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The effect of audible sound on the kinetics of an inorganic and an enzyme catalyzed reaction

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ABSTRACT

The application of audible sound to biological systems has shown a variety of results in biological systems, such as seed germination of plants and specific enzymatic activity of rice-koji. This study focuses on two types of chemical reactions, one inorganic and the other biological, the effects of audible sound on the kinetics of the Iodine Clock reaction and the Potato Acid Phosphatase reaction. Audible sound in the form of white noise at 104 dB produced no significant effects on the rate of the Iodine Clock reaction. Potato acid phosphatase reactions irradiated under the frequencies of 100, 1000, 6300 Hz, as well as under white noise conditions also showed no significant differences in reaction rates. The reactions produced statistical values ranging from P=0.337 to P=0.966. Thus the conclusion is that under the conditions of this study audible sound has no effect on the reaction rates of Iodine Clock and Potato Acid Phosphatase reactions.

Keywords: *Iodine Clock Reaction, Potato Acid Phosphatase, Audible Sound*

INTRODUCTION

The application of sound to biological systems has shown many results over a variety of fields. Many studies have been conducted on the effects of audible sound on plants. Studies have shown that a seed germination bioassay produced more sprouted seeds when exposed to music or noise. (Creath K. Schwartz G. 2004) The application of sound waves to plants has been shown to increase yield and strengthen the plant immune system. (Hassanien R. Hou T.Z. 2014) When treated with acoustic frequency strawberry leaves have shown an increase in net photosynthetic rate as well as augmentation of chlorophyll content and the number of fruits and flowers. (Meng Q. Zhou Q. et al. 2012) A study found that sound waves have effects on the specific enzymatic activity of rice-koji. Although the mechanism of imposed frequency and its impact on rice-koji was not determined, the study suggests that sound may play an important environmental role in the making of rice-koji. (Saigusa N. Imayama S. et al. 2015) Each of these biological systems must involve chemistry in some manner.

There is little literature indicating that the effect of audible sound on chemical reaction systems has been significantly studied. Sound has been shown to affect reaction rates. Early literature has shown that the addition of sonic energy to the hydrolysis of potassium persulfate will increase the reaction rate as well as the concentration of persulfate. (Schumb W. Rittner E. 1940) In oxidations using audible frequencies it was confirmed that chemical activation occurs during sonic cavitation. (Flosdorf E. Chambers L. et al. 1936) Ultrasound waves can expand and compress the medium they travel through, allowing the waves to physically act on cells and biomolecules. (Pitt W. Husseini G. et al. 2006)

More recent literature has shown that application of single frequency computer generated sounds will increase crystal yield as well as crystal purity in the protein crystallization process. (Zhang C.Y. Wang Y. et al. 2016) Further research has shown that this increased yield and purity also takes place under exposure to “real-world” sounds found in the everyday environment. This is proposed to be due to an increase in the nucleation rate of the protein. (Zhang C.Y. Liu Y. et al. 2018)

Sound is produced by soundwaves. As a sound wave travels molecules and other elements of air are compressed and moved from equilibrium. Changes in pressure and density are also observed following the wave motion direction. There are three main categories of sound waves: audible, infrasonic, and ultrasound. Sound waves are composed of a frequency, a wavelength, and an amplitude. White noise is a continuum of frequencies equally distributed over the whole hearing range. (Serway R.A. Jewett J.W. 2016)

It was decided to probe the effects of audible sound on the reaction rates of an inorganic reaction as well as biochemical reaction. The two reactions chosen were the Iodine clock reaction and Potato Acid Phosphatase cleavage of substrates.

Iodine clock reactions are well known for their easily visible color change. They serve as a good example to observe solution kinetics.

$$5HSO_3^- + 2IO_3^- \rightarrow I_2 + 5SO_4^{2-} + H_2O + 3H^+$$

There has been research done on the mechanisms for these reactions. The reduction of iodate by bisulfate in the reaction produces an iodate species. One study suggests that the rate determining step of the reaction is related to migration and reaction of these species.

(Lambert J.L. Fina G.T. 1984)

Experiments have been formulated to study the enzymatic assay of potato acid phosphatase. (Bergmeyer H.U. Gawehn K. et al. 1974

$$\text{p-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{Phosphatase, Acid}} \text{p-Nitrophenol} + P_i$$

These experiments use the method of quenching to stop the reaction in the samples taken out for analysis. Quenching is useful when running spectrophotometric analysis.

MATERIALS AND METHODS

Iodine Clock Reaction

A solution of 0.036 M KIO_3 was prepared in a 250 mL volumetric flask by dissolving 1.926 g of KIO_3 (Sigma-Aldrich) into water. Using approximately 200 mL of water and 2 g of starch, a starch water solution was made. A solution of 0.016 M $NaHSO_3$ was then made by dissolving 0.1664 g of $NaHSO_3$ (Mallinckrodt) into the starch water in a 100 mL volumetric flask. (Hall J.F. 1997) A three place scale was used to measure all the weights.

Method 1: Generating independent solutions for comparison of means

Initial trials were run to determine the average kinetic rate of the iodine clock reaction in a normal room environment. The reaction was prepared by adding 25.00 g of water, 20.00 g of the KIO_3 solution into a beaker. The beaker was set up in front of a phone camera and the recording started. 5.00 g of HSO_3 solution was then added followed by thirty seconds of stirring with a stir rod. When a deep blue color began to appear, the camera was stopped, and another trial was set up. Four trials were run simultaneously in front of the camera. The length of the trial was determined by the time from when the HSO_3 solution was added to the second the reaction began to turn blue. (Hall J.F. 1997)

A similar experimental set up was utilized in the sound irradiation trials. The prepared beaker and solution were placed into a plastic container (Walmart) with two powered speakers (Chemistry Lab) set up on either side of the beaker. The speakers were turned to ¾ on the volume setting. Decibels were measured on an iPhone using the Decibel X app. A computer was connected to the speakers and the website mynoise.net used for generating white noise (20-20,000 Hz). At the end of the reaction the temperature of each solution was measured.

Method 2: Generating and splitting a single solution for individual paired t-tests

A new method of data collection was devised which increased precision. Generating one solution and splitting it proved to be the best method to produce and compare identical solutions exposed to different environments. In this method, 12.5 g of water and 2.50 g of HSO_3 solution were measured into one large test tube and vortexed. 10.00 g of KIO_3 solution was measured into a second large test tube. The camera was started and the KIO_3 solution was poured into the first test tube and the entire solution was poured back into the second test tube and vortexed for ten seconds. This solution was split evenly between two medium sized test tubes with one placed into an insulated high sound environment and the other into an insulated low sound environment. It took approximately thirty seconds to transfer the test tubes into the two environments. The length of the trial was determined by the time from when the HSO3 solution was added to the second the reaction began to turn blue.

A variety of methods were determined to test pH, gas content, and stirring. These methods allowed each run to be tested as an individual. The test tubes were acid washed in 3 M HCl for thirty minutes before tests were run. Trials were run with stirring and without stirring. The reactions were sparged with Helium (99.9%) and compressed air (Melhorn Science Lab).

Potato Acid Phosphatase Reaction

A 100mL of 90mM citrate buffer of pH 4.8 (2.6465g Citric Acid (Sigma-Aldrich) in 100 mL of DI water, adjusted to pH 4.8 using HCl) along with a 15.2 mM solution of p-nitrophenyl phosphate (PNPP) (6.08 mL of 0.05 M stock PNPP solution diluted to 20 mL with DI water) and 1 L of a 100 mM NaOH (4g NaOH (Fischer) in 1 L of DI water) solution were prepared. An enzyme solution containing 0.15-0.25 unit/mL of acid phosphatase was prepared by diluting 1mg of lyophilized potato acid phosphatase (Sigma-Aldrich) in 12 mL of cold deionized water. Fifteen test tubes, seven for each sound environment and one for a blank, were filled with 2.5 mL of the NaOH solution.

In a large test tube, a solution was made using 3 mL of the citrate buffer and 3 mL of the PNPP solutions. Enzyme solution, 0.60 mL, was added to the test tube and the solution was vortexed thoroughly. Immediately, 0.50 mL of the solution was removed and added into one of the test tubes containing the NaOH solution to quench the reaction. This solution served as the blank for both sound environments. The solution was evenly split between two test tubes and placed into the two different sound environments. Aliquots of 0.50 mL were removed from each sound environment and quenched at five-minute intervals. In the sound environment, the samples were surrounded by the two speakers and insulation and exposed to white noise generated by the website mynoise.com. The low sound environment was an insulated container placed on a different countertop in a separate part of the room. Absorbances were measured at 410 nm in plastic cuvettes using a Genesys 10S UV-VIS Spectrophotometer (Thermo Scientific). (Bergmeyer H.U. Gawehn K. et al. 1974)

Trials were run in a similar manner at the frequencies of 100, 1000, and 6300 Hz generated by the website szynalski.com. Frequencies were confirmed by the app Piano Tuner.

The rates of the two different types of reactions were calculated by plotting Absorbance vs Time and the LINEST function on Excel was used to determine slope and standard deviation of the slope.

RESULTS

Iodine Clock Reaction

Method 1: Generating independent solutions for comparison of means

Forty initial trials of the Iodine clock reaction were run by generating each test solution independently. These trials were used to develop and increase precision as well as the appropriate method of dispensing solutions. Initial trials were run using volumetric addition of reagents but did not yield results that were reproducible enough to show statistical differences. The solutions were then measured out gravimetrically rather than volumetrically as weighing out the reagents to two decimal places yielded more consistent results but were still not precise enough to draw any statistical conclusions.

Trials were run with ambient sound on the lab table and others were exposed to sound via speakers in a closed box. The sound environment was measured at 104 dB and the low sound environment was measured at 60 dB. Three groups of trials were run, with different batches of solutions made for each of the groups. None of the groups showed a significant statistical difference in the reaction rate. Although they were often within a minute of each other, there was very little consistency in reaction rates as some trials showed sound took longer and some trials showed low sound took longer.

Method 2: Generating and splitting a single solution for individual paired t-tests

Initial trials proved irreproducible, so a new method of data collection was devised to increase precision. The Low sound environment was changed to an insulated box measured at 33 dB to reduce ambient sound. The high sound environment remained the same.

These results minimized the error of the previous dispensing methods. There was still variation between runs, but the new method helped to produce greater consistency within each run. This method of splitting the solution allowed for the results to be evaluated with paired t tests (assuming equal variance).

Table 1 showcases the results of the first set of concentrations of KIO_3 and IO_3. These trials ran for a shorter period of time, approximately four and a half minutes. Group 1 is the set of trials run in beakers, p-value of 0.923. The trials of Groups 2-6 were run in test tubes. The p-values were 0.966, 0.874, 0.337, 0.889, and 0.506 respectively.

Table 2 showcases the results of the trials run at the second set of concentrations of KIO_3 and IO_3. These trials ran for a longer period of time, around eight minutes.

Table 1. Groups represent trials done with a single batch of reagents. Group 1 was run in beakers, groups 2-6 were run in test tubes. The mean difference is LS-S. Time is in seconds and temperature is in °C. LS-Low sound, S- Sound

Group	1	2	3	4
# runs	5	4	2	4
[KIO_3]	0.0144	0.0144	0.0144	0.0144
[HSO_3]	0.0016	0.0016	0.0016	0.0016
Average Time S	288.2	252	246.5	245.5
Average Time LS	289	252.5	243	243.25
Average Temp	20.3	20.83	20.8	21.7
Mean Difference	0.8	0.5	-3.5	-2.25
p-values	0.923	0.966	0.874	0.337

Table 2. Groups represent trials done with a single batch of reagents. The mean difference is LS-S. Time is in seconds and temperature is in °C.

Group	5	6
# runs	2	8
[KIO_3]	0.0072	0.0072
[HSO_3]	0.0016	0.0016
Average Time Sound	503.5	491.625
Average Time Low Sound	501	495.125
Average Temp	20.95	21.113
Mean Difference	-2.5	3.5
p-values	0.889	0.506

The p-values of each of these trials are close to 1.0

which indicates that they support the null hypothesis and show very little difference in the reaction rates between the sound and low sound environments.

Pooling all of the twenty-eight trials, both shorter and longer times, resulted in a p-value of 0.980.

Attempts were made to understand and reduce error by sparging the solution, stirring the solution, and controlling the pH of the glassware. These results were inconclusive.

Potato Acid Phosphatase Reaction

An initial trial of the potato acid phosphatase reaction was run at 37°C. The same procedure was followed to run trials at room temperature. These trials were run under white noise conditions as well as at 100, 1000, and 6300 Hz. The slopes were then analyzed using the LINEST function. These trials produced no significant statistical differences in the rate of kinetics of low sound versus sound.

Table 3 showcases the rates (slopes) and standard deviations of the potato acid phosphatase reactions under different treatments. The first two trials were run with one enzyme solution. A new one was made for trials three through six. Trial 7 was run with a new enzyme solution on a different date.

Table 3. The reaction rates (slope) and standard deviations (SD) of potato acid phosphatase reactions. Slope and SD generated with LINEST function. Slope and SD reported in (10^{-4} absorbance per second).

Trials	Treatment	Slope(st. dev.)
1 - Sound	White noise	6.791 (0.233)
1 - Low Sound	White noise	6.625 (0.085)
2 - Sound	White noise	7.622 (0.185)
2 - Low Sound	White noise	7.494 (0.150)
3 - Sound	100 Hz	4.311 (0.234)
3 - Low Sound	100 Hz	4.294 (0.032)
4 - Sound	1000 Hz	3.724 (0.308)
4 - Low Sound	1000 Hz	4.141 (0.075)
5 - Sound	1000 Hz	4.105 (0.065)
5 - Low Sound	1000 Hz	4.150 (0.131)
6 - Sound	6300 Hz	3.527 (0.103)
6 - Low Sound	6300 Hz	3.911 (0.101)
New Enzyme	**Solution**	
7 - Sound	6300 Hz	16.45 (0.222)
7 - Low Sound	6300 Hz	15.95 (0.334)

DISCUSSION

Iodine Reaction

The experimental results indicate that under the conditions of this study the Iodine Clock Reaction is not sensitive to audible sound. Throughout this experiment, multiple variations of data collection were devised to increase precision. The method of generating and splitting a single solution was adapted from the technique developed for the potato acid phosphatase reaction. The data indicates that at both lower and higher concentrations of iodate, there seems to be no effect of audible sound on the reaction.

Further research might be done on longer reactions of the Iodine Clock Reaction as previous studies required significantly longer reaction times before they showed any significant results.

Enzyme Reaction

The experimental results indicate that the Potato Acid Phosphatase reaction is not sensitive to audible sound. When the slopes (rates) and standard deviations of the runs produced by the LINEST functions were compared, one frequency level (6300 Hz) appeared to show a potential statistical difference. A second run at this frequency was conducted and showed the reverse trend indicating the first deviation was probably due to random effects. The sound and low sound trials mostly seemed to be within a standard deviation of each other.

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