The Optimum Conditions for Yield of Ethanol by Fermentation

Name: Jieqiong Yang
SQA number: 020496096
Centre: Harris Academy
Centre number: 5329531)

CONTENTS

Summary	**3**
Introduction	**4**
Procedures	**7**
Apparatus and Set Up	7
Particle Size	8
Concentration	9
Temperature	9
pH of Solution	10
Results	**11**
Particle Size	11
Concentration	12
Temperature	14
pH of Solution	16
Discussion	**18**
Conclusion	18
Evaluation of Procedure	19
Evaluation of Results	21
Suggestions for Further Work	22
Raw Data	**23**
Appendix	**29**
References	**30**
Acknowledgements	**31**

SUMMARY

The aim of the investigation was to find the optimum conditions for yield of ethanol by fermentation.

The optimum conditions for yield of ethanol by fermentation were found to be:

- Small particle size
- 0.5M (concentration of solution)
- 40°C (temperature)
- pH 5.65
- Inhibition of reaction at after 1 hour

INTRODUCTION

The aim of the investigation was to find the optimum conditions for yield of ethanol by fermentation.

Such findings would allow industries to rapidly speed up and increase the efficiency of ethanol production. Therefore saving money and time. Higher production efficiency also decreases the strain on resources. When in competition, these factors become the basis of the company’s survival.

The fermentation method is used to make alcoholic drinks. Fruit juices such as grape juice contain the sugar glucose, $C_6H_{12}O_6$ *(Figure 1). When yeast is added, the sugar ‘ferments’ to form wine (a solution of ethanol) and carbon dioxide:* (Ramsden 1994)

$$\mathbf{C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2\ C_2H_5OH + 2\ CO_2}$$

Glucose   Ethanol   Carbon Dioxide

Yeasts are unicellular fungi that reproduce asexually by budding or fission. From the yeast's point of view, alcohol and carbon dioxide are waste products, and as the yeast continues to grow and metabolise in the sugar solution, the accumulation of alcohol will become toxic when it reaches a concentration between 14-18%, thereby killing the yeast cells. (Wong 2003)

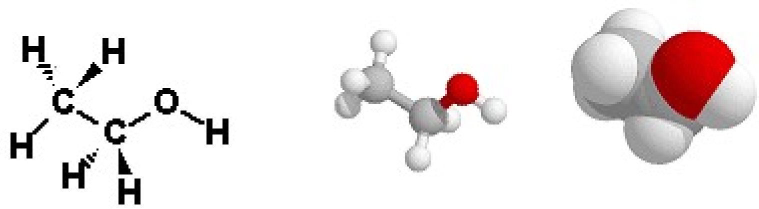

Figure 1
Ethanol Molecule

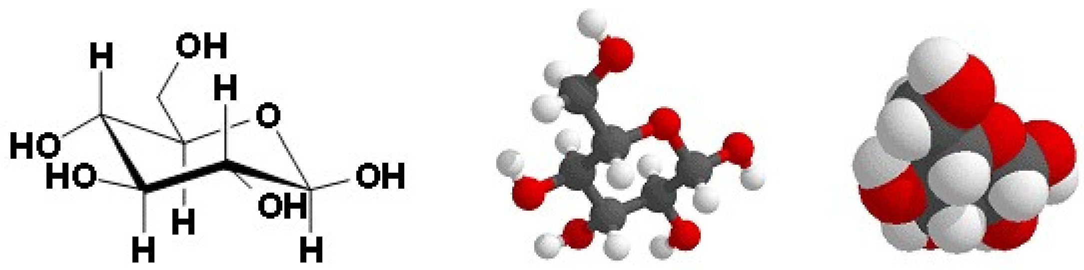

Figure 2
Glucose Molecule

The experiments were carried out where one condition was altered each time to determine the value of the condition at which maximal ethanol synthesis occurs. Each time, this maximum value was kept constant thoughout the rest of the experiment whilst other conditions were tested for its most effective point in producing ethanol. In the end, maximum ethanol synthesis was achieved by a collection of maximum conditions.

Particle size of yeast
As particle size of a substance decrease i.e. from granules to powder, the total surface area of that substance will increase. Therefore, the chances of collision increases, which results in an increase in the number of collisions. Hence, speeding up the rate of reaction. This means, theoretically, the rate of reaction should be faster when powdered yeast is used instead of yeast granules.

Concentration of overall solution
As the concentration of a solution increases, the number of reactant molecules within the solution increases (whilst the volume stays the same). Consequently, the number of collisions will increase, speeding up the rate of reaction. Hypothetically the rate of reaction should increase as the concentration increases. However, there may be an optimum concentration above of which the rate starts to decrease.

Temperature
Temperature control during alcoholic fermentation is necessary to (1) facilitate yeast growth, (2) extract flavours and colours from the skins, (3) permit accumulation of desirable by-products, and (4) prevent undue rise in temperature, killing the yeast cells. (Uva 2005)
Therefore, yeast's sensitivity to temperature means that temperature is an important factor of ethanol production.
Since yeast is an enzyme (zymase), it will denature at a certain temperature, above which the enzyme will stop working as a catalyst and the reaction will slow down or stop completely.

pH of solution
When Pasteur realised that yeast was responsible for the conversion of glucose to alcohol, he also revealed that the metabolism of yeast was pH-dependent. (Uva 2005)
Therefore, the pH of the yeast solutions used was measured to determine the optimum pH for ethanol production.

Another important factor of industrial ethanol production efficiency would be the point at which fermentation is stopped. In most chemical reactions, the rate of reaction decreases as the reaction proceeds. This means higher efficiency might be achieved by not allowing the reaction to reach its endpoint (completion). Instead, the reaction is inhibited before the rate of reaction becomes too slow, because it may be more this way as ethanol is constantly produced at the highest possible rates.

PROCEDURE

Apparatus and Set Up

Equipment

Water bath
4x 50ml beaker
10ml Measuring cylinder
Glass stirring rod
Weight balance
4x test tube
4x 100ml measuring cylinder
4x gas delivery tube
4x rubber test tube stopper
Thermometer
Spatula

Assembled apparatus

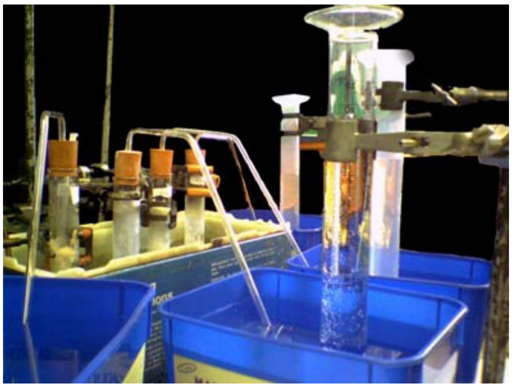

Figure 3.1
Assembled Apparatus (1)

Figure 3.2
Assembled Apparatus (2)

Figure 4
Diagram of Experimental Set Up

Particle size

Experiment 1
Granules of Yeast - Bigger Particle size
Four different concentrations of glucose solutions were made. 0.4g of glucose was dissolved in 9.2ml, 9.9ml, 10.9ml, and 12.3ml of deionised water to make the solutions of concentrations 0.22M, 0.204M, 0.185M and 0.164M respectively. The glucose solutions were poured into test tubes and 0.4g of yeast granules were added to each concentration of glucose solution. The test tubes were placed inside a water bath set at 35°C. The CO_2 gas was collected as shown in Figure 4.

Experiment 2
Yeast powder – Smaller Particle size
0.4g of yeast granules were dissolved in 4ml of deionised water. A glass-stirring rod was used to whisk the solution to help the yeast granules to dissolve faster. Four of the same yeast solution was made in separate beakers and placed in a preheated bath water of 35°C for 10 minutes. Later, the beakers were taken out of the water bath and 0.4 grams of glucose were added to each solution. 5.2ml, 5.9ml, 6.9ml and 8.3ml of deionised water were added to make the overall solutions of concentrations 0.22M, 0.204M, 0.185M and 0.164M respectively. Each solution was poured into a test tube, placed in the water bath set at 35°C. The CO_2 gas was collected using the same method as Experiment 1.

Concentration

Experiment 3
Four yeast solutions were made using the same method and quantity as Experiment 2. 0.4g of glucose was added to each solution. 5.9ml, 5.6ml, 5.2ml and 2.7ml of deionised water were added to make the overall solutions of concentrations 0.204M, 0.21M, 0.22M and 0.3M respectively. Each solution was poured into a test tube and the same procedures were used for the collection of CO_2 gas. The water bath was heated to 35°C.

Experiment 4
The exact same procedures were repeated for the concentrations 0.3M, 0.4M and 0.5M. 2.7ml and 1.1ml of deionised water were added to make the 0.3M and 0.4M solutions respectively. No more deionised water was added to the third solution because it was already 0.5M.

Calculation 1

Temperature

Experiment 5
Four yeast solutions were made using the same method and quantity again. 0.4g of glucose was added to each solution. Two of each of the highest concentrations tested (from Experiment 4) were used this time. 1.1ml of deionised water were added to make the 0.4M solutions and none were added for the 0.5M solutions. The solutions were poured into test tubes and set up for collection of CO_2 gas. The water bath was set to 40°C.

Another exact same batch of solutions were made and placed in a water bath of 45°C. The same procedures were repeated for the temperatures 50°C, 60°C, then 55°C.

pH of solution

Experiment 7
Four different pH of solutions were made from different proportions of 0.1M citric acid, 0.2M disodium phosphate and distilled water each time. 0.4g of yeast was added to each solution and then heated in a water bath of 35°C for 10 minutes. 0.4g of glucose was then added to each solution and the pH of each solution was measured using a pH meter. The solutions ranged from pH3.9 to pH7.3. The water bath was set to 45°C.

Experiment 6 was reapeatd with a different batch of pHs of solutions. This time, the pHs ranged around the pH that produced CO_2 gas at the highest rate (from Experiment 6). The same experimental procedures were repeated another two times to narrow down to the pH of which prompted the highest CO_2 production rate, since this would be the optimum pH for ethanol synthesis.

See Appendix (page 29) for recipe of pH solutions

Best time for discontinuation of reaction

The rate of reaction for each fermenting solution was monitored throughout all the experiments to determine the preferable timing for the discontinuation of the reaction. (See Introduction page 4 for reason)

This specific time can be determined by the measurement of rate of reaction over a period of time or simply the inspection of graph 9. (See Results page 16)

Measurements and observations

The quantity of ethanol produced in each experiment was obtained by measuring the volume of CO_2 gas released. The set up shown in Figure 4 was used to collect the carbon dioxide gas

The volume of CO_2 released was recorded at regular intervals of every ten to twenty minutes.

A thermometer was placed inside the water bath to show the degree of fluctuation in temperature. This was used to account for the errors during the analysis of results.

Air was already trapped in the measuring cylinder before CO_2 gas collection in most cases. Therefore, raw data had to be processed before graphs can be plotted.

RESULTS

Particle Size

Concentration

Temperature

pH of solution

DISCUSSION

Conclusion

The optimum conditions for yield of ethanol by fermentation are:

- Small particle size
- 0.5M (concentration of solution)
- 40°C (temperature)
- pH 5.65
- Inhibition of reaction at after 1 hour

However, these may not be the most favourable conditions when synthesizing ethanol industrially as experiments would need to be carried out using greater quantities of reactants. And scaling up the quantities of substrate may alter the optimum conditions.

Evaluation of experimental procedures

The first trial of fermentation involved the following set up:

Figure 5

This did not produce any CO_2 gas when left overnight. For the second trial, the glucose concentration was increased from the previous 0.11M to 0.31M. However this alteration did not produce any CO_2 either. By the third trial, the glucose solution was heated to 45°C then cooled to 35°C before adding the yeast, but still no CO_2 was produced. The solution was left overnight with paper towel wrapped around the test tube containing the solution as a form of insulation. This was unsuccessful. However when the solution was heated with a Bunsen burner the next day to 45°C, 7cm^3 of gas was produced. Unfortunately, this could have been the expansion of the air that was already trapped in the test tube due to the increase in temperature.

For the fourth trial, a water bath was incorporated into the set up and a different method of CO_2 gas collection was also used:

Figure 6

Both methods of gas collection worked this time. But since the syringe did not work the previous times, the measuring cylinder was considered to be a more reliable way for gas collection and was used for all future experiments. The success of CO_2 gas production was due to the use of a water bath, which maintained the temperature of 35°C over night. Hence, the usage of a water bath for further experiments.

Errors occurred when measurements and observations were made:

- A 10ml-measuring cylinder was used to measure the volume of deionised water needed to make the yeast solutions. Measurements were recorded to the nearest 0.1ml. Therefore the degree of error was 1%.
- When the volume of CO2 gas was recorded, the volumes were read off a 100ml-measuring cylinder. The measurements were recorded to the nearest 0.5ml. Therefore the degree of error was 0.5%.
- The same four beakers were used to make the solutions before poured into a test tube. Sometimes there was not enough time to wait for the beakers to dry off completely after wash. Therefore when the wet beakers were used to make another batch of solutions, the small quantity of deionised water that was left in the beaker diluted the new solutions slightly. This induced an error in the concentration of solutions.
- The mixture of yeast and glucose solution was slightly viscous. Consequently when the solution was poured from the beaker into a test tube, some of the solution was left in the beaker. This caused a reduction in the total quantity of substrate from the intended quantity, therefore, inducing a slight error.
- The thermometer in the water bath showed that there was a fluctuation in the temperature of water within the water bath. +- 5°C of the intended temperature occurred as the heater switched on and off (to maintain a fairly constant temperature). This great variation in temperature induced an error of which in particularly reduced the reliability of the results obtained from the experiments testing for the optimum temperature for yield of ethanol.
- 4ml of deionised water was added to the yeast granules and glucose powder. The solutions should have been made up to 4ml. This induced a considerable error to the overall investigation, as the total volumes of solutions were unknown.

<u>Possible improvements</u>

The biggest limitation to this investigation was time. The fermentation process is a comparably slow reaction; each batch of solutions required 2 to 3 hours to produce results that were analysable. Greater allowance of time would aid a greater number of repetitions of experiments. This would significantly increase the reliability of the concluded values of optimum conditions.

The experiments lacked replications because for each variable, there was a wide range to be covered. It was more important to obtain results from a wider range of each variable and have an extensive appreciation of how the rate of ethanol production varied with each condition than to obtain fewer specific values from replicated experiments.

Narrower measuring cylinders could be used to measure the volume of CO_2 gas produced to gain more precise measurements. Thus, increasing the accuracy of the results.

Evaluation of results

Particle Size
Graphs 1 and 2 show different concentrations of solutions. But both graphs show that the rate of CO_2 production was greater when yeast was used instead of yeast granules.

However, an average of 10 minutes was needed to made yeast solutions. Consequently, this time factor should be taken into consideration when deciding which is the most efficient way of obtaining ethanol.

Concentration
Graph 3 shows that the 0.5M solution produced CO2 gas with the highest rate.

Graph 4 and 5 show the as the concentration of solution increased from 0.3M to 0.5M, the rate of reaction increased.

The pink dots were disregarded because they were results of an early experiment – when techniques were not perfectly mastered.

Higher concentrations should have been tested to identify the optimum concentration for ethanol production.

Temperature
Graph 6 shows that the rate of reaction was fastest at 40°C for a period of 90 minutes. However, the initial rate of reaction was the greatest at 45°C (the first 55 minutes).

Graphs 7 and 8 show the optimum temperature is around 40°C to 42°C. Therefore, if the reaction was allowed to proceed for a short period of time (around 40 minutes), a temperature close to 45°C should be used for the most efficient ethanol attainment.

pH
Graph 9 shows that pH4.94 produced the largest volume of CO_2 after 160 minutes. But the initial rate of reaction at pH5.65 was much higher. Therefore, according to Graph 9, if the reaction was inhibited any time before 130 minutes, pH 5.65 is the optimum pH. Graphs 10 and 11 confirm the optimum pH is around 5.65.

Best time for discontinuation of reaction
This was determined using Graph 9, because it represented an experiment carried out nearest to the optimum conditions. The rate of reaction differs drastically between the first hour and the second hour of the reaction. Almost 50% of maximum ethanol production was achieved by the end of the first hour. There was only an increase of around 16 percent during the next hour as the rate of reaction began to decrease. This may be a result of high concentrations of ethanol poisoning the yeast. Therefore it may be wise to stop the reaction after 1 hour.

Suggestions for further work

Different substrates (such as sucrose or fructose) could have been used whilst keeping the same kind of yeast to find out which reactant prompted a greater rate of alcohol synthesis.

Different types of yeast could have been used to examine which type of yeast maximised ethanol production.

RAW DATA

Particle size
Graph 1 / Graph 2

Time (minutes)	Volume of Gas (cm³)			
	0.164M	0.185M	0.204M	0.22M
0	25.0	7.0	8.0	16.0
120	61.0	45.0	50.0	55.0
130	65.0	48.0	52.0	56.0
140	68.0	52.0	55.0	60.0
150	73.0	56.0	59.0	64.0
160	76.0	59.0	62.0	68.0

Trial 6 / 35°C
Table 1

Time (minutes)	Volume of Gas (cm³)			
	0.164M	0.185M	0.204M	0.22M
0	0.0	0.0	5.0	6.0
25	3.0	6.0	8.5	14.0
90	12.0	27.0	30.0	35.0
110	17.0	34.0	36.0	43.0
125	21.0	38.0	40.0	47.0
135	23.0	40.0	42.5	49.0
150	27.0	44.0	45.0	53.0
165	31.0	47.0	48.0	57.0
210	32.0	49.0	49.0	59.0
180	34.0	52.0	51.0	61.0

Trial 7 / 35°C
Table 2

Concentration
Graph 3 / Graph 4 / Graph 5

Time (minutes)	Volume of Gas (cm^3)		
	0.30M	0.40M	0.50M
0	8.0	8.0	13.0
20	9.5.0	11.0	16.5
30	12.0	13.5	19.5
40	15.0	17.0	24.0
50	20.0	21.0	28.0
60	23.0	25.0	33.0
70	28.0	28.5	36.0
80	33.0	34.0	42.0
100	39.0	40.0	48.0
110	44.0	44.0	52.0
130	52.0	51.0	59.0

Trial 9 / 35°C
Table 3

Time (minutes)	Volume of Gas (cm^3)			
	0.20M	0.21M	0.22M	0.3M
0	9	6.5	6	-*
15	10	8	8	-
25	13	12	11	-
35	15.5	16	16	-
45	19	19.5	21	-
60	25	27	28	17
70	28.5	30	32	19
80	32	35	36	22.5
90	36	40	41	26
100	40	44	45	30

Trial 8 / 35°C
*column disregarded due to faulty apparatus
Table 4

Temperature
Graph 6 / Graph 7 / Graph 8

Time (minutes)	Volume of Gas (cm³)		
	0.30M	0.40M	0.50M
0	8.0	8.0	13.0
20	9.5.0	11.0	16.5
30	12.0	13.5	19.5
40	15.0	17.0	24.0
50	20.0	21.0	28.0
60	23.0	25.0	33.0
70	28.0	28.5	36.0
80	33.0	34.0	42.0
100	39.0	40.0	48.0
110	44.0	44.0	52.0
130	52.0	51.0	59.0

Trial 9 / 35°C
Table 5

Time (minutes)	Volume of Gas (cm³)				Average Volume of Processed Data (cm³)
0	10.0*	15.0	8.0	10.0	0.0
10	14.0	17.0	10.0	12.0	2.0
20	18.0	21.0	14.0	16.0	6.0
30	23.0	26.0	19.0	21.0	11.0
45	29.0	31.0	24.0	26.0	16.0
50	31.0	34.0	26.0	28.0	18.3
60	34.5	37.0	30.0	31.0	21.7
70	38.0	40.0	32.0	34.0	24.3
82	42.0	45.0	37.0	38.0	29.0
90	45.0	47.0	40.0	40.0	31.3

Trial 16 / 40°C / 0.5M
*column disregarded due to faulty apparatus
Table 6

Time (minutes)	Volume of Gas (cm^3)				Average Volume of Processed Data (cm^3)
0	8.0	8.0	8.0	11.0	0
10	11.5	11.0	13.0	13.5	3.5
25	18.5	15.0	20.0	18.0	9.1
30	20.5	16.5	21.5	20.0	10.9
40	25.0	20.0	26.0	23.0	14.8
50	28.0	23.0	29.0	25.0	17.5
60	30.0	25.0	31.0	27.0	19.5
70	33.0	27.5	34.0	29.5	22.2
80	35.0	29.5	36.0	32.0	24.4
95	39.0	33.0	40.0	34.5	28.8
110	42.0	36.0	43.0	38.0	31.0
160	49.0	43.0	49.5	45.0	37.9
175	50.0	45.0	51.0	47.0	39.5
185	51.0	46.0	52.0	48.0	40.5

Trial 12 / 45°C / 0.5M
Table 7

Time (minutes)	Volume of Gas (cm^3)				Average Volume of Processed Data (cm^3)
0	8.0	10.0	11.0	7.0	0
10	11.0	11.0	12.0	8.0	1.5
20	13.0	13.0	13.0	10.0	3.3
30	15.0	15.0	15.0	12.0	5.3
50	20.0	20.0	18.0	16.5	9.6
60	21.0	21.0	20.0	17.5	10.9
70	23.0	23.0	21.0	19.0	12.5
80	25.0	25.0	23.0	21.0	14.5

Trial 22 / 50°C / 0.5M
Table 8

Time (minutes)	Volume of Gas (cm^3)				Average Volume of Processed Data (cm^3)
0	25.0	26.0	23.0	23.0	0
10	28.0	28.0	25.0	24.0	2.0
20	29.0	28.5	25.5	24.0	2.5
30	29.0	29.0	26.0	24.0	2.8
40	29.0	29.0	26.0	24.0	2.8

Trial 23 / 60°C / 0.5M
Table 9

pH of Solution
Graph 9 /Graph 10 / Graph 11

Time (minutes)	Volume of Gas (cm^3)			
	pH 3.90	pH 4.96	pH 7.23	pH 7.27
0	14.0	23.0	17.0	14.0
10	14.0	26.0	17.0	14.0
20	14.0	28.0	18.0	14.0
30	14.0	32.0	19.0	14.0
120	14.0	55.0	40.0	37.0
130	15.0	58.0	44.5	41.5
140	15.5	60.5	48.0	45.0
150	16.0	62.0	51.0	47.0
160	16.5	64.0	54.0	50.0
170	17.0	65.5	56.0	52.0

Trial 18 / 45°C / 0.5M
Table 10

Time (minutes)	Volume of Gas (cm^3)			
	pH 3.86	pH 4.12	pH 4.62	pH 4.98
0	14.0	17.0	9.0	13.0
10	14.0	17.0	11.0	19.0
20	14.0	17.0	14.0	24.0
30	14.0	17.0	17.0	28.0
40	14.0	17.0	20.0	32.5
50	14.0	17.0	23.0	36.0
60	14.0	17.0	26.0	40.5
70	14.0	17.0	29.0	43.5
85	15.0	17.0	33.0	48.5
90	15.0	17.0	35.0	51.0
100	15.0	17.0	37.0	53.5

Trial 19 / 45°C / 0.5M
Table 11

Time (minutes)	Volume of Gas (cm^3)			
	pH 4.53	pH 4.94	pH 5.65	pH 6.25
0	16.0	15.0	37.0	12.0
10	17.0	18.0	48.0	19.0
20	22.0	31.0	60.0	28.0
30	26.0	38.0	68.0	35.5
40	30.0	43.0	75.0	41.5
50	34.5	49.0	82.0	48.0
60	38.0	53.5	87.0	53.5
70	43.0	59.0	92.0	59.0
80	46.0	62.5	97.0/7.0*	63.0
135	65.0	83.0	15.0	74.0
145	67.0	85.0	15.0	74.0
150	68.5	86.0	16.0	74.5
160	71.0	87.0	16.0	75.0

Trial 20 / 45°C / 0.5M
*Changed measuring cylinder, started with 7cm^3
Table 12

Time (minutes)	Volume of Gas (cm^3)			
	pH 5.31	pH 5.71	pH 6.08	pH 6.22
0	12.0	6.0	11.0	11.0
10	20.0	17.0	19.0	16.0
20	26.5	25.0	26.5	22.0
30	34.0	34.0	35.0	27.0
40	39.0	39.0	39.0	31.0
50	43.0	45.0	45.0	35.0
60	47.0	49.0	50.0	39.0
70	50.0	53.0	54.0	41.0
80	54.5	56.0	58.0	45.0

Trial 21 / 45°C / 0.5M
Table 13

APPENDIX

pH	vol. of c.a. (ml)	vol. of d.h.p (ml)	vol. of distilled water (ml)
9.2	0	3.2	0.8
8.1	0.1	3.1	0.8
7.1	0.7	2.5	0.8
6.1	1.1	2.1	0.8
5.2	1.6	1.6	0.8
3.9	2.1	1.1	0.8
2.5	3.2	0	0.8

c.a. – 0.1 M citric acid
d.h.p. – 0.2 M disodium phosphate

These are general guideline to the proportion of each solution to make an overall 4ml solution.

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Ethanol and Glucose molecules (photo) Figure1 and Figure 2
URL: http://chimge.epfl.ch/En/scnat/1scnat28.htm

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