2.1*0.2
    km_ALDH2 = 1.4
if ALDH2_type == 'mutant':
    v_max_ALDH2 = 2.1*0.01
    km_ALDH2 = 1.4

def dX_dt(t, X):
    A1, A2, A4, E = X
```

```
def dX_dt(t, X):
    A1, A2, A4, E = X
    #Can adjust the variable to change input enzyme concentration (unit is μM)
    #0.083 for wine mild en punto, 0.07 too little, 0.1 too much
    #current_enzyme_conc = 0.07
    v_ADH = -(v_max_ADH*E)/(km_ADH + E)
    v_ALDH2 = (v_max_ALDH2*A2)/(km_ALDH2+A2)
    #This is the equation for the wild type aldh2 genotype
    v_ALDH2_wild = (v_max_ALDH2_wild*A1)/(km_ALDH2_wild+A1)
    #Below is enzymatic activity of 0.222μM ALDH2*1
    #Can easily alter enzymatic activity based on the input
    #V_ALDH2_1 = (4.5*A3)/(0.2+A3)*0.15/0.222
    V_ALDH2_2 = (4.5*A4)/(0.2+A4)*0.103/0.222
    #V_ALDH2_3 = (4.5*A5)/(0.2+A5)*0.05/0.222
    #array[0] is wild type genotype, array[1] is mutant genotype, array[2]-[4] are corrected, array[5] is ethanol
    return np.array([60*(-v_ADH - (v_ALDH2_wild)), 60*(-v_ADH - (v_ALDH2)),
                     60*(-v_ADH - (v_ALDH2)-V_ALDH2_2), 60*v_ADH])

total_time = 15
num_steps = 100
t = np.linspace(0, total_time, num_steps)
result = solve_ivp(dX_dt, (t.min(), t.max()), [ACH_conc, ACH_conc, ACH_conc, ethanol_conc], t_eval = t, method = "LSODA", max_step = total_time/num_steps)

title = "Best Modeling"

fig = plt.figure()
#ax = fig.add_subplot(111)
ax = fig.add_subplot(111, xlim=(0,15), ylim=(-10, 600))
ax.set_xlabel('Time(min)')
ax.set_ylabel('Concentration(μM)')
ax.set_title(title)



ax.plot(result.t, result.y[0], 'r-', label = 'ACH (wild type genotype)')
ax.plot(result.t, result.y[1], 'g-', label = 'ACH (mutant-before)')
ax.plot(result.t, result.y[2], 'k.', label = 'ACH (mutant-after, 0.103μM)')

ax.legend()
```