Bio-Rusting: Effects of Facultative Anaerobic Bacteria on the Formation of Rust

Science 151 Capstone Research Project

Final Report

Submitted by

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Introduction

A comparative study was conducted to observe the results of facultative anaerobic bacteria on the formation of rust. This experiment is modified from an experiment previously conducted by Andrew T. Ostrom in 2013 (California State Science Fair, 2013). Rusting is a redox reaction, specifically, an oxidation reaction in which iron + oxygen and water are consumed to produce rust. (Equation 1) (BBC n/a). The following hypothesis is established: If yogurt is added into distilled water, it will slow down or inhibit the formation of rust due to the aerobic qualities of the bacteria present which will consume the available oxygen to slow the process of rusting. The accompanying null hypothesis is as follows: If yogurt is added into water, it will not slow down or inhibit the formation of rust due to the aerobic qualities of the bacteria present which will consume the available oxygen to slow the process of rusting. This experiment sets out to answer the following research question: How the effects of aerobic bacteria and whether it slows down the rusting process on nails?

In yogurt the main bacterium present is *Lactobacillus bulgaricus* which is used during the fermentation process of yogurt. (Hammes and Vogel 1995) *Lactobacillus bulgaricus* is a facultative anaerobic bacterium. Unlike plants, this bacterium makes ATP by aerobic respiration if oxygen is present but switches to fermentation or *anaerobic* respiration if oxygen is absent. Due to the presence of *Lactobacillus Bulgaricus*, theoretically if placed into a solution with a nail these bacteria will consume the oxygen needed to complete the oxidation reaction and thus the process of the oxidation of the nails is slowed and less rust is formed. In contrast, there should be and increase in mass in the control group, due to the lack of oxygen consuming bacteria; allowing the oxidation process to occur more readily. Therefore, the larger the concentration of O_2 gas the more readily the reaction proceeds resulting in more H_2O in the chemical makeup of rust (Hydrated iron(III) oxides Fe_2O_3) and an increase in mass. (Equation 1). This will be calculated by measuring the pre and post mass of nails, then taking the mean change in mass of both groups. Rust prevention inhabits a large market, theoretically if information regarding the use of biological agents such as bacteria could be used to prevent rust more research could be conducted to a more widespread application. The statistical analysis used to analyse the data due to the two factor levels, was a two-sample t-tools test, relative frequency histograms, a side-by-side boxplot and QQ plots.

$$\underset{\text{Iron}}{4Fe} + \underset{\text{Oxygen}}{3O_2} + \underset{\text{Water}}{2H_2O} \longrightarrow \underset{\substack{\text{Hydrated iron (III) oxide} \\ \text{(Rust)}}}{2Fe_2O_3 \cdot H_2O}$$

Equation 1: Oxygen and water are crucial in the production of rust.

Methods:

60 nails were placed in numbered plastic bags then weighed using a balance. 30 condiment containers were labeled “control” and a number from 1-30. The same was repeated to 30 condiment containers to form the experimental group. The nails were then placed in their

corresponding containers. In the containers labeled control, the nails were immersed in 40 mL of distilled water. In contrast, for the experimental group the nails were immersed in a solution of 20 mL yogurt and 20 mL of distilled water to result in a total volume of 40 mL per container. Covers were place on top of all samples. (Figure 1). The samples were stacked and placed in Tupperware for the duration of the week. After one week the nails were rinsed using distilled water and dried in their corresponding containers. After a day the nails were weighed using a balance and recorded. The mean change in mass was calculated using the post nail mass subtracting the pre-nail mass.

Experimental Set-up

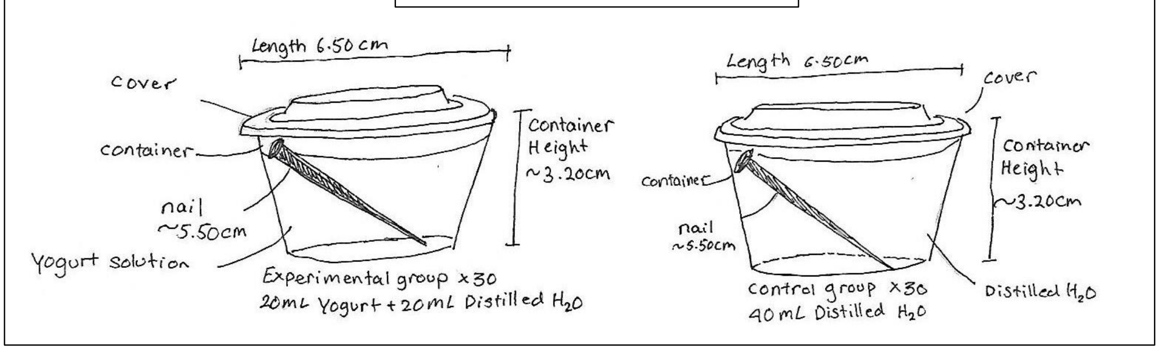

Figure 1: Experimental design set-up of the control group and the experimental group and the specific measurements for the solution set-up and number of trials in each group.

Results:

After one week the post weight of the nails was collected using a balance. The following data was achieved by using the mean change in the control and experimental group masses. This was achieved by taking the post groups masses of each group and subtracting the pre-nails masses on *Excel* to achieve the mean change in both groups. Raw data is included in appendix 1 and appendix 2along with a sample calculation.

Table 1: Relevant summary statistics created using *Statcrunch.*

Column	n	Mean	Std. dev.
Change in Control Group Mass (g)	30	0	0.0090972
Change in Experimental Group Mass (g)	30	-0.020	0.0071840

The summary statistics in (table 1) display the appropriate descriptive statistics for a two-factor level experiment. It shows the number of trials in each group. Based on a preliminary review of the pre and post masses of the nails it was decided that none of the trials would be omitted. Therefore, all the trials were included in the latter statistical analyses.

Table 2: Two sample T hypothesis test conducted on stat crunch at a significance level 0.05.

Two sample T hypothesis test:
μ_1 : Mean of Change in Control Group Mass (g)
μ_2 : Mean of Change in Experimental Group Mass (g)
$\mu_1 - \mu_2$: Difference between two means
$H_0 : \mu_1 - \mu_2 = 0$ $H_A : \mu_1 - \mu_2 > 0$ (without pooled variances)
Hypothesis test results:

Difference	Sample Diff.	Std. Err.	DF	T-Stat	P-value
$\mu_1 - \mu_2$	0.0203	0.0021	55	9.608	<0.0001

Table 3: Two sample T confidence interval test conducted at 95% confidence and a significance level of 0.05.

Two sample T confidence interval:
μ_1 : Mean of Change in Control Group Mass (g)
μ_2 : Mean of Change in Experimental Group Mass (g)
$\mu_1 - \mu_2$: Difference between two means (without pooled variances)
95% confidence interval results:

Difference	Sample Diff.	Std. Err.	DF	L. Limit	U. Limit
$\mu_1 - \mu_2$	0.0203	0.0021	55	0.0161	0.0246

This experiment has one dependent variable and one independent variable. The dependent variable is the change in mass of the nails and the independent variable is numerical, "the addition of yogurt (bacteria) into a solution". The overall results are qualitative. Since the experiment presents only two factor levels, two independent sample t-tools was used to conduct the statistical analysis as exhibited in (table 2 and table 3).

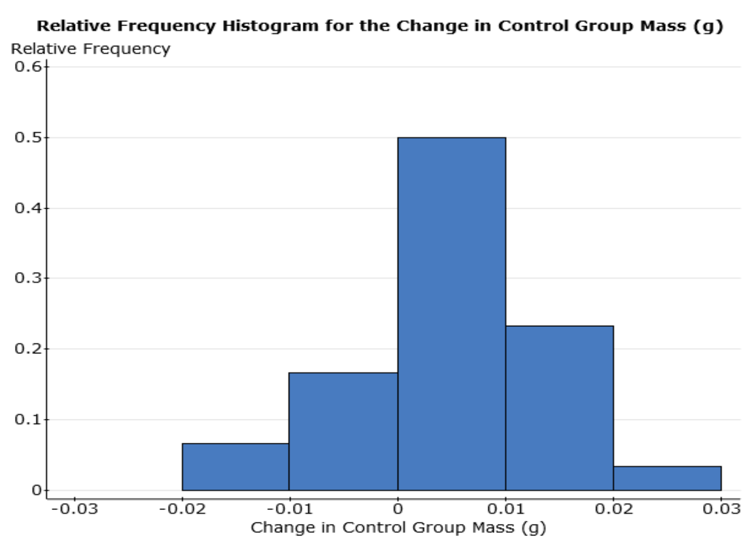

Figure 2: Relative frequency histogram for the change in the control group mass in grams conducted using *Statcrunch*

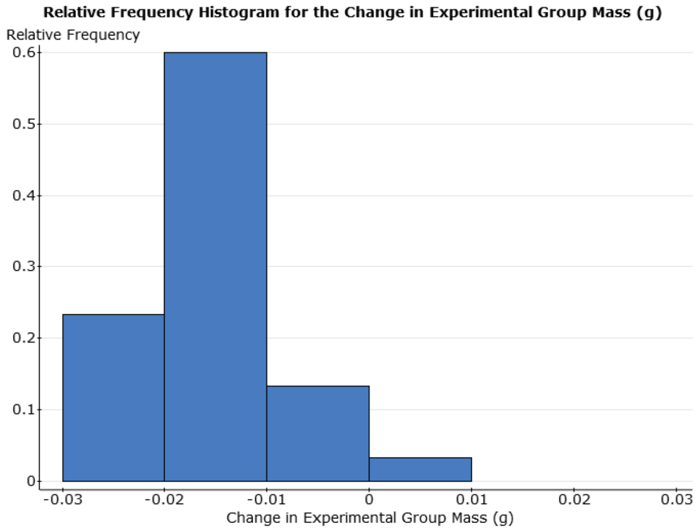

Figure 2: Relative frequency histogram for the change in the experimental group mass in grams conducted using *Statcrunch*.

Relative Frequency histograms were chosen to show the spread of the overall data as well as the distribution of the data in specific bins. These were created to compare the distribution and the spread of the change in control and experimental group masses. Another reason as to why these histograms were created was to check one of the assumptions associated with t-tools. By checking the histograms, it is apparent that the data for the control group is symmetric and unimodal. On the other hand, the experimental group histogram demonstrates the data to be skewed to the right. Overall, suggesting that there is no evidence of a lack of normality. Therefore, the normality assumption is satisfied.

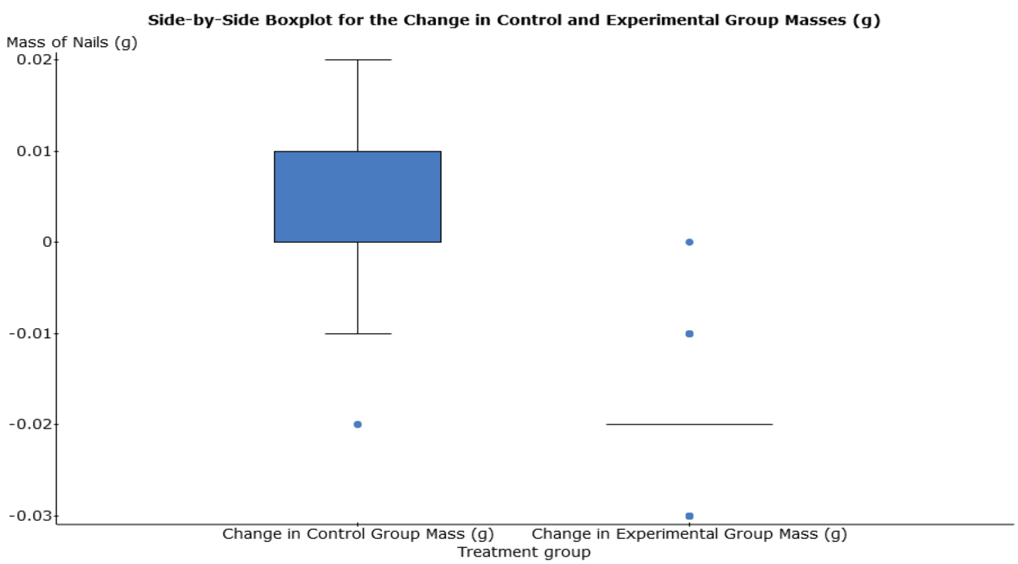

Figure 3: Side-by-side boxplot to compare variance and spread of the change in control and experimental group mass.

The side-by-side boxplot was used alongside the histograms to truly compare the spread of the data, the distribution and the lack of variance in the experimental group data. One of the assumption for t-tools (constant variance) is not applicable in this situation as it is abundantly clear that there is a significant difference of variance between the control and the experimental group.

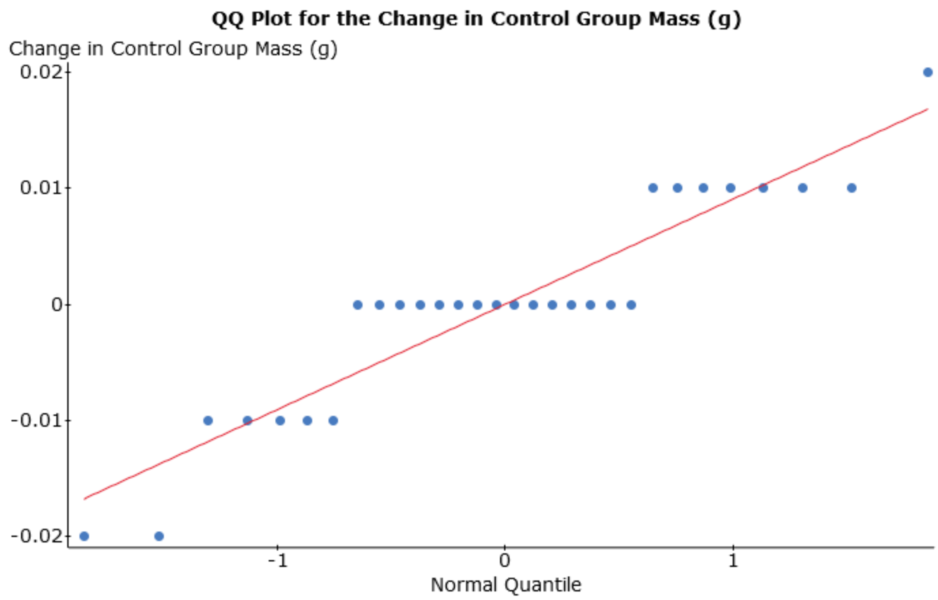

Figure 4: QQ plot for the change in control group mass to test the normality assumption.

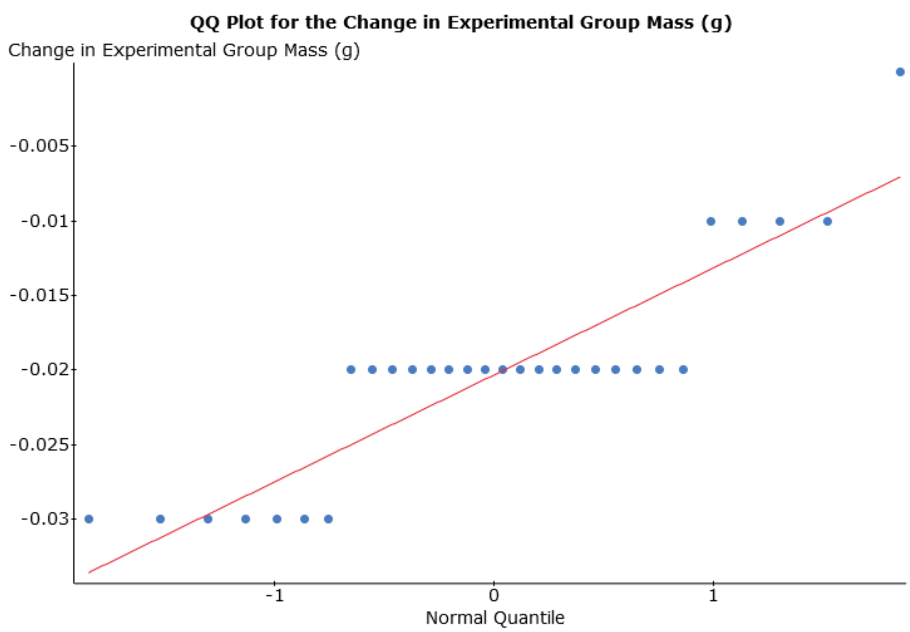

Figure 5: QQ plot for the change in experimental group mass to test the normality assumption.

Although not essential to the overall statistical analysis of the experiment the QQ plots were used to test the normality assumption required to complete a t-tools test. The QQ plot suggest symmetry about the line, therefore there is little evidence to suggest that the distribution is not normal. Based on this evidence the normality assumption is satisfied.

As mentioned above, variability is not essential to the interpretation of the data in this experiment. It is assumed that since all samples were prepared in the same environment and were in individually closed containers, the samples are assumed to be independent. Therefore, the independence assumption is satisfied.

Important Trends:

After weighing the nails post experiment, the control group mass showed little change. The data was inconsistent as there were some nails that increase and decreased in mass as well. In the experimental group the overall trend when compared to the control group is that the mass of the nail decreased with the exception of one outlier where it increased.

Figure 6: White oxidation characterized as white rust from the galvanized coating of the zinc.

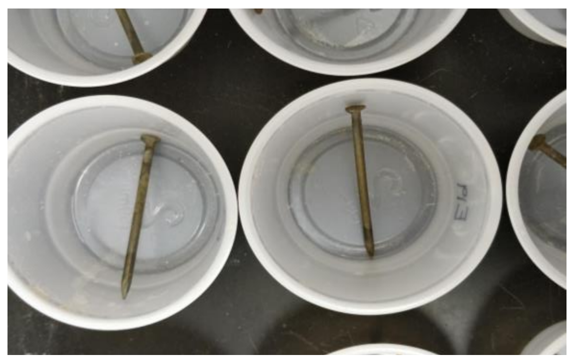

Figure 7: Red brown iron oxidation of the post experiment experimental group.

Discussion:

The overall objective of this experiment is to see whether the addition of a facultative anaerobic bacteria from yogurt aided in reducing the amount of rust formed on a nail, this means that the overall change in mean mass of experimental group nail would be less then the mean change in mass in the control group mass because of the lack of oxygen consuming bacteria.

However, unlike the hypothesis would suggest after rinsing both groups in the lab qualitatively deviations were noted. A white oxidation was coating the nails of the control group (Figure 6). This was intriguing and thus spurred further investigation. Upon further research the white oxidation may have been white rust. The following information was found: “galvanized products are frequently treated with formulations based on chromate or dichromate ions to prevent 'white rusting' (corrosion product of zinc) during storage in damp conditions." (Hinton and Wilson 1989). The nails that were used in the experiment were galvanized. Characterized by the shiny chrome-like finish to the nails. Although not confirmed we assume that the nails have been treated with chromate or dichromate ions as they were bought from a hardware store. The zinc coating is used to act as the "sacrificial anode". (Hinton and Wilson 1989) since it will oxidize first before the iron underneath which will prevent the oxidation of iron. With this information the white oxidation can therefore, be characterized as white rust. Due to the aqueous environment of the control group the zinc oxidized first before the iron underneath.

In the experimental group the coating was removed by the bacteria. Exhibited by no galvanized shiny coating present (Figure 7). The removal of the galvanization allowed the iron underneath to be exposed. The red-brown oxidation is characterized as hydrated iron oxide due to it's traditional rust colour.

After weighing the post experiment nails the weight of the experimental group generally decreased while the control group mass of the nails stayed relatively unchanged with certain nails increasing or decreasing in mass. As described in the previous paragraphs the likely reason for these occurrences is as follows. The mass of the experimental group decreased due to the removal of the galvanized coating and little to do with actual rusting. On the other hand, the control group stayed relatively the same as the galvanized nature of the nails resisted the rusting process. (Raw data in Appendix 1 and 2)

The summary statistics (table 1) indicates that the standard deviation for the change in control group and experimental group mass is very small. A low standard deviation means that a large majority of the data points in each group are very close to the mean.

When examining the histogram for the change in control group mass the distribution shows a unimodal generally symmetrical spread. The bin with the largest number of data point is 0-0.01. This means that the overall change of the majority of the data points exhibited no change. On the other hand, if the histogram for the change in experimental group mass is examined a different distribution is noted. This histogram exhibits a right skewed distribution. The bin containing the largest distribution of data points is -0.02 to -0.01. This means that the overall change in experimental group mass decreased within that interval.

Despite the generally good spread exhibited in both of the histograms the side-by-side boxplot (figure 3) sheds real light into the actual distribution of the data. In the boxplot for the control group there is generally good variance as exhibited by the fences and the overall distribution from 0.02 to -0.0. With a majority of the data between 0 and 0.01. This is consistent with the data shown in the corresponding histogram (figure 2). In contrast, despite the generally good spread in the histogram for the change in experimental group mass the corresponding boxplot does not exhibit the same variance in data. The line at -0.02 suggest that majority of the data falls at that point of -0.02. The three outliers at 0.01, 0 and 0.03 are represented as the bins in the corresponding histogram. The histogram is misleading especially, in the experimental group as it tricks the reader into thinking that there is generally good spread of data. When in fact the boxplot reveals that there is little variance in to overall data.

The two-independent sample t-tools was deemed an appropriate choice due to the qualitative nature of the data and the two factor levels. The t-hypothesis test (table 2) was conducted to test the following null hypothesis H_0: $\mu_1 - \mu_2 = 0$. Where μ_1 is the mean of change in control group mass (g), μ_2 is the mean of change in experimental group mass (g) and $\mu_1 - \mu_2$ is the difference between the two means. The t tool results conducted at a significance level of 0.05 exhibited the following p value: <0.0001. The large take away from this test is that since the p value of <0.0001 is definitely smaller than a significance level of 0.05, this shows that there is significant evidence to reject the null hypothesis of H_0: $\mu_1 - \mu_2 = 0$ in favour of the alternative hypothesis H_A: $\mu_1 - \mu_2 > 0$. This ultimately means that there is significant evidence that the difference between the means of the change in control group mass and the change in experimental group mass is larger then zero.

To accompany the hypothesis test, a 95% confidence interval test was also conducted (table 3). Where μ_1 is the mean of change in control group mass (g), μ_2 is the mean of change in experimental group mass (g) and $\mu_1 - \mu_2$ is the difference between the two means. The sample difference between the two means is 0.0203. This means that with 95% confidence the difference in mean change in mass falls between 0.01609 and 0.02457.

The QQ plots (figure 4 and figure 5) once again show a significant lack of variance in both of the control group and the experimental group as many of the data points line up. This shows that they are the same. In an ideal QQ plot the data would show significant scatter that is symmetrical about the line. In this case there is little evidence to suggest that the distribution is not normal based in the symmetrical nature about the line, despite lack of variance. Based on this evidence the normality assumption is satisfied.

There are several questions that have arisen after completing the experiment such as: How can variance in the data points be increased? How did galvanization affect the result? And finally, was the trial too short?

The overall data gathered exhibited significant lack of variability. In hindsight, there are definite modification to the methods and the procedures if this experiment was repeated to achieve more variance in the results. Firstly, an analytical scale could have been used to increase accuracy and decrease rounding error of the data point. It would do this because and analytical scale is more sensitive and may detect changes in mass more accurately. One of the largest factors that should be modified is running the experiment using ungalvanized nails. This should be changed in order to compare the oxidation of solely iron. After conducting the experiment, it was difficult to make comparison as the oxidation in both groups were different, as the comparison was between zinc and iron. By removing the galvanization of the nails, a more appropriate comparison of the effects of facultative anaerobic bacteria on rust could be conducted, and the error associated with different metals eliminated. Finally, the last modification that would be made is increasing the run time for the overall experiment to allow a larger time period for oxidation to occur. By increasing the run time this would theoretically allow more rust to form, which would allow for more variability within the data.

Conclusion:

The white oxidation in the control group is concluded to be zinc, because of the galvanized nature of the nails. The red/brown oxidation in the experimental group is concluded to iron, the coating was removed and the iron was exposed to be oxidized. The results suggest significant evidence that the difference between the means of the control and experimental group is larger than zero. However, because of the lack of variance exhibited in the data of both the control and the experimental group it can be concluded that the addition of facultative anaerobic bacteria from yogurt did not slow the rusting process. Since the experimental group mass did not exhibit a decrease in the mean change of group mass relative to an increase in the mean change in control group mass. The experiment was unfortunately unable to confirm the validity of the hypothesis due to the lack of variance in the data and error in the methods.

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Appendix:

Appendix 1

Capstone Project: Pre-experiment Nail Masses of Group C and E in Grams

Nail Number	Control Group (g)	Experimental Group (g)
1	1.95	2.06
2	2.04	2.05
3	2.01	2.07
4	1.98	2.06
5	2.06	2.07
6	2.05	1.95
7	2.05	2.02
8	2.05	2.08
9	2.07	2.05
10	2.04	2.05
11	2.04	2.04
12	2.03	1.94
13	2.08	2.00
14	2.01	1.98
15	2.06	1.95
16	2.04	2.01
17	2.02	2.11
18	1.95	2.07
19	2.07	2.00
20	2.01	2.01
21	1.97	2.00
22	2.03	2.05
23	2.08	2.06
24	2.08	2.04
25	2.06	2.04
26	2.00	2.09
27	1.90	2.03
28	2.06	2.07
29	2.06	2.01
30	2.01	2.04

Appendix 2

Capstone Project: Post-experiment nail masses of Group C and E in Grams

Nail Number	Control group (g)	Experimental group (g)
1	1.96	2.04
2	2.04	2.03
3	2.01	2.04
4	1.98	2.04
5	2.06	2.04
6	2.05	1.93
7	2.05	2.01
8	2.04	2.07
9	2.08	2.04
10	2.04	2.03
11	2.05	2.02
12	2.03	1.92
13	2.07	1.99
14	2.01	1.96
15	2.06	1.92
16	2.04	1.99
17	2.04	2.09
18	1.96	2.05
19	2.08	1.98
20	2.00	1.99
21	1.97	2.00
22	2.03	2.03
23	2.09	2.03
24	2.09	2.02
25	2.05	2.02
26	2.00	2.06
27	1.90	2.00
28	2.05	2.05
29	2.04	1.99
30	1.99	2.01

Sample Calculation:

Mean change in control group mass nail 1:

Post-experiment nail mass subtract pre-experiment nail mass

(i.e.) 1.96 (g) - 1.95 (g) = 0.01 (g)