

# Yeast catalysis of hydrogen peroxide as an enhanced chemical treatment method for harvested rainwater

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#### **SUMMARY**

Rainwater is frequently collected at home for many reasons, so household methods of rainwater treatment are necessary for convenience. Usually, the chlorination process with hypochlorite (CIO<sup>-</sup>) bleach is used, but hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) has been proposed as a home treatment method. Catalyzed H,O, treatment is already used for wastewater pollution, so it may also be applied to harvested rainwater. A home-accessible catalyst is yeast. This study aimed to investigate whether catalyzed H<sub>2</sub>O<sub>2</sub> treatment is a viable way to chemically treat homecollected rainwater. Our main hypothesis was that H<sub>2</sub>O<sub>2</sub> would decrease concentrations of chemical contaminants more effectively than CIO due to its stronger oxidation capabilities and catalysis would quicken treatment speed. Rainwater samples were treated with varying concentrations of CIO<sup>-</sup>, pure H<sub>2</sub>O<sub>2</sub>, or yeast-catalyzed H<sub>2</sub>O<sub>2</sub>. We assessed the samples with pH probes, dissolved oxygen (DO) probes, and chemical contaminant test strips before, during, and after treatment. We found that in terms of pH, CIO treated water the most effectively, bringing the pH closest to 7; but in terms of DO, yeast-catalyzed H,O, treatment was the most effective, resulting in the healthiest DO levels. The test strips assessing chemical contaminants had uncertainties too high to form many conclusions. Therefore, either chlorination or yeast-catalyzed H<sub>2</sub>O<sub>2</sub> treatment show potential as the best methods investigated for home rainwater treatment, but further research is needed to assess their effects on specific contaminants. These findings imply that yeast-catalyzed H<sub>2</sub>O<sub>2</sub> treatment could be a novel method of home rainwater treatment that is both cost-effective and eco-friendly.

# INTRODUCTION

Many people collect rainwater at home for a variety of reasons. Sometimes it is for conservation purposes, and sometimes it is the household's only water source. Either way, this water has lots of uses, treated or not – it is often used for watering plants, outdoor ponds, bathing, cleaning, and sometimes drinking (1). Some applications, such as drinking, require collected rainwater to be purer than others. In any case, it is important to treat the water so its quality is the highest it can be. A key step in water treatment is chemical treatment, in which potentially harmful chemicals, such as manganese, are reduced in concentration, either by oxidation out of the water

or change in speciation to form precipitates (2). Even though rainwater does occasionally contain microorganisms, they are more easily eliminated than chemical contaminants (3). Water is normally treated at home by chlorination with hypochlorite (CIO-) and/or boiling. Occasionally, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is used (4). Chlorination both kills microorganisms and chemically reduces concentrations of harmful chemicals, and boiling kills microorganisms well but is less effective at cleaning chemicals out - many chemical contaminants' boiling points are above that of water (metals like manganese and zinc, for example) and thus will remain in the water after boiling (4). There is evidence that H<sub>2</sub>O<sub>2</sub>, like ClO<sup>-</sup>, both kills microorganisms and chemically treats water (5,6). In fact, it may be just as effective, if not more, than CIO- at decreasing harmful chemicals since it is a more potent oxidizer than CIO-(5). That said, there is no current experimental data to indicate whether its strength of treating chemical contaminants is comparable to that of CIO-.

A reason to use  $\rm H_2O_2$  for home water treatment is that even though both  $\rm H_2O_2$  and CIO-take a long time to treat water,  $\rm H_2O_2$  can be catalyzed to act much faster than CIO-, only needing a few hours as opposed to days for CIO- (7,8). Additionally, chlorinated water requires disposal, dilution, or some other additional chemical treatment to be safe, because chlorine remains in water after bleach treatment (8). In contrast, water treated with  $\rm H_2O_2$  does not need additional chemical treatment as  $\rm H_2O_2$  will decompose into harmless oxygen gas and water. At most, biomatter and precipitates would need to be filtered from water treated with  $\rm H_2O_2$ , but additional chemical treatment is not necessary (9).

The power of  $\rm H_2O_2$  is that it is a strong oxidizer. Its normal reaction pathway produces reactive hydroxyl and superoxide radicals, and it can be catalyzed by iron in its 2+ oxidation state, Fe(II), to a much faster pathway that also involves reactive free radicals, such as hydroxyl and hydroperoxyl (2,10). Fe(II) is a powerful catalyst of  $\rm H_2O_2$  and is sometimes known as the Fenton reagent. The Fenton process, in which an iron catalyst is directly put in solution with  $\rm H_2O_2$ , is already widely used to treat wastewater pollution (9). The Fenton reagent commercially comes in the form of FeSO<sub>4</sub>, but this chemical is inconvenient for households, as it does not have many household applications. A more accessible form is yeast, which contains Fe(II) in its cells (10).

The purpose of this investigation was to test whether yeast-catalyzed  $\rm H_2O_2$  is viable to treat home-collected rainwater in 24 hours, especially when compared to CIO-, which is currently the standard for this type of chemical treatment. We decided to use a 24-hour time frame because when stored for longer periods, water is prone to acidification and contamination. We tested the pH, dissolved oxygen (DO), and several common

water contaminant concentrations of all samples to assess water quality before, during, and after treatment. We used probes to test pH and DO to ensure precision.

We tested pH and DO because they are important water quality indicators. pH is a measurement of acidity or alkalinity based on the concentration of hydronium ions, and the pH value of pure water is exactly 7 at 25°C (neutral), meaning that any deviation from this value means the water is impure, allowing us to roughly quantify the treated water's proximity to cleanliness. DO measures the concentration of oxygen dissolved in the water. Lower levels show the presence of more organic waste, because most oxygen-demanding waste is organic waste. A DO level of less than 5 mg/L would indicate an undesirable level of organic waste, and most healthy water has DO levels above 6.5-8 mg/L (11,12).

As water quality can also be determined by the concentrations of chemical contaminants within, we measured nine additional parameters: total alkalinity, zinc, sulfate, nitrite + nitrate, combined chlorine, manganese, iron, hydrogen sulfide, and hardness. All of these parameters were assessed with test strips, as they are an accessible and typical method of approximating water quality. A total alkalinity range of approximately 60-180 mg/L is preferred (13). We monitored combined chlorine in hopes to be aligned with the concentrations of CIO- added, as a general measure of the test strips' accuracy. Lower concentrations of zinc, sulfate, nitrite + nitrate, manganese, iron, and hydrogen sulfide are preferred (in addition to lower values of hardness), because they negatively affect water quality, potentially causing sickness and unpleasant odors, colors, and side effects on the skin (14-16).

We hypothesized that both catalyzed and pure  $H_2O_2$  would reduce concentrations of chemical contaminants more effectively than CIO in 24 hours because of the stronger oxidation capabilities of  $H_2O_2$ , and that catalyzing  $H_2O_2$  would quicken its treatment speed compared to its pure form. We found that CIO was more successful than  $H_2O_2$  at water treatment in terms of pH, causing it to be the closest to 7. However, catalyzed  $H_2O_2$  treatment was more successful than CIO in terms of dissolved oxygen (DO), producing the healthiest DO levels. The key takeaway is that CIO or catalyzed  $H_2O_2$  treatment appear to be more successful for rainwater in 24 hours than pure  $H_2O_2$ .

## **RESULTS**

We collected rainwater once and then split it into samples of equal volumes, on which we performed water treatment. This was to eliminate regional and seasonal variation in the chemicals within the rainwater. We treated it with varying concentrations of three chemicals: CIO-, pure H2O2, and catalyzed H<sub>2</sub>O<sub>2</sub>. The concentrations of these chemicals were varied based on a range of recommended concentrations in the literature, to increase the possibility of using the optimal concentration of that chemical (8,17-20). We assessed water quality based on standard parameters such as pH and DO, as well as the presence of more specific chemical contaminants such as zinc, sulfate, and manganese. These specific parameters are commonly used to assess water quality because they are EPA-regulated, meaning if they exceed a certain concentration, the water is considered unsafe (14). We tested all samples at varying times to track the progression of their chemical treatment, stopping at the 24-hour mark.

As CIO $^{-}$  is still the standard for home rainwater treatment, we used it as a comparison to the potential catalyzed  $H_2O_2$ method. In addition, as the water treatment ability of pure  $H_2O_2$  compared to CIO $^{-}$  is relatively unknown, an assessment of it was necessary. Further, treatment with pure  $H_2O_2$  would provide a direct comparison with the treatment ability of the catalyzed version of itself, allowing for an assessment of how much catalysis enhances the speed and quality of  $H_2O_2$  water treatment. We also included a control group to represent the natural fluctuation of the parameters within rainwater.

We observed that rainwater pH tended to decrease over time, even in the control group (**Figure 1**). The lowest percentage of CIO tested, 0.05% v/v, produced the pH closest to 7 of all treatments (**Figures 2 - 4**). Treatment with pure  $H_2O_2$  and yeast-catalyzed  $H_2O_2$  always resulted in pH levels above the control's, though for CIO 0.15% v/v yielded the same final pH as the control (**Figures 2 - 4**). Higher concentrations of CIO generally resulted in lower pH levels (**Figure 2**). For pure  $H_2O_2$ , the pH was generally higher as more was added, but the trend is not strongly followed, notably with the highest concentration tested, 1.5% v/v, which had a different pH-overtime trend than the other concentrations (**Figure 3**). The pH for yeast-catalyzed  $H_2O_2$  treatment fluctuated greatly across time, though its mean value was similar to that of the other treatment agents (**Figures 1, 4**).

In the control, DO (dissolved oxygen, higher levels preferred) decreased over time before increasing slightly at 24 hours, but for all treatment agents, DO tended to reach its highest value before the end (**Figure 5**). CIO generally produced lower final DO levels than the control, with more CIO often resulting in lower DO levels during the treatment process—at 1 hour, for example, 0.05% v/v CIO resulted in a DO of 8.7 mg/L, compared to 0.1% resulting in 7.2 mg/L and 0.15% resulting in 3.5 mg/L (**Figure 6**). All of the pure H<sub>2</sub>O<sub>2</sub> DO levels ended higher than that of the control (**Figure 7**). Their highest DO values across the entirety of their treatment time were also higher than that of CIO notably 1.5% v/v H<sub>2</sub>O<sub>2</sub> at 1 hour, which had a DO of 13 mg/L compared to 8.7 mg/L for CIO (**Figures 6, 7**). For the catalyzed H<sub>2</sub>O<sub>2</sub>

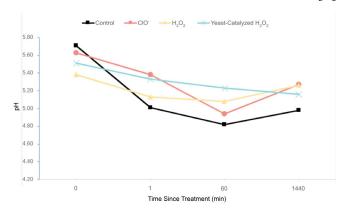


Figure 1. Mean pH of all concentrations of treatment agents vs. time. Mean pH values over time (each line represents n=9 measurements). 0.05mL, 0.1mL, or 0.15 mL of 7.5% CIO $^\circ$  bleach; 0.5 mL, 1 mL, or 1.5 mL of 3%  $\rm H_2O_2$  solution; 0.5 mL, 1 mL, or 1.5 mL of 3%  $\rm H_2O_2$  solution with 0.02 g, 0.04 g, or 0.06 g of dry baker's yeast (respectively) were added to home-collected rainwater (n=3 for each amount) and pH was assessed at different times after treatment agent addition with a digital probe. In the control, nothing was added but pH was still assessed at similar times to the treated samples.

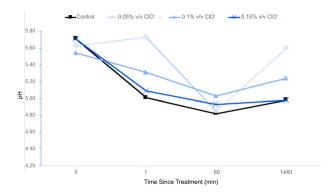


Figure 2. pH of varying concentrations of hypochlorite-treated rainwater vs. time. Mean pH values over time (each line represents n=3 measurements). 0.05 mL, 0.1 mL, or 0.15 mL of 7.5% CIO bleach was added to home-collected rainwater and pH was assessed at different times after CIO addition with a digital probe. In the control, nothing was added but pH was still assessed at similar times to the CIO samples.

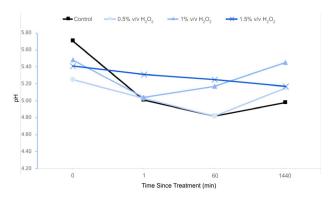


Figure 3. pH of varying concentrations of hydrogen peroxide-treated rainwater vs. time. Mean pH values over time (each line represents n=3 measurements). 0.5mL, 1mL, or 1.5mL of 3%  $\rm H_2O_2$  solution was added to home-collected rainwater and pH was assessed at different times after  $\rm H_2O_2$  addition with a digital probe. In the control, nothing was added but pH was still assessed at similar times to the  $\rm H_2O_2$  samples.

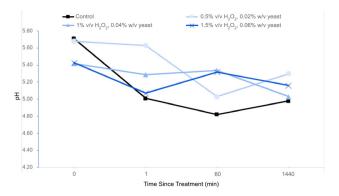


Figure 4. pH of varying concentrations of yeast-catalyzed hydrogen peroxide-treated rainwater vs. time. Mean pH values over time (each line represents n=3 measurements). 0.5mL, 1mL, or 1.5mL of 3%  $\rm H_2O_2$  solution was added with 0.02g, 0.04g, or 0.06g of dry baker's yeast (respectively) to home-collected rainwater and pH was assessed at different times after  $\rm H_2O_2$  and yeast addition with a digital probe. In the control, nothing was added but pH was still assessed at similar times to the catalyzed  $\rm H_2O_2$  treatment samples.

treatment, every concentration's highest DO value across time was much higher than that of the other treatment agents (**Figures 6-8**). The DO levels were higher when more  $H_2O_2$  and yeast was added, though by the end of the 24 hours, this relationship was less evident as the highest DO value at 24 hours belonged to the lowest concentration of  $H_2O_2$  and yeast. It was also the highest final DO out of all treatment agents tested, at 9.5 mg/L for 0.5% v/v  $H_2O_2$  and 0.02% w/v yeast, indicating that catalyzed  $H_2O_2$  treatment was the most effective at inducing preferable DO levels (**Figure 8**).

We assessed the chemical parameters with test strips. However, after performing operations such as averaging and summing the data, the propagated measurement uncertainties for all test strip parameters became too high to draw many valid conclusions.

## **DISCUSSION**

We performed our analysis under the assumption that the closer the pH was to 7, the generally more well-treated the water was, because pure water has a pH of 7. Our observation that pH tended to decrease over time agrees with the literature because theoretically, when water is exposed to air, the carbon dioxide in the air reacts with water to form carbonic acid which should decrease the pH (21). In terms of pH, we found CIO to be the most effective, because it was the treatment method that produced a pH closest to 7. We observed H<sub>2</sub>O<sub>2</sub> to have the second-closest pH to 7, followed by catalyzed H<sub>2</sub>O<sub>2</sub> treatment, though both catalyzed and uncatalyzed H2O2 treatments' pH graphs decreased similarly over time. Theoretically, the decomposition of H<sub>2</sub>O<sub>2</sub> should have decreased the pH from the starting value. This is because it is an exothermic reaction which raises the temperature, and as temperature increases, pH decreases due to a shift in the solution's equilibrium (22). Therefore, the decreasing pH values we observed for both catalyzed and uncatalyzed H<sub>2</sub>O<sub>2</sub> treatment agreed with the literature.

With respect to DO (dissolved oxygen, higher levels preferred), we found catalyzed H2O2 treatment to be most effective. This is because it generally yielded the highest final DO levels, with a maximum of 9.5mg/L after 24 hours with 0.5% v/v H<sub>2</sub>O<sub>2</sub> and 0.02% w/v yeast. We observed that CIO tended to decrease the DO, with levels ranging from unhealthy (<5 mg/L) to acceptable but not recommended (5-6.5 mg/L). We expected chlorination to do the opposite and increase the DO, because chlorination has been proven to reduce biological oxygen demand, which is inversely proportional to DO (23). We found that both catalyzed and uncatalyzed H<sub>2</sub>O<sub>2</sub>treatment gave acceptable DO values for all concentrations (≥5mg/L), and both had far higher maximum values than CIO- across their entire treatment process. This aligned with our theoretical understanding, because as H<sub>2</sub>O<sub>3</sub> decomposes into water and oxygen gas, some of the oxygen gas should remain dissolved in solution, making the reaction raise the DO levels simply by progressing. Therefore, our observation of catalyzed H<sub>2</sub>O<sub>2</sub> treatment having even higher maximum values than its uncatalyzed version also aligned with our understanding-catalysis had greatly increased the reaction rate, so in 24 hours, much more oxygen was produced under catalyzed conditions than non-catalyzed conditions. Overall, we found catalyzed H2O2 treatment to yield the healthiest DO levels, followed by uncatalyzed H<sub>2</sub>O<sub>2</sub> and then CIO-, which sometimes even decreased the DO to

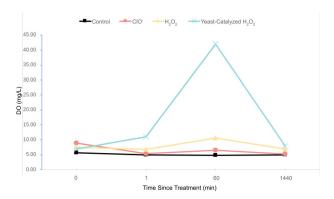


Figure 5. Mean DO of all concentrations of treatment agents vs. time. Mean DO values over time (each line represents n=9 measurements). 0.05mL, 0.1mL, or 0.15mL of 7.5% ClO $^\circ$  bleach; 0.5mL, 1mL, or 1.5mL of 3%  $\rm H_2O_2$  solution; 0.5mL, 1mL, or 1.5mL of 3%  $\rm H_2O_2$  solution with 0.02g, 0.04g, or 0.06g of dry baker's yeast (respectively) were added to home-collected rainwater (n=3 for each amount) and DO was assessed at different times after treatment agent addition with a digital probe. In the control, nothing was added but DO was still assessed at similar times to the treated samples.

below control levels. Therefore, based on DO, catalyzed  $\rm H_2O_2$  treatment was the most effective method tested.

We could not form many conclusions from the test strip parameters due to their high measurement uncertainties. The uncertainties overlapped and thus potentially nullified any relationship that seemed visible. We found that the various treatment methods did not seem to greatly change many of the parameters, though it is difficult to have much confidence in claiming any correlation between them.

We hypothesized that the water treated with  $\rm H_2O_2$  would have a larger reduction in concentrations of chemical contaminants than the water treated with  $\rm ClO^-$ , but with our data it was difficult to prove or disprove our hypothesis due to the high test strip uncertainties. On the other hand, our data agreed with our hypothesis that the catalyzed  $\rm H_2O_2$  treatment would be faster than the uncatalyzed version—we observed catalyzed  $\rm H_2O_2$  treatment to have higher maximum DO values than uncatalyzed  $\rm H_2O_2$ , a sign that the reaction was progressing quicker than the uncatalyzed version.

This investigation was not without limitations. A key one was our test strips having uncertainties too high to accurately measure chemical contaminant concentrations. When investigating contaminants, we should have used more precise equipment such as probes instead. In addition, our procedure required yeast with weights to the hundredths place, but the mass scale we borrowed from an institution was only precise to the tenths, so when massing out the yeast, we had to estimate the appropriate amounts. This may have caused some inconsistencies in the catalyzed H<sub>2</sub>O<sub>2</sub>treatment data and decreased our confidence in it. Next time, when borrowing equipment, it would be beneficial to double-check its precision. Thirdly, there were some errors in the process of data collection which required us to discard some data points. As we only had access to laboratory materials for a limited time, we were unable to repeat these data points which decreased our sample size. Collecting data for more time points would have helped increase the sample size. Finally, DO was not the most effective measurement to indicate water quality in the investigation because it is only

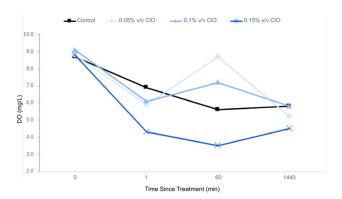


Figure 6. DO of varying concentrations of hypochlorite-treated rainwater vs. time. Mean DO values over time (each line represents n=3 measurements). 0.05mL, 0.1mL, or 0.15mL of 7.5% CIO¹ bleach was added to home-collected rainwater and DO was assessed at different times after CIO¹ addition with a digital probe. In the control, nothing was added but DO was still assessed at similar times to the CIO¹ samples.

an indirect indicator of organic waste (11). For example, as DO increased due to chemically-produced oxygen from  $\rm H_2O_2$  decomposition, a CIO<sup>-</sup>-treated sample and a  $\rm H_2O_2$ -treated sample could have had the same amount of organic waste but displayed different DO levels.

From our results, CIO- seemed to generally treat water more effectively than pure H2O2 in 24 hours. However, yeast catalysis greatly enhanced the speed and efficiency of H<sub>2</sub>O<sub>2</sub>chemical treatment, making catalyzed H<sub>2</sub>O<sub>2</sub> treatment a possible competitor to CIO as a harvested rainwater treatment method. If catalyzed H2O2 treatment is proven to be viable for that use, it would additionally have possible applications in providing clean water to those who cannot access it, emergency situations, outdoor portable water treatment kits, and more. It would also be more eco-friendly than CIO as a water treatment agent, because H2O2 decomposes into water and oxygen gas, leaving no trace in the water; yeast remnants can be filtered out and are biodegradable. In contrast, CIOremains in the water and must be discarded after treatment. That said, more research needs to be done on the effects of catalyzed H<sub>2</sub>O<sub>2</sub> treatment on specific chemical contaminants, ideally using precise tools such as probes. It would also be beneficial to continue comparing it to CIO, which is the established standard for rainwater treatment, and to find catalyzed-H<sub>2</sub>O<sub>2</sub>-to-water ratios that optimize its effectiveness at chemical treatment.

We observed  $H_2O_2$  to produce similar DO and pH levels to traditional CIO $^-$  treatments. Therefore, it has potential to be an eco-friendly alternate rainwater treatment method, though further testing is required to assess its viability in that regard. If proven successful, yeast-catalyzed  $H_2O_2$  treatment could expand the possibilities for home rainwater treatment, using treatment agents that are biodegradable over non-biodegradable agents such as CIO $^-$ .

## **MATERIALS AND METHODS**

Important safety note: Do not mix acids or household cleaners like  $H_2O_2$  with bleach, as they can react strongly and produce dangerous substances such as chloramine and chlorine gas (24).

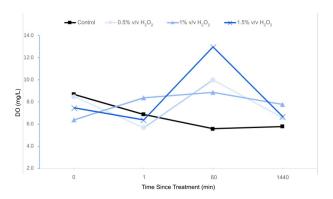


Figure 7. DO of varying concentrations of hydrogen peroxide-treated rainwater vs. time. Mean DO values over time (each line represents n=3 measurements). 0.5mL, 1mL, or 1.5mL of 3%  $\rm H_2O_2$  solution was added to home-collected rainwater and DO was assessed at different times after  $\rm H_2O_2$  addition with a digital probe. In the control, nothing was added but DO was still assessed at similar times to the  $\rm H_2O_3$  samples.

# **Preparing the samples**

A clean metal bowl (IKEA, Cat# 20922) was placed directly under the sky, elevated so rainwater would not splash into it from its surroundings. It was left outside overnight until it collected 3 L of rainwater. Organic matter within was removed by hand and with a pipette. The water was poured into clean 2 L bottles (Coca-Cola) and the excess was disposed of. The bottles were closed and then shaken to mix the water into as homogeneous a state as possible. A 100mL plastic graduated cylinder (Eisco, Cat# CH0354G) was used to measure 100mL water samples, and the samples were placed into 30 glass beakers, with 100mL of water given to each beaker.

#### Setting up for data collection

The 30 samples were divided into 4 groups—one group of 3 and three groups of 9. The group of 3 was the control, and each group of 9 was treated with a different agent (CIO $^{-}$ , H $_2$ O $_2$ , or yeast-catalyzed H $_2$ O $_2$  treatment). Within each group of 9, there were 3 subgroups of varying concentrations (CIO $^{-}$ : 0.05% v/v, 0.1% v/v, 0.15% v/v. H $_2$ O $_2$ : 0.5% v/v, 1% v/v, 1.5% v/v. Yeast-catalyzed H $_2$ O $_2$  treatment: 0.5% v/v H $_2$ O $_2$  + 0.02% w/v yeast, 1% v/v H2O2 + 0.04% w/v yeast, 1.5% v/v H $_2$ O $_2$  + 0.06% w/v yeast). Three trials were performed for each concentration.

# **Data collection before treatment**

A pH probe (Vernier) connected to a LabQuest 2 (Vernier) was used to determine the pH of all samples. The pH probe was rinsed and recalibrated before each measurement. A DO probe (RCYAGO, Cat# X002PU6JZJ) was used to determine the DO in mg/L of all samples. If timed data was being collected, the DO probe was submerged at the same time as the pH probe. The probe was rinsed and re-calibrated for each measurement. One unused water test strip and its parameter color reference sheet (Vansful) were used to determine the levels of every chemical parameter needed for each sample. A photo of the test strip against the reference sheet was taken by a smartphone (Apple, Cat# F17DQC070D9H) for future checking. The strip was then discarded.

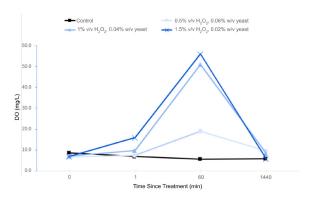


Figure 8. DO of varying concentrations of yeast-catalyzed hydrogen peroxide-treated rainwater vs. time. Mean DO values over time (each line represents n=3 measurements). 0.5mL, 1mL, or 1.5mL of  $3\%~H_2O_2$  solution was added with 0.02g, 0.04g, or 0.06g of dry baker's yeast (respectively) to home-collected rainwater and DO was assessed at different times after  $H_2O_2$  and yeast addition with a digital probe. In the control, nothing was added but DO was still assessed at similar times to the catalyzed  $H_2O_2$  treatment samples.

#### Data collection for 1-minute mark

On the smartphone, multiple stopwatches were set and labeled the same as the 30 samples. The timer for Untreated #1 was started first. When it almost reached 1 minute, the data collection procedure was repeated for that sample. We attempted to synchronize the time of data collection to the exact time the stopwatch reached 1 minute. After the data for Untreated #1 was collected, the timer was started for Untreated #2 for its data collection at 1 minute, and so on. All samples' 1-minute data collection were performed in their order of appearance in the order they were previously listed. The timers of the chemical treatment agents were started as soon as the treatment agents touched the water.

For 0.05% v/v CIO<sup>-</sup>, a plastic pipette was used to add 1 drop (0.05 mL) of 7.5% CIO<sup>-</sup> bleach (Up&Up, Cat# 059647850608) to the sample. For 0.1% v/v CIO<sup>-</sup>, the pipette was used to add 2 drops (0.1 mL) of 7.5% CIO<sup>-</sup> bleach to the sample. For 0.15% v/v CIO<sup>-</sup>, the pipette was used to add 3 drops (0.15 mL) of 7.5% CIO<sup>-</sup> to the sample.

For 0.5% v/v  $\rm H_2O_2$ , a plastic pipette was used to add 0.5 mL of 3%  $\rm H_2O_2$  solution (Up&Up, Cat# 349580268333) to the sample. For 1% v/v H2O2, the pipette was used to add 1 mL of 3%  $\rm H_2O_2$  solution to the sample. For 1.5% v/v  $\rm H_2O_2$ , the pipette was used to add 1.5 mL of 3%  $\rm H_2O_2$  solution to the sample.

For catalyzed  $\rm H_2O_2$  treatment (0.5% v/v  $\rm H_2O_2$  and 0.02% w/v yeast), a plastic pipette was used to measure 0.5 mL of 3%  $\rm H_2O_2$  solution. A mass scale (Ohaus Scout H-5852) was used to measure 0.02g of dry baker's yeast (Fleischmann's, Cat# 04072915) in a small paper cup. For 1% v/v  $\rm H_2O_2$  and 0.04% w/v yeast, this was repeated, but with 1 mL of 3%  $\rm H_2O_2$  solution and 0.04 g of yeast. For 1.5% v/v  $\rm H_2O_2$  and 0.06% w/v yeast, this was repeated, but with 1.5 mL of 3%  $\rm H_2O_2$  solution and 0.06 g of yeast. For all catalyzed  $\rm H_2O_2$  treatment samples, both the  $\rm H_2O_2$  and yeast were added to the sample at the same time.

#### **Further data collection**

Data was collected for each of the 30 samples around the time each stopwatch reached 1 hour and then 24 hours after treatment.

At 24 hours, and for the catalyzed  $\rm H_2O_2$  treatment group only, a funnel (Nalgene, Cat# 4250-0100) and pieces of filter paper (Ahlstrom, Cat# 6150-1250) were used to filter the yeast out of each sample twice. The 24-hour data was then collected for each catalyzed  $\rm H_2O_2$  treatment sample.

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