Gas Segmented Continuous Flow Colorimetric Analysis

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Abstract

Oxidation followed by colorimetric analysis is a popular method for determining the nutrient levels (nitrates, phosphates, silicates, etc.) in water sources because concentrations of nutrients can be determined in parts per billion, a concentration undetectable by other methods. The purpose of this experiment was to determine if the original method parameters are optimal in a saltwater matrix or if alternative parameters would be more efficient. The first section of this experiment aimed at finding the amount of time needed to oxidize each type of sample, with the second half testing how hydrogen peroxide concentration affected test results. It was found that brackish water (from brackish ponds near NELHA) reaches maximum oxidation at 1 hour, deep sea water (ft) reaches maximum oxidation at 1 1/2 – 2 hours and surface sea water reaches maximum oxidation at 2 – 2 ½ hours. Furthermore it was concluded that hydrogen peroxide was needed to oxidize samples, but 0.6 mL hydrogen peroxide did not increase rate of oxidation to an appreciable extent in comparison to 0.3 mL hydrogen peroxide.

Introduction

When nutrients are in oxidized states, reactions occur with other reagents added to the oxidized sample to create color in the run sample. The color of the sample is then linearly related to the amount of nutrients in the sample, hence a standard can be run to calibrate a standard curve, and nutrient concentration in the sample can be determined. The colorimetric analysis executed in this experiment was multi-pass, which enables the sample being run to be tested for multiple nutrient concentrations. Color of the sample was determined by a colorimetric apparatus that emits light at a wavelength of 540 nm through the sample. The light becomes absorbed moving through the solution, which then is measured on the other side of the solution by the apparatus. The two nutrients examined were nitrates and phosphates, which are most indicative of pollution including fertilizer runoff and sewage spills. These two nutrients are also required by plants, and are closely monitored to prevent algae blooms offshore.

Total nitrates and total phosphates were calculated and are reflective of all nitrogen and phosphorous in samples, including nutrients contributed from microorganisms and particulates. There was no filtration in the experiment, and thus changes in nutrient levels detected by methods are expected to be more observable than with filtration. Surface sea water was used for the same purpose in the dosage experiment, since this water was found to oxidize slowest of the three samples. In this experiment nitrate and nitrite levels were graphed without ammonia levels. Ammonia would usually contribute to nitrogen levels in samples, but because of oxidation ammonia levels were almost completely converted to nitrate or nitrite with almost undetectable levels of ammonia after peak oxidation, and so ammonia levels are not graphed for the experiment runs.

Methods

All solutions were prepared in accordance with EPA method 353.4 (Zhong Zhang, Ortner, Fischer, 1997). This includes:

- calibration standards
- laboratory fortified blanks
- low nutrient sea water
- prepared standard solutions of differing concentrations
- reagent water used to run the colorimetric apparatus.
- Brij-35 solution
- sulfanilamide solution
- NED solution
- imidazole buffer solution
- copper sulfate solution
- colored SYNC peak solution

High purity nitrogen gas and a cadmium column were also used for monitoring nitrogen levels, with the cadmium column sufficiently being activated before sample runs. All data was recorded by a computer based data acquisition systems with carry over being accounted for and automated analysis by autosampler and colorimetric apparatus being monitored by intern and supervisor. No filtration was used on samples and samples were stored in polyethylene bottles in a designated refrigerator at about 4° C. Method detection limit was calculated and deemed acceptable for the task at hand.

The standard curve used to analyze the samples in the experiment was constructed from five calibration standards, and an acceptable correlation coefficient of less than .995 was obtained Zhong Zhang, Ortner, Fischer, 1997). Blanks and washes were utilized to clear out the colorimetric apparatus before, after and in between runs of samples.

All materials used in this experiment were thoroughly cleaned before use. Sampling tubes were rinsed out with low nutrient sea water three times each to avoid contamination before running the analysis, glassware were acid washed followed by detergent and rinsing three times with deionized water. Colorimetric analysis apparatus was washed out with high molarity hydrochloric acid, then run with reagent water for 20 minutes.

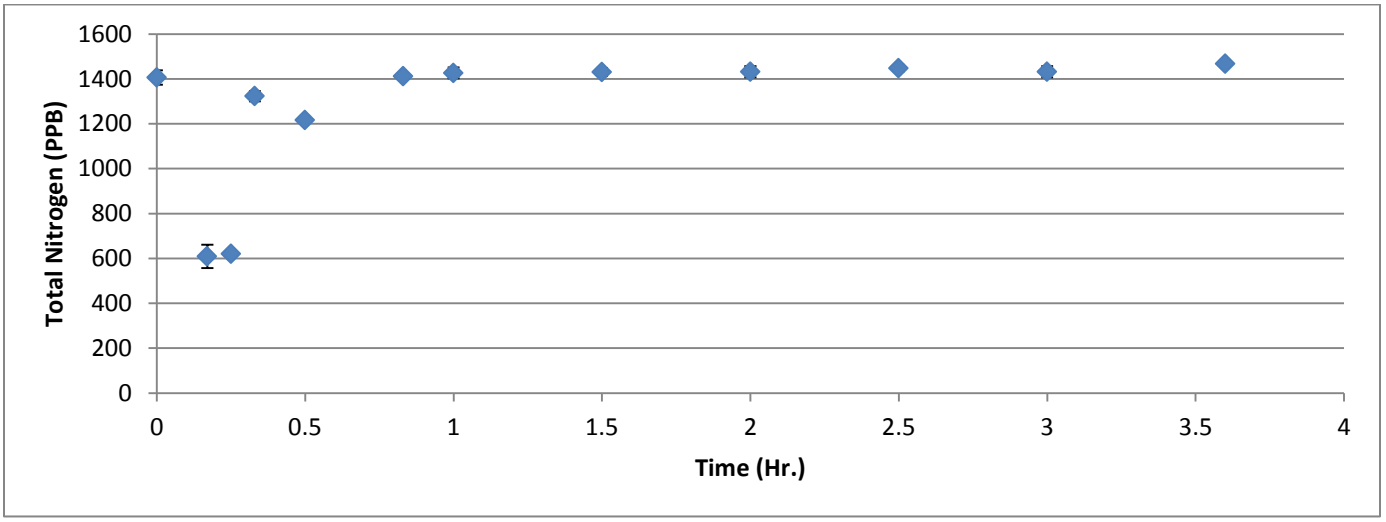
In the oxidation time test surface sea water, deep sea water, and brackish water samples were oxidized in increasing intervals from 15 minutes to an hour (refer to figures 1, 2, 3, 4, 5 and 6) with 0.3 mL hydrogen peroxide. Samples were run from 15 minutes to 3.6 hours, which were graphed to find the time at which maximum (plateau of oxidized nutrients) oxidation was reached. Samples were done in duplicates and triplicates, depending on the available space in the

oxidation chamber, and the data points from these were then averaged (arithmetic mean) to find the data point at the specified time.

Surface sea water samples were oxidized with 0, 0.3 and 0.6 mL hydrogen peroxide for the hydrogen peroxide dosage test, which were graphed to find the time at which maximum (plateau of oxidized nutrients) oxidation was reached and if dosage needed to be revised. The same time intervals were used as in the oxidation time test.

Results

It was shown that at one hour brackish water samples reached peak oxidation for both phosphate and nitrate and nitrite levels (refer to fig. 1 and 2). Deep sea water reached peak oxidation at around one and one half hours to two hours (refer to fig. 3 and 4, and surface sea water was last to reach maximal oxidation at two to two and a half hours for total nitrogen (refer to fig. 5 and 6 respectively).



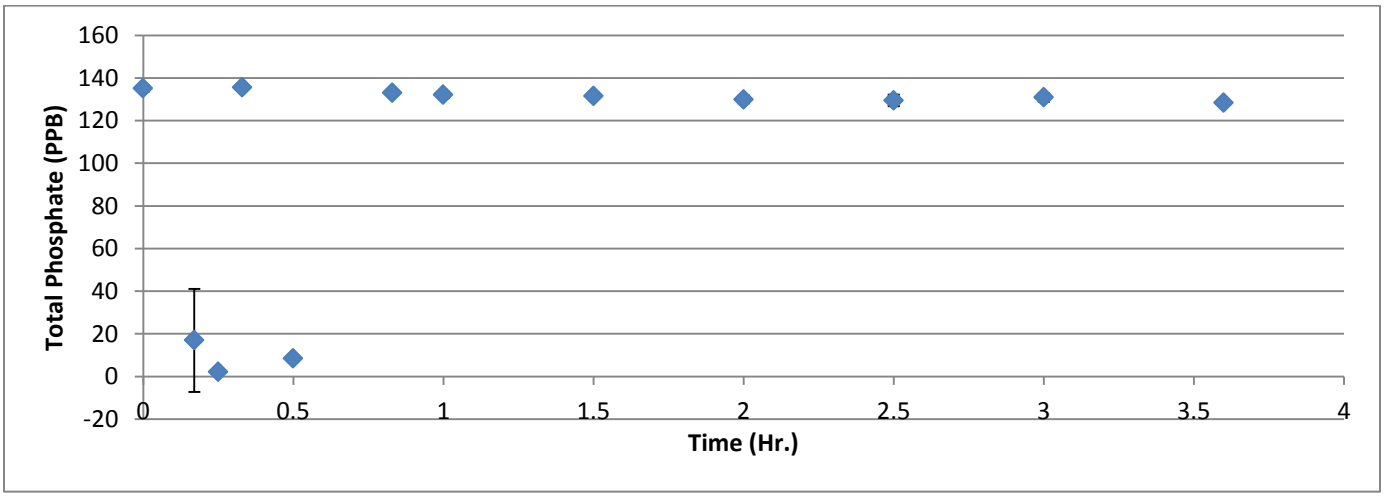
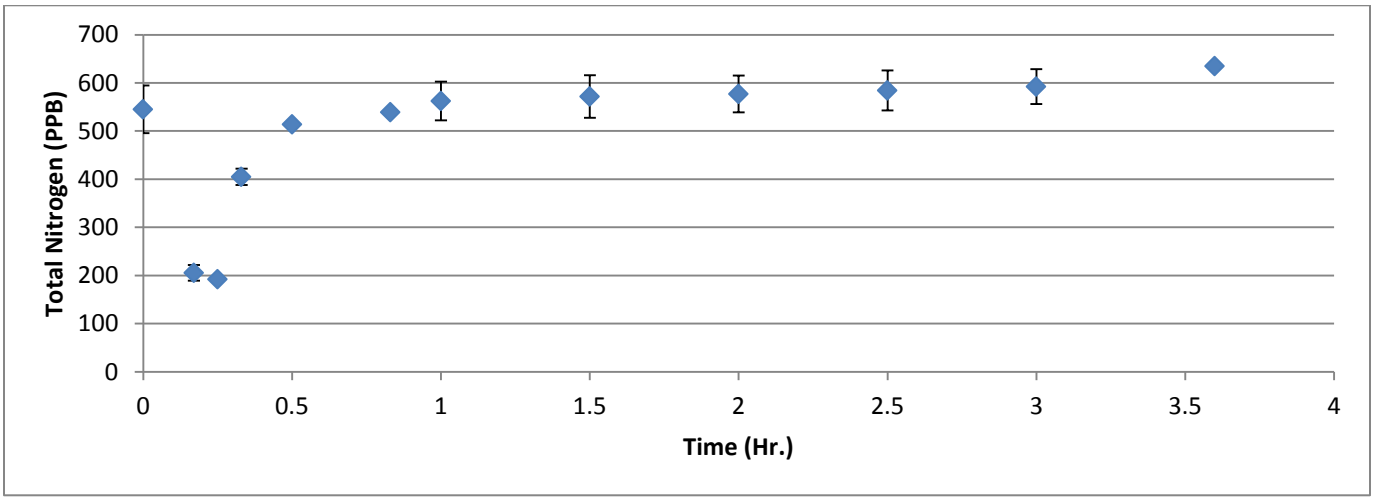


Figure 1 and figure 2 showing total nitrogen and total phosphate levels in brackish water samples at oxidation times. Levels of nutrients detected stabilized after one hour, which indicate the earliest time at which a reading could be taken.



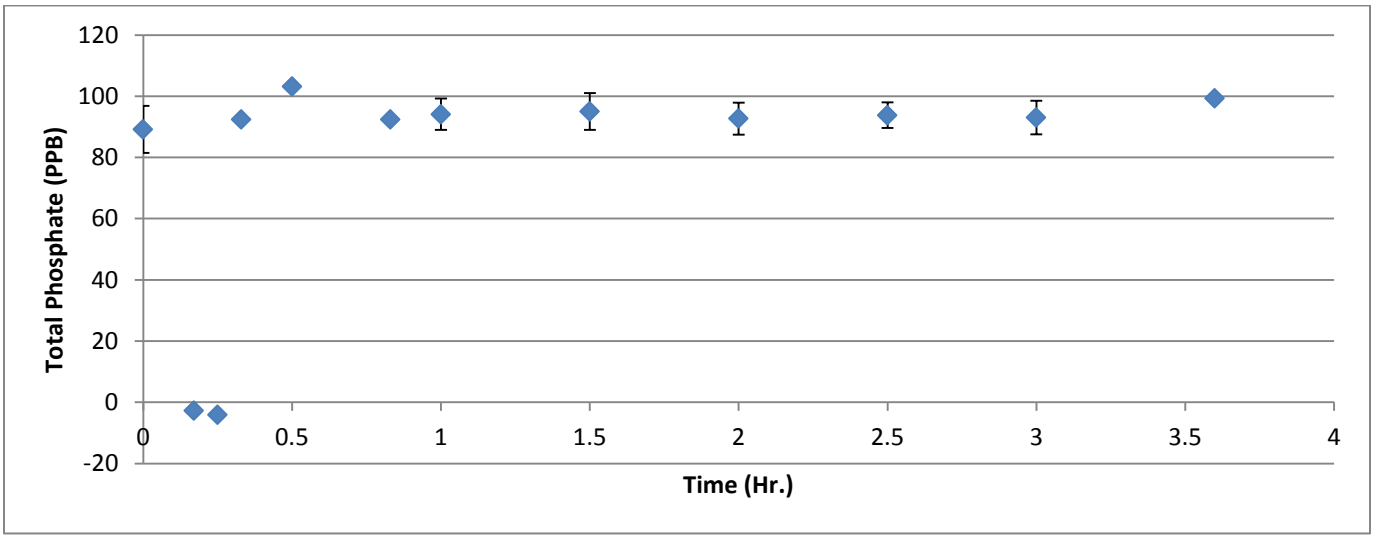
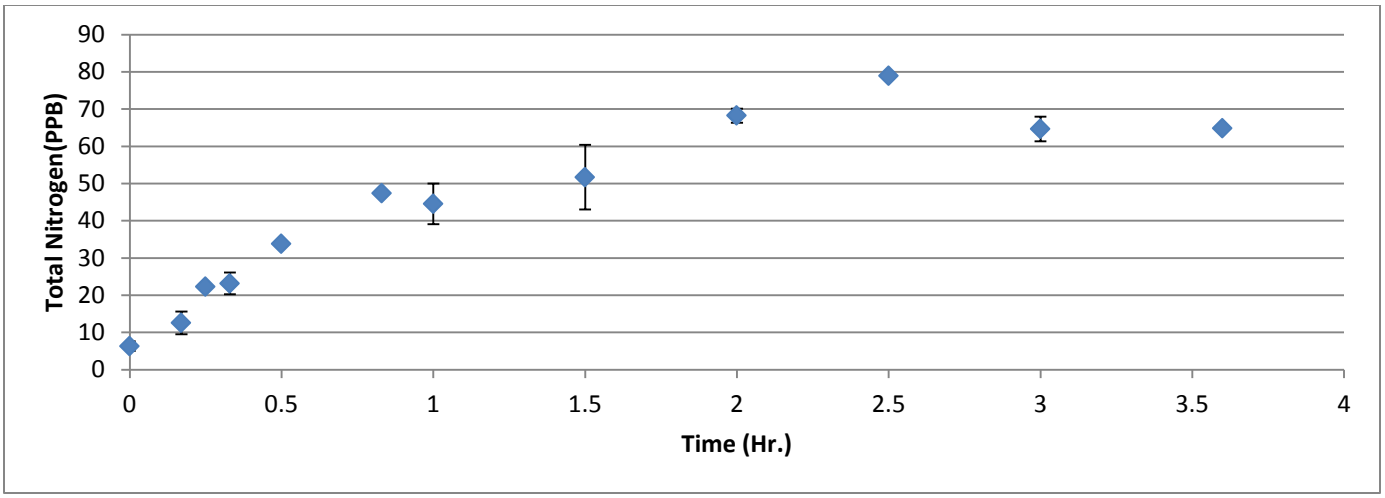


Figure 3 and figure 4 showing nutrient levels in deep sea water samples taken from NELHA deep sea waterlines. Levels of nitrogen stabilize after 1 ½ hours, and phosphate levels stabilize after half an hour, which implies analysis of both nutrients requires oxidation for 1 ½ hours.



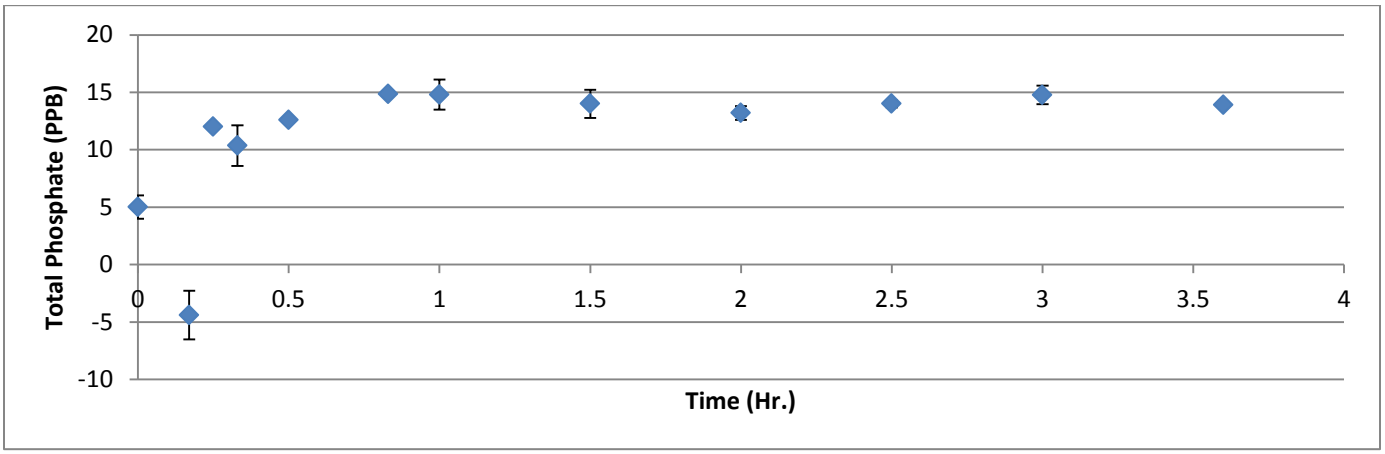
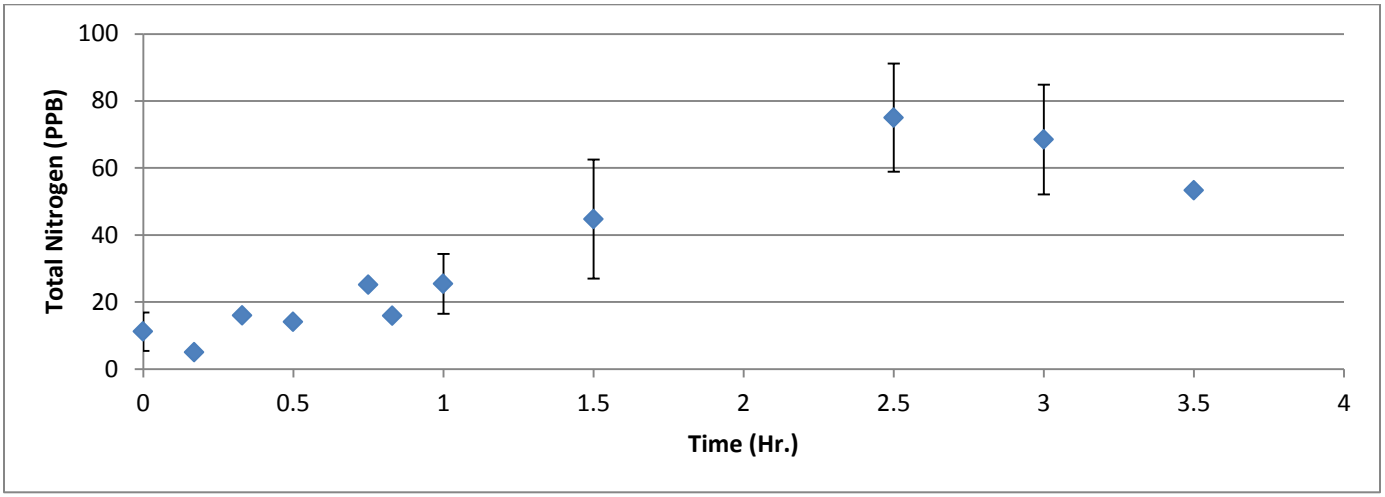
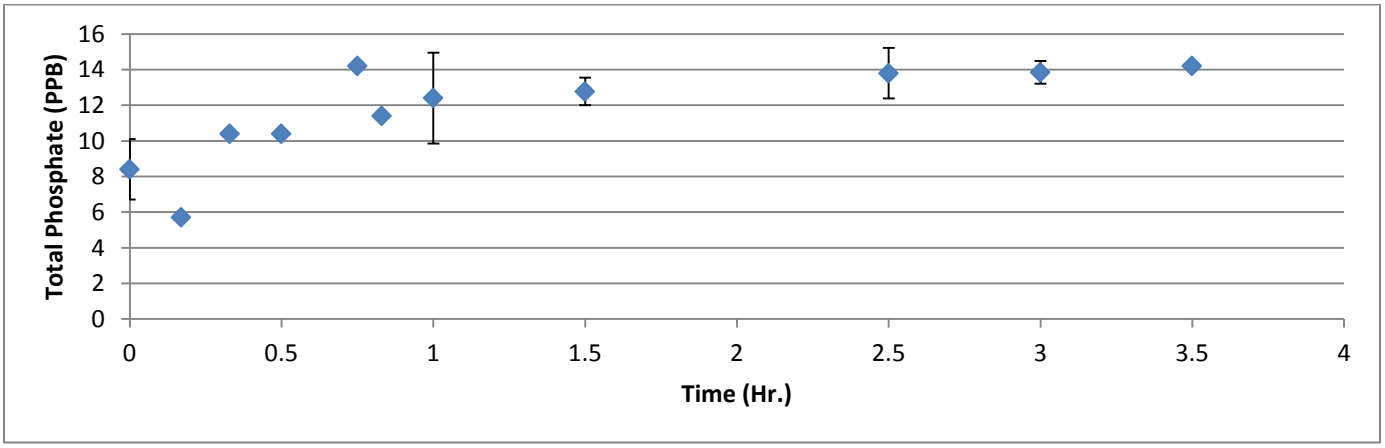


Figure 5 and figure 6 showing nutrient levels in surface sea water samples taken from NELHA surface sea waterlines. Levels of nitrogen stabilize after 2 to 2 ½ hours, and phosphate levels stabilize after half an hour, which implies analysis of both nutrients requires oxidation for at least two hours.

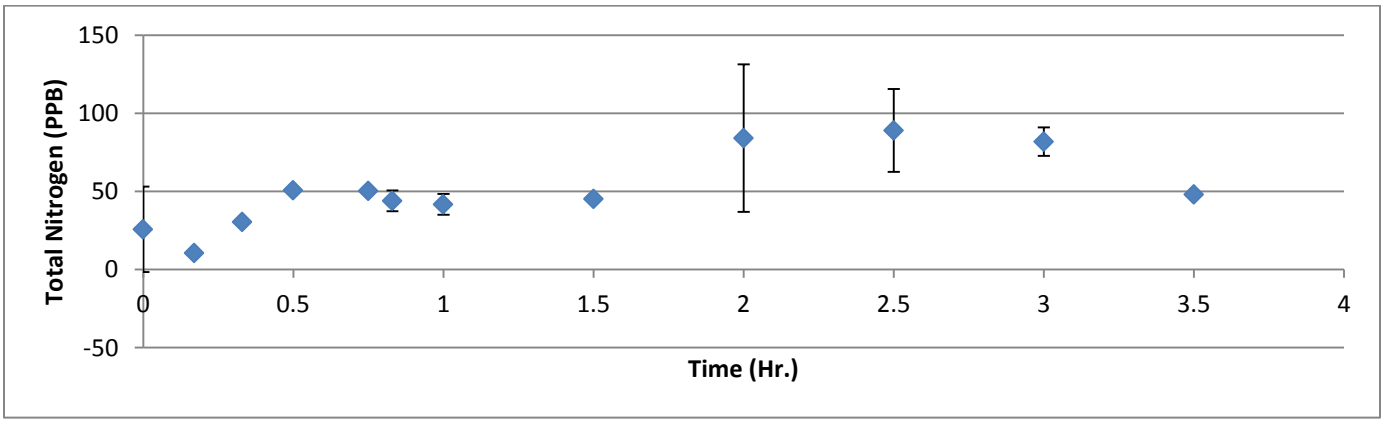
In the hydrogen peroxide test, it was observed (by comparing the three runs), that there was a notable loss of oxidation in the test without hydrogen peroxide, while in the surface sea water run with 0.6 mL hydrogen peroxide, the time gained was not observable or at least not large enough to warrant adding more than the recommended 0.3 mL (refer to figures 7, 8, 9, 10, 11 and 12). The results indicated that there was sufficient hydrogen peroxide in the sample at the normal 0.3 mL dosage and the 0.6 mL dosage to incur oxidation of all (excepting trace amounts of nutrients) nutrients in samples, since ammonia was also plotted and reached near zero values in each of these tests. Loss of nitrogen in the 0.3 mL hydrogen peroxide run at 3.5 hours is unexplained, but serves as further evidence as to why the three hour oxidation time was used in the test facility (refer to fig. 9). In the 0.6 mL hydrogen peroxide run at 0.17 hours, there was an outlier (399 parts per billion) which was not graphed, and the sample at 1.5 hours was not recorded because of human error involved in the sample (sample was not put into oxidation

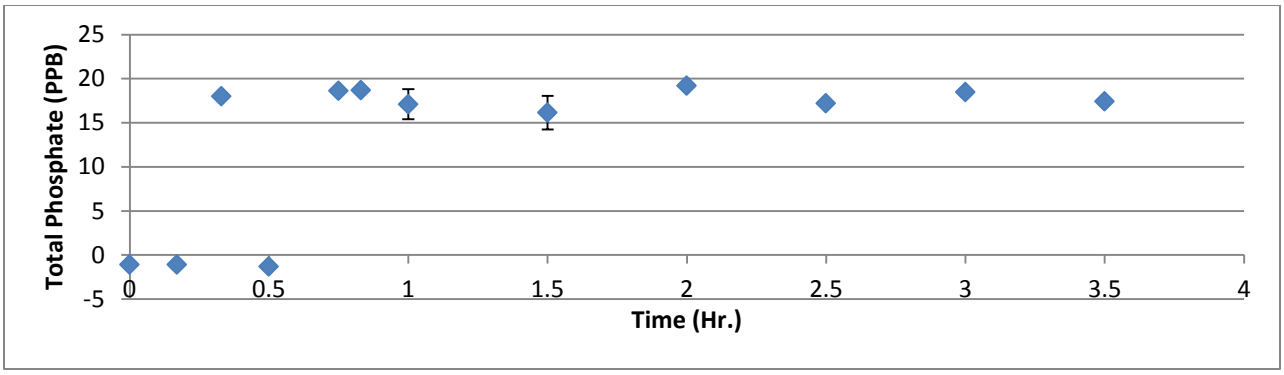
chamber). These two errors in the data were thought to be isolated, and thus were extrapolated to have no effect on other data points or overall trend in data.



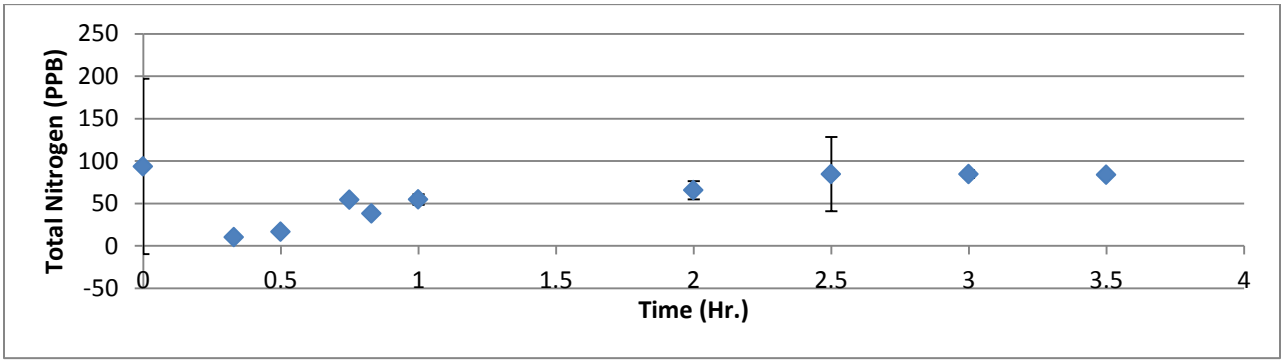


Figures 7 and 8 (above) showing nitrate and phosphate levels in surface sea water runs with no hydrogen peroxide added prior to oxidation. Nutrient levels are much lower than expected maximal values of nutrients for the sample run.





Figures 9 and 10 (above and bottom of page 7) showing nitrate and phosphate levels in surface sea water runs with 0.3 mL hydrogen peroxide added prior to oxidation. Phosphate levels reached maximum at around 0.75 hours, but nitrogen levels did not plateau until around two hours. Reason for loss of nitrogen after three hours is unknown.



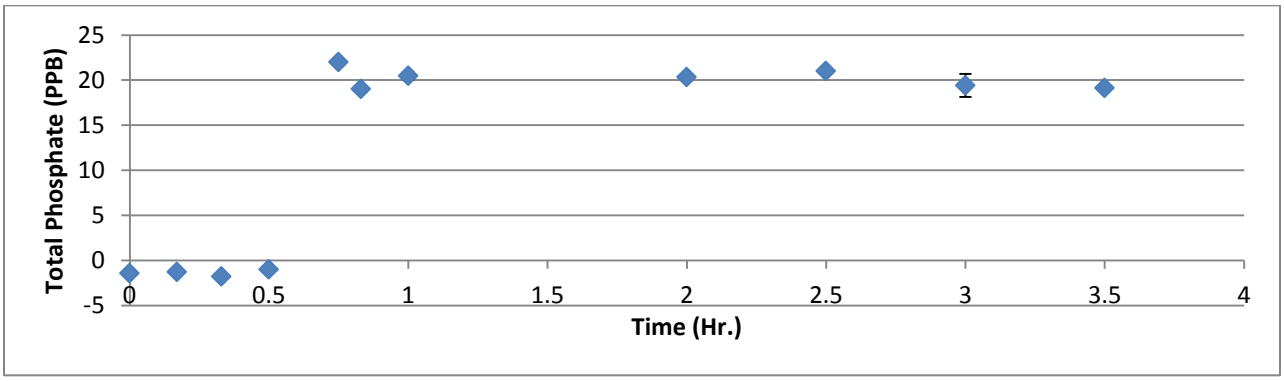


Figure 11 and 12 showing nitrate and phosphate levels for surface sea water runs with 0.6 mL hydrogen peroxide. An outlier at 0.17 hours was not graphed (399 ppb) and the sample at 1.5 hours was not run due to human error.

Samples in the test facility before this experiment were placed in the oxidation chamber for three hours with hydrogen peroxide. This amount of time oxidizes all samples to maximal oxidation levels, as found in this experiment.

Conclusion

Colorimetric analysis revealed differences in time to oxidation between brackish, deep, and surface sea water, which were thought to be caused by differences in (1) nutrients, (2) microorganisms, and (3) particulates. Microorganisms and particulates take longer to oxidize, thus we should observe longer oxidation times in waters with more of the listed impurities. Surface sea water has large amounts of microorganisms with respect to other samples (due to photosynthesis and more active microorganism communities), and it is believed that this is why surface sea water samples take the longest to reach a maximum oxidation. Deep sea water has lower amounts of microorganisms and particulates, and thus is second to reach maximum oxidation. Brackish water is the fastest to oxidize, probably because of the low amount of microorganisms and particulates. This would explain the higher amount of nutrients in the brackish water and lower oxidation time since free nutrients would oxidize faster than other materials in the sample. More research is needed to ascertain the exact reasoning behind oxidation times.

The surface sea water was then taken (because of its long oxidation time) and tested to examine the effects of a double dose of hydrogen peroxide and lack of hydrogen peroxide with respect to the normal dosage in samples. The results implied that hydrogen peroxide was needed for results to be indicative of nutrient levels (there was oxidation without hydrogen peroxide, but lower amounts than replicate samples with more hydrogen peroxide), but adding more than one 0.3 mL dose did not speed up oxidation, probably because the reaction is at a maximum rate for sample size at this point. The reaction is believed to be at a maximum rate because the hydrogen peroxide serves as an oxygen source for oxidation of nutrients, and at 0.3 mL there is enough oxygen being supplied to the reaction to maximize the rate.

Admittedly, there was some error in a few of the data points in this experiment, one being displayed as an outlier (refer to fig. 11 and 12, page 7) and one sample not being run (refer to fig. 11 and 12, page 7). These errors did not seem to throw off the general trend of the graphs. There are however a few large error bars which indicate error of some sort as in the zero time total nitrogen levels of the double dosage hydrogen peroxide test (refer to fig. 11, page 7). Also noted is that about half of the points do not have standard deviations, which is from only one sample being run at that point. The points without standard deviations were thought to be less important to the experiment since these were transition areas or after nutrient levels evened out.

Colorimetric analysis is a temperamental method which is the reason for EPA methods stating that the experimenter should have at least six months training in the method before execution, and this is reflected in some of the uncertainties in the project. More data is needed to plot a mathematical smooth curve for the relationship between variables across the whole time frame. Nonetheless, there are general trends that can be observed from the data gathered, trends which can be explained within reason, so the results from this experiment are believed to be credible.

References

1. United States of America. EPA. Office of Research and Development. *Determination of Nitrate and Nitrite in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis*. By Jia-Zhong Zhang, Peter Ortner, and Charles Fischer. Sept. 1997. Web. 8 Jan. 2012. <http://www.epa.gov/nerlcwww/m353_4.pdf>.

2. United States of America. EPA. Office of Research and Development. *Determination of Ammonia in Estuarine and Coastal Waters by Gas Segmented Continuous Flow Colorimetric Analysis.* By Jia-Zhong Zhang, Peter Ortner, Charles Fischer, and Lloyd Moore. Sept. 1997. Web. 8 Jan. 2012. <http://www.epa.gov/microbes/m349_0.pdf>.

Appendix A

Plotted Oxidation Time Test Averages

Time (hr.)	Pond Water Nitrogen (PPB)	Std. Dev.	Time (hr.)	Surface Sea Water Nitrogen (PPB)	Std. Dev.	Time (hr.)	Deep Sea Water Nitrogen (PPB)	Std. Dev.
0	1406.333	31.89566	0	6.3	1.30767	0	545.1333	49.59691
0.17	609	52.3259	0.17	12.55	3.040559	0.17	205.5	16.26346
0.25	621	0	0.25	22.3	0	0.25	192	0
0.33	1323	22.62742	0.33	23.15	2.899138	0.33	405	16.97056
0.5	1217	0	0.5	33.8	0	0.5	514	0
0.83	1411.5	2.12132	0.83	47.35	0.636396	0.83	539.5	0.707107
1	1426.667	24.54248	1	44.53333	5.430776	1	562.6667	40.15387
1.5	1430.333	17.92577	1.5	51.7	8.674676	1.5	572	44.19276
2	1432	25.15949	2	68.23333	1.871719	2	577	38.15757
2.5	1448	13.89244	2.5	78.93333	0.873689	2.5	584.3333	41.30779
3	1432	25.15949	3	64.63333	3.300505	3	592.3333	36.08786
3.6	1468	0	3.6	64.8	0	3.6	635	0

Time (hr.)	Pond Water Phosphate (PPB)	Std. Dev.	Time (hr.)	Surface Sea Water Phosphate (PPB)	Std. Dev.	Time (hr.)	Deep Sea Water Phosphate (PPB)	Std. Dev.
0	135.0667	1.616581	0	5	1.014889	0	89.13333	7.68917
0.17	16.9	24.18305	0.17	-4.4	2.12132	0.17	-2.75	0.212132
0.25	2.1	0	0.25	12	0	0.25	-4.1	0
0.33	135.5	0.707107	0.33	10.35	1.767767	0.33	92.45	0.070711
0.5	8.4	0	0.5	12.6	0	0.5	103.2	0
0.83	133	0	0.83	14.85	0.070711	0.83	92.4	0.565685
1	132.1	1.558846	1	14.8	1.30767	1	94.1	5.139066
1.5	131.5333	0.723418	1.5	14	1.228821	1.5	95	5.975784
2	129.9	1.852026	2	13.2	0.6	2	92.66667	5.236729
2.5	129.4333	2.713546	2.5	14	0.3	2.5	93.8	4.158125
3	130.8667	1.96299	3	14.76667	0.80829	3	93.03333	5.46015

		1						9
3.6	128.4	0	3.6	13.9	0	3.6	99.3	0

Plotted Hydrogen Peroxide Test Averages

Time (hr.)	Blank Dose Nitrogen (PPB)	Std. Dev.	Time (hr.)	Single Dose Nitrogen (PPB)	Std. Dev.	Time (hr.)	Double Dose Nitrogen (PPB)	Std. Dev.
0	11.15	5.727565	0	25.7	27.43574	0	93.65	103.3083
0.17	5	0	0.17	10.5	0	0.17	399	(outlier)
0.33	16	0	0.33	30.4	0	0.33	10	0
0.5	14	0	0.5	50.7	0	0.5	16.5	0
0.75	25.1	0	0.75	50.3	0	0.75	54.2	0
0.83	15.9	0	0.83	43.9	6.717514	0.83	38.2	0
1	25.4	8.909545	1	41.65	6.717514	1	54.63333	6.185736
1.5	44.725	17.76183	1.5	45.15	1.626346	2	65.53333	10.8657
2.5	75	16.12203	2	84.05	47.16402	2.5	84.55	43.62849
3	68.5	16.40488	2.5	88.95	26.5165	3	84.55	4.454773
3.5	53.3	0	3	81.85	9.121677	3.5	83.8	0
			3.5	48.1	0			

Time (hr.)	Blank Dose Phosphate (PPB)	Std. Dev.	Time (hr.)	Single Dose Phosphate (PPB)	Std. Dev.	Time (hr.)	Double Dose Phosphate (PPB)	Std. Dev.
0	8.4	1.697056	0	-1.1	0	0	-1.45	0.212132
0.17	5.7	0	0.17	-1.1	0	0.17	-1.3	0
0.33	10.4	0	0.33	18	0	0.33	-1.8	0
0.5	10.4	0	0.5	-1.3	0	0.5	-1	0
0.75	14.2	0	0.75	18.6	0	0.75	22	0
0.83	11.4	0	0.83	18.7	0	0.83	19	0
1	12.4	2.545584	1	17.1	1.697056	1	20.46667	0.450925
1.5	12.775	0.771902	1.5	16.15	1.909188	2	20.33333	0.351188
2.5	13.8	1.414214	2	19.2	0	2.5	21	0.282843
3	13.85	0.636396	2.5	17.2	0.282843	3	19.4	1.272792
3.5	14.2	0	3	18.45	0.070711	3.5	19.1	0

			3.5	17.4	0		

Appendix B

Oxidation Time Test

Time (hr.)	Pond Water Nitrogen (PPB)	Time (hr.)	Surface Sea Water Nitrogen (PPB)	Time (hr.)	Deep Sea Water Nitrogen (PPB)
0	1391	0	7.2	0	516
0	1385	0	6.9	0	517
0	1443	0	4.8	0	602.4
0.17	572	0.17	14.7	0.17	217
0.17	646	0.17	10.4	0.17	194
0.25	621	0.25	22.3	0.25	192
0.33	1339	0.33	25.2	0.33	393
0.33	1307	0.33	21.1	0.33	417
0.5	1217	0.5	33.8	0.5	514
0.83	1413	0.83	47.8	0.83	539
0.83	1410	0.83	46.9	0.83	540
1	1412	1	41.2	1	538
1	1413	1	41.6	1	541
1	1455	1	50.8	1	609
1.5	1421	1.5	47.2	1.5	548
1.5	1419	1.5	46.2	1.5	545
1.5	1451	1.5	61.7	1.5	623
2	1419	2	69	2	553
2	1416	2	69.6	2	557
2	1461	2	66.1	2	621
2.5	1439	2.5	78.7	2.5	562
2.5	1441	2.5	78.2	2.5	559
2.5	1464	2.5	79.9	2.5	632
3	1419	3	64.7	3	571
3	1416	3	67.9	3	572
3	1461	3	61.3	3	634
3.6	1468	3.6	64.8	3.6	635

Time (hr.)	Pond Water Phosphate (PPB)	Time (hr.)	Surface Sea Water Phosphate (PPB)	Time (hr.)	Deep Sea Water Phosphate (PPB)
0	136	0	5.9	0	84.3
0	136	0	5.2	0	85.1
0	133.2	0	3.9	0	98

0.17	-0.2	0.17	-2.9	0.17	-2.9
0.17	34	0.17	-5.9	0.17	-2.6
0.25	2.1	0.25	12	0.25	-4.1
0.33	136	0.33	9.1	0.33	92.4
0.33	135	0.33	11.6	0.33	92.5
0.5	8.4	0.5	12.6	0.5	103.2
0.83	133	0.83	14.9	0.83	92.8
0.83	133	0.83	14.8	0.83	92
1	133	1	15.4	1	90.6
1	133	1	15.7	1	91.7
1	130.3	1	13.3	1	100
1.5	132	1.5	15.4	1.5	91.6
1.5	131.9	1.5	13.1	1.5	91.5
1.5	130.7	1.5	13.5	1.5	101.9
2	131.7	2	12.6	2	89.3
2	130	2	13.2	2	90
2	128	2	13.8	2	98.7
2.5	131	2.5	13.7	2.5	91.5
2.5	131	2.5	14.3	2.5	91.3
2.5	126.3	2.5	14	2.5	98.6
3	132	3	15.5	3	90.5
3	132	3	14.9	3	89.3
3	128.6	3	13.9	3	99.3
3.6	128.4	3.6	13.9	3.6	99.3

Hydrogen Peroxide Dosage Test

Time (hr.)	**Blank Dose Nitrogen (PPB)**	**Time (hr.)**	**Single Dose Nitrogen (PPB)**	**Time (hr.)**	**Double Dose Nitrogen (PPB)**
0	7.1	0	45.1	0	166.7
0	15.2	0	6.3	0	20.6
0.17	5	0.17	10.5	0.17	399.6
0.33	16	0.33	30.4	0.33	10
0.5	14	0.5	14.3	0.5	16.5
0.75	25.1	0.75	50.7	0.75	54.2
0.83	15.9	0.83	43.9	0.83	38.2
1	19.1	1	36.9	1	61.7
1	31.7	1	46.4	1	50.2
1.5	32.1	1.5	44	1	52
1.5	70.9	1.5	46.3	2	72.3

1.5	35.7	2	117.4	2	71.3
1.5	40.2	2	50.7	2	53
2.5	63.6	2.5	107.7	2.5	115.4
2.5	86.4	2.5	70.2	2.5	53.7
3	80.1	3	75.4	3	81.4
3	56.9	3	88.3	3	87.7
3.5	53.3	3.5	48.1	3.5	83.8

Time (hr.)	Blank Dose Phosphate (PPB)	Time (hr.)	Single Dose Phosphate (PPB)	Time (hr.)	Double Dose Phosphate (PPB)
0	7.2	0	-1.1	0	-1.3
0	9.6	0	-1.1	0	-1.6
0.17	5.7	0.17	-1.1	0.17	-1.3
0.33	10.4	0.33	18	0.33	-1.8
0.5	10.4	0.5	-1.3	0.5	-1
0.75	14.2	0.75	18.6	0.75	22
0.83	11.4	0.83	18.7	0.83	19
1	10.6	1	15.9	1	20
1	14.2	1	18.3	1	20.5
1.5	12	1.5	14.8	1	20.9
1.5	12.3	1.5	17.5	2	20.3
1.5	13.1	2	19.2	2	20
1.5	13.7	2	19.2	2	20.7
2.5	12.8	2.5	17.4	2.5	21.2
2.5	14.8	2.5	17	2.5	20.8
3	13.4	3	18.5	3	18.5
3	14.3	3	18.4	3	20.3
3.5	14.2	3.5	17.4	3.5	19.1