(1) Introduction

The aim of the first experiment is to produce our own ethanol solution through fermentation. The aim of the second experiment is to determine the concentration of the unknown sample of ethanol (i.e. the sample of ethanol we produced). In order to do so, we plan to generate a calibration curve using standard solutions of ethanol, such that we can derive the unknown concentration of ethanol from the same curve.

The independent variable is the ethanol concentration, with standard solutions of ethanol concentrations of 2%, 4%, 6%, 8%, 10%, 12%, 14% and 16% prepared for us in the Eppendorf tubes already. The dependent variable is the absorbance value that is obtained through spectrophotometry. The controlled variables are the amount of ethanol (i.e. 10 µl of each standard solution), potassium dichromate (i.e. 100 µl of 0.25M $K_2Cr_2O_7$) and sulfuric acid (i.e. 100 µl of 6M H_2SO_4) added to each well, as well as the time of reaction (i.e. 5 minutes) between ethanol, and the potassium dichromate and sulfuric acid mixture.

(2) Results and Discussions

<u>First experiment</u>

Glucose was added to 40ºC H_2O to prepare a suitable environment for yeast to respire. The anaerobic respiration of yeast produces carbon dioxide and ethanol. After adding yeast to the glucose solution in our conical flask, we quickly swirled to mix the mixture, then promptly placed a balloon over the mouth of the conical flask. This is to prevent oxygen gas from entering so that only anaerobic respiration takes place (instead of aerobic respiration).

Observations:

The balloon inflated after being incubated at 30ºC for about 24 hours. This shows that gas was being produced by the mixture in the conical flask.

Second experiment

30ml from the mixture from the first experiment was transferred to a 50 ml Falcon tube and centrifuged for 5 minutes at 4200rpm so that the yeast settles down at the bottom of the tube. After centrifuging, 1ml of the supernatant (i.e. ethanol) is transferred into an Eppendorf tube. The ethanol concentration in this tube is meant to be determined. 15ml of the remaining supernatant is transferred into another 15ml Falcon tube to be used as fuel for the rocket launch. Into 2 rows of 10 wells on the 96 well-plate, we added 100 µl of 0.25M potassium dichromate and 100 µl of 6M sulfuric acid to each well. Next, we proceeded to add 10 µl of the different concentrations of standard solutions to each well, with the first well as a blank. The unknown concentration of ethanol was added to the last well.

Observations:

$2\ K_2Cr_2O_7(aq) + 8\ H_2SO_4(aq) + 3\ C_2H_5OH\ (aq) \rightarrow 2\ Cr_2(SO4)_3(aq) + 2\ K_2SO_4(aq) +$
$3\ CH_3COOH\ (aq) + 11\ H_2O(l)$

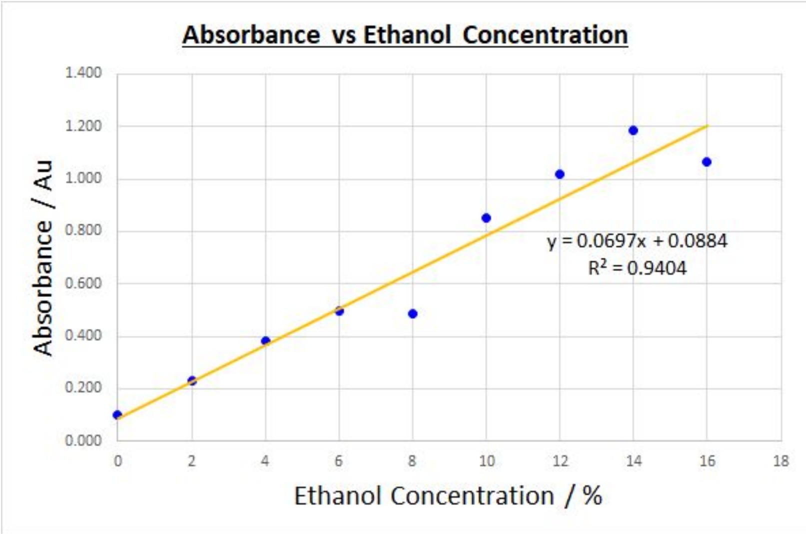
There is a colour gradient from the left to the right, with increasing colour intensity from orange to green. This is because the first well has no ethanol added to it, hence there is no oxidation of ethanol into ethanoic acid which results in the colour change, thus it remains orange. To each well on the right, we added standard solutions of increasing concentrations of ethanol, hence, the colour change became more prominent across the row, with the exception of the last well, as that contains an unknown concentration of ethanol.

Tables:

Standard Number	1	2	3	4	5	6	7	8	9
Standard Concentration/%	0 (Blank)	2	4	6	8	10	12	14	16

	Ethanol concentration/ %	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**Unknown**
Absorbance/Au	**Reading 1**	0.099	0.192	0.373	0.518	0.563	0.829	1.029	1.228	0.999	1.241
	Reading 2	0.098	0.267	0.393	0.471	0.405	0.876	1.008	1.144	1.132	1.625
	Average reading	0.099	0.230	0.383	0.495	0.484	0.853	1.019	1.186	1.066	1.433

Graphs:



We used a linear fit for our graph as it is the best fit. It takes into account the whole range of data points. From linear regression, $y = 0.0697x + 0.0884$, where y is absorbance and x is concentration, $R^2 = 0.9404$, $x = \frac{(y - 0.0884)}{0.0697}$, we derived that the absorbance of the unknown is 1.433. Substituting this value of y in the above equation to get the value of the unknown concentration, $x = \frac{(1.433 - 0.0884)}{0.0697} = 19.291$. Assuming that our graph will continue linearly beyond 16% ethanol concentration, the concentration of our unknown sample of ethanol is 19.291%.

We performed a blank subtraction to determine the background readings, corresponding to the buffer alone with the reagents. We also prepared 2 wells of the same sample, known as technical replicates, to account for technical error (e.g. pipetting error) and reproducibility between the data points, so that the reliability of the data is increased. However, we could have improved the experiment by doing triplicates instead. This is because with duplicates, we are not able to tell which data points are more reliable when 2 data points are far apart. By doing triplicates, it is possible to identify which of the data points may be the outlier. In addition, we used a microplate instead of a cuvette for this experiment as we needed to record the absorbance of many solutions at the same time. Moreover, only a small volume of the solution is needed, and the 96-well plate method allows high throughput measurements, often required in laboratory research involving hundreds of samples.

(3) Summary

From the second experiment, we have determined the concentration of the unknown sample of ethanol to be 19.291% using the calibration curve we have plotted using the absorbance values obtained from conducting spectrophotometry for the solutions of potassium dichromate and sulfuric acid that we have added the standard solutions and the unknown to. We have also tested the efficiency of the ethanol solution we have produced through the first experiment during the rocket launch.

(4) Questions

Q1) The oxidized and reduced form of nicotinamide adenine dinucleotide (NAD) are NAD_{ox} (NAD^+) and NAD_{red} (NADH) respectively. In the absence of oxygen, pyruvate will undergo either lactic acid fermentation or alcohol fermentation. In the process of fermentation, no ATP is produced and NAD_{red} is oxidized to become NAD_{ox}. The regenerated NAD_{ox} is used in glycolysis to yield 2 net ATP per glucose molecule. In alcohol fermentation, pyruvate is converted to acetaldehyde first, releasing CO_2, before being converted into ethanol and in this process, regenerating NAD_{ox} from NAD_{red}.

Glycolysis	Glucose + $2NAD^+$ + 2ADP + 2Pi → 2Pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$
Fermentation	2Pyruvate → 2Acetaldehyde + $2CO_2$
	2Acetaldehyde + 2NADH → $2C_2H_5OH$ + $2NAD^+$

Q2)

$\Delta H°rxn$

$= \Delta H°products - \Delta H°reactants$

$= \Delta H°(2Pyruvate + 2ATP + 2NADH + 2H^+ + 2H_2O) - \Delta H°(Glucose + 2NAD^+ + 2ADP + 2Pi)$

$= (2 \times -597.04 + 2 \times -2995.59 + 2 \times -41.38 + 0 + 2 \times -286.65) -$

$(-1267.11 + 2 \times -10.26 + 2 \times -2005.92 + 2 \times -1299.39)$

$= 56.93\ kJmol^{-1}$

$\Delta G°rxn$

$= \Delta G°products - \Delta G°reactants$

$= \Delta G°(2Pyruvate + 2ATP + 2NADH + 2H^+ + 2H_2O) - \Delta G°(Glucose + 2NAD^+ + 2ADP + 2Pi)$

$= \Delta G°(2 \times -350.78 + 2 \times -2097.89 + 2 \times 1120.0 + 0 + 2 \times -155.66) -$

$\Delta G°(-426.71 + 2 \times 1059.11 + 2 \times -1230.12 + 2 \times -1059.49)$

$= -80.77\ kJK^{-1}mol^{-1}$

$\Delta G° = \Delta H° - T\Delta S°$

$-80.77 = 56.93 - (273 + 30)\Delta S°$

$\Delta S°rxn = \frac{(-80.77-56.93)}{(-303)} = 0.45446\ kJK^{-1} = 454.46\ JK^{-1}$

The entropy has a positive sign at 30℃. This is because there is an increase in disorder in the system as glycolysis occurs. This is because there are more moles of products as compared to reactants as seen from the equation of the glycolysis reaction.

Q3) No, the alcohol fermentation was not done in perfectly anaerobic conditions. In the experimental set-up, the glucose was still exposed to oxygen before the balloon was put around the mouth of the conical flask. Moreover, the water added to the mixture has dissolved oxygen in it. Pyrogallol can be added to make the conditions more anaerobic as it reacts with oxygen to form benzoquinone and water. Alternatively, nitrogen gas can also be added to make the conditions more anaerobic as it also reacts with oxygen to form nitric oxide. Thus, the above two chemical compounds will be able to remove oxygen from the experimental set-up, making the conditions more anaerobic.

Q4) To ensure the redox reaction is a reliable way to estimate alcohol content, we can repeat the experiment for a minimum of 3 times. If the results are relatively constant, then the reliability of the redox reaction is ensured. In the redox reaction, ethanol is oxidized to ethanoic acid while the dichromate ions are reduced to chromium 3^+ ions.

Another way to estimate ethanol would be to burn ethanol through a combustion reaction and calculate its amount through calorimetry.

$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$

In the combustion reaction, the carbon in ethanol is oxidised, with its oxidation number increasing from -2 in C_2H_5OH to +4 in CO_2, while oxygen is reduced, with its oxidation number decreasing from 0 in O_2 to -2 in both CO_2 and H_2O. In the combustion reaction, ethanol is burned and the temperature increase is measured. The equation $Q = mc\Delta T$, where m is the mass of ethanol burnt, c is the specific heat capacity of water and ΔT is the change in temperature, can be used to calculate the amount of heat energy released when burning ethanol, and the equation $\Delta H = \frac{Q}{n} = \frac{mc\Delta T}{n}$, where n is the number of moles of ethanol, can be used to calculate the enthalpy change of combustion of ethanol. As the enthalpy change of combustion is proportional to the quantities of reactants and products, the amount of ethanol can be estimated through this method.

Q5) Carbon dioxide. Colorless gas bubbles formed during the experiment. This indicated that a gas was being produced. In addition, at the end of the experiment, the balloon expanded and became upright as it was filled with the gas that was produced. The gas can be identified by adding it into calcium hydroxide. If a white precipitate (i.e. calcium carbonate) forms, then the gas is CO_2.

Q6)

0.76 torr = 0.001atm

$KH = \frac{0.001atm}{[X(aq)]} = 29$

$[X(aq)] = 3.448 \times 10^{-5} molL^{-1}$

Reaction	H_2O (*l*) + X (*aq*) ↔ H^+ (*aq*) + HXO^- (*aq*)			
Initial	-	3.448×10^{-5}	0	0
Change	-	$-x$	$+x$	$+x$
Equilibrium	-	$3.448 \times 10^{-5} - x$	x	x

$$\frac{x^2}{(3.448\times10^{-5}-x)} = 4.5 \times 10^{-7}$$
$$x = 3.72045 \times 10^{-6} = 4.5 \times 10^{-7} = [HXO^{-}] = [H^{+}]$$
$$pH =- log[H^{+}] =- log(3.72045 \times 10^{-6}) = 5.4294 < 6$$
Fermentation would cease as pH is below 6.

Q7) The greater the O_2 concentration, the lower the rate of ethanol production. 0% dissolved oxygen has the highest rate of ethanol production while 100% dissolved oxygen has the lowest rate of ethanol production. This is because the greater the O_2 concentration, the more likely glucose will undergo aerobic respiration, and thus the rate of anaerobic respiration to produce ethanol would decrease. There is a saturation point for ethanol production as there is a limited amount of glucose molecules available to act as the substrate for glycolysis, in turn producing a limited amount of pyruvate, the substrate for alcohol fermentation.

Q8b) No, the ethanol that we produced in the laboratory was not able to launch the rocket. This is because the ethanol produced was too diluted (i.e. had a very low concentration). It is given that the yeast we used for the experiment to synthesize alcohol is primarily used for brewing alcohol. This could possibly be why the concentration of ethanol we produced was very low as on average, the amount of alcohol content in alcoholic drinks is not very high (i.e. 3.5 - 9% for beer and 5 - 21% for wine), hence when using the same type of yeast to synthesize ethanol, a similar concentration of ethanol would be produced (i.e. 19.3%). A different type of yeast that has a higher rate of anaerobic respiration can be used during the fermentation process. In this way, the higher rate of anaerobic respiration would lead to a higher rate of pyruvate getting converted into acetaldehyde and eventually ethanol, increasing the ethanol concentration, making it more likely for the rocket to be launched using it. On the other hand, the amount of water added to the glucose at the start can be decreased, or even removed. If it is removed, the conical flask of glucose and yeast can be put in a water bath of 40ºC instead to provide yeast with a suitable environment to respire. In this way, the concentration of ethanol would increase as well, increasing the likelihood of the rocket being launched using it.

Appendix

Reference: https://winefolly.com/tutorial/alcohol-content-in-wine/