The Effect of pH and Time on Aerobic CO_2 Production in *S. cerevisiae*

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Abstract

This experiment sets out to find the effect of pH and time on aerobic respiration in *Saccharomyces cerevisiae* blooming in different pH treatments. *S. cerevisiae* were added to four bottles with different pH mediums – a pH of 2, 4, 8 and a control (pH 7). Balloons attached to the bottles would collect the CO_2 gas released by the yeast, and the balloon height would be considered a measure of respiration. Data was collected in two intervals separated by an hour to measure the effect of time. Yeast in the control (pH 7) had significantly greater aerobic respiration compared to the other pH groups ($p < 0.0001$). Only the yeast in the control (pH 7) group showed a significant increase in respiration with time ($p < 0.0001$), while the rest showed no change with time. These results are discordant with what is known in studies conducted for anaerobic respiration. Consequently, this suggests that the baking industry (which focuses on aerobic respiration) should follow different guidelines for the usage of yeast than the liquor industry (which focuses on anaerobic respiration) in order to maximize profit.

Introduction

Humans have been utilizing yeast for centuries. Yeast was the essential ingredient in producing wines stored in Egyptian tombs 5,100 years ago (Frazer, 2013). Yeast was also used as a leavening agent for bread – hieroglyphics depict dough being left out before baking, possibly to allow airborne yeast spores to leaven it (Sitwell, 2015). Nowadays, the baking industry has a noteworthy impact on economy. The US comprises of approximately 6,000 bakeries (LaMarco, 2019), thus competition is fierce, and there's high demand for research in optimizing leavening. *S. cerevisiae* makes bread rise because of the release of CO_2 via aerobic respiration (Lawandi, 2019). One feature not well studied is the effect of pH on aerobic respiration, though it has been extensively studied for yeast anaerobic respiration.

Consequently, I measured aerobic respiration in four pH groups – pH 2, 4, 8 and a control (pH 7) – by collecting CO_2 gas in balloons. I measured balloon heights as a way of quantifying CO_2 levels. As previously stated, most studies focus on anaerobic respiration, which also results in CO_2 release as a by-product. Reddy et al. (2020) found that anaerobic fermentation in yeast was highest with an acidic pH, but lowest at a pH of 7. Similarly, a study by Liu et al. (2015) found fermentation rates to be highest at pH 4.5. For these reasons, I hypothesize that balloon heights in the pH 7 (control) and pH 8 treatments will be lower than the heights achieved by yeast in more acidic pH, as long as aerobic respiration parallels anaerobic respiration. I do however predict that a pH of 2 will result in less CO_2 production than a pH of 4. This is because Liu et al. (2015) found that yeast growth was greatest at a pH of 4 – 5, but growth was

stunted at a pH less than 3. Arrested growth means less respiration, therefore less CO_2 production. I also conducted the experiment in two time intervals to see the effect of time on aerobic respiration. As bread dough is left to rise, the pH drops from the initial pH due to the formation of lactic and acetic acid ("The science of fermentation," 2015) and so yeast initially in the pH 2, pH 4 solutions would be in even more acidic conditions after time has passed. However, yeast in pH 7 would be in more favourable acidic conditions. With this, I expect yeast respiration to not change with time in pH 2 and pH 4, increase with time in pH 7, but not be changed with time in pH 8 (the drop in pH is likely too small to shift into acidic conditions). The null hypothesis is that time and pH would have no effect.

METHODS:

Pre-Experiment Preparation:

The following experiment was conducted in my home kitchen on November 9, 2020. Before preparing the pH solutions, I measured and recorded the temperature of the tap water (about 2 litres) that had been left sitting out in a pitcher for at least a few hours. All equipment was calibrated, including the pH meter (with calibration solutions).

Creating the pH Solutions:

I measured 250 ml of the water from the pitcher transferred it into a plastic cup. I repeated this for three other cups (four cups in total, each with 250 ml of water). To each cup, I added 1 tablespoon of sugar and mixed it in until fully dissolved. Sugar cannot change the pH of the solutions, but it is vital for cellular respiration in yeast. I assigned and labelled the plastic cups with a pH group – a pH of 2, 4, 8 or the control. For the cup labelled "pH 2," I slowly added and stirred in citric acid (in powdered form) with a micro spatula until the pH meter read a pH of 2. I repeated this for the cup labelled "pH 4," but added a little less citric acid. I then took and recorded the pH measurement of the cup labelled as the "control." For the cup labelled "pH 8," I slowly added and stirred in baking soda with a micro spatula until the pH meter read a pH of 8.

Experimental Set-up and Addition of the Yeast:

I assigned and labelled four 1-litre bottles with a pH group. With a clean funnel, I transferred the prepared pH solutions within the plastic cups to their respectively labelled bottle. One bottle at a time, I added in two tablespoons of yeast to the bottle, swirled it in gently, and put on a balloon over the opening of the bottle. Once all four bottles were done, I used electrical tape to seal the lip of the balloon to the neck of the bottle, ensuring no air escaped. I put the bottles against a wall, and attached vertical strips of masking tape from the lip of the bottle upwards (see Figure 1). I marked the height of the balloon after one hour, and then again once two hours had passed on the strips of masking tape. I measured and recorded the height of the balloon from the lip of the bottle to the markings on the masking tape with a ruler. I repeated this experiment eight times, to get a total of 8 data points per pH group at t = 1 hour (total of 32 data points in the first hour), and another 8 data points at t = 2 hours, per pH group (a total of 32 data points in the second hour).

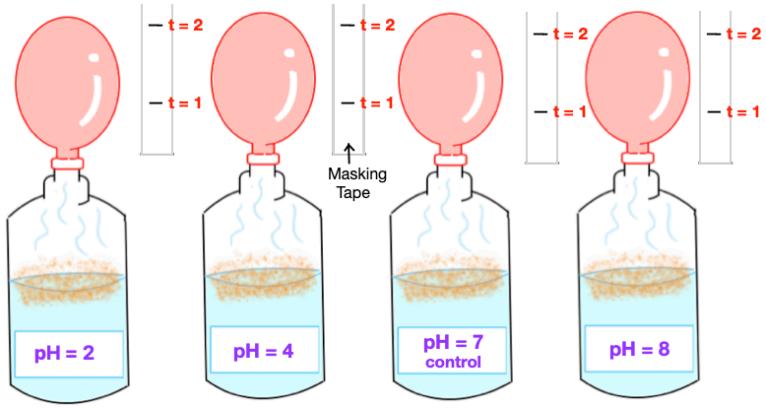

Figure 1. Shown here is a diagram of the experimental set-up of a single trial (8 trials were done in total).

Statistics:

I conducted a two-way ANOVA to find the effect of pH on balloon height, the effect of time on balloon height, and whether time and pH interact with one another to effect balloon height. I used a Tukey post-hoc test to find which pH groups had significant effects on balloon height (at t = 1 hour and also at t = 2 hours). I used a Šidák's post-hoc test to find which pH groups differed significantly between their measurements at t = 1 hour and t = 2 hours. All my statistical tests were conducted by using Graphpad Prism version 8.4.3.

RESULTS:

I measured the temperature of the water to be $26°C$, and the pH of the control was exactly 7.0. The effect of pH on balloon height was significantly different in at least one pH group in both time intervals (ANOVA, $F_{3, 56} = 109.6$, $p < 0.0001$). In the first hour, balloon heights of the pH 2, 4 and 8 treatments were similar to one another, and any pairwise combination of these three treatments were non-significant. In contrast, the yeast in the control (pH 7) treatment reached greater heights, see Figure 2A(i), and this was significant (Tukey, $p < 0.001$ for all pairwise combinations with the control). These same patterns were paralleled in the second hour, see Figure 2A(ii). Again, the mean balloon height in the pH 8, 4 and 2 treatments weren't significantly different from one another. Conversely, the mean height in the control (pH 7) was significantly higher than any other pH treatment (Tukey, $p < 0.0001$ for all pairwise combinations with the control group).

Balloon height changed with time significantly in at least one pH treatment (ANOVA, $F_{1, 56} = 19.65$, $p < 0.0001$). The mean height in the second hour was significantly greater than the mean height in the first hour in the control treatment (Šidák, $p < 0.001$), but balloon heights weren't significantly different among the other pH groups with time, see Figure 2B. Time and pH interacted together to significantly cause an effect on balloon height (ANOVA, $F_{3, 56} = 3.995$, $p = 0.0120$).

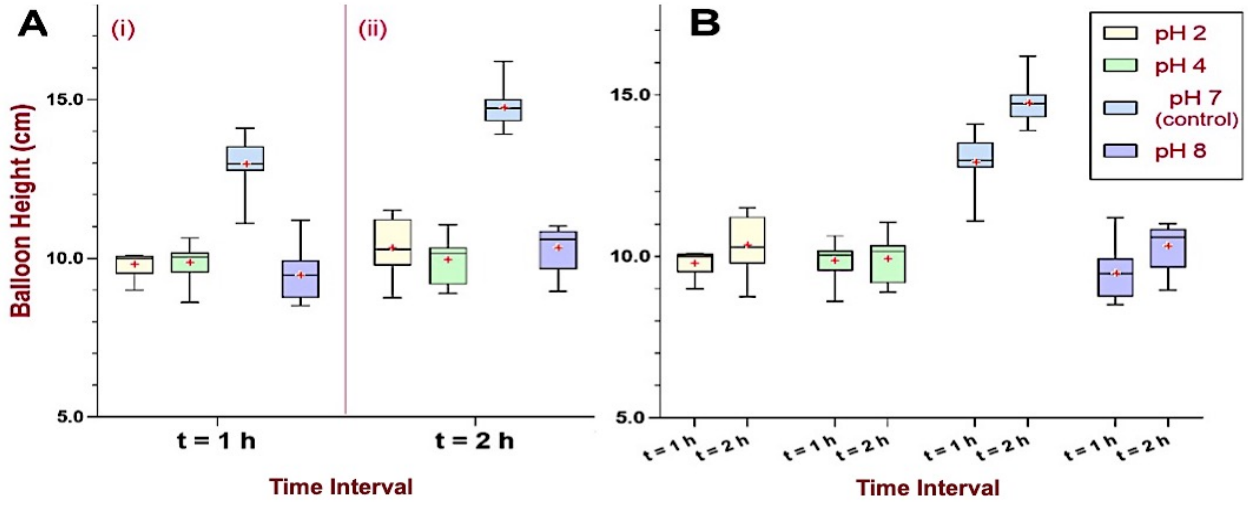

Figure 2. Boxes represent interquartile ranges, and whiskers stretch from the minimum to maximum data points. Red plus signs represent the means. [A] The effect of pH on balloon height (cm) in t = 1h and t = 2h. Mean balloon heights in the control don't overlap the data range of any other treatment, and the mean height in the control is significantly higher when compared to all other treatments ($p<0.0001$) in both t = 1h [i] and t = 2h [ii]. [B] The effect of time on balloon height (cm). The means of all pH groups in the two time periods overlap, except in the control (pH 7), where the mean height is significantly greater in t = 2h than in t = 1h ($p < 0.0001$). N = 8 per group per hour.

DISCUSSION:

Here I assessed the effect of pH and time on yeast aerobic CO_2 production. I connected CO_2 production to increases in balloon height. My results rejected the null hypothesis that neither time nor pH would have an effect. My first alternative hypothesis suggested that gas production (and thus balloon heights) would be lowest in the control (pH 7) and pH 8 groups. However, gas production was highest (significantly, $p < 0.0001$) in the control (pH 7) when it's compared to the non-control groups, and the non-control groups didn't significantly differ in gas production when compared to one another. The disconnect between my results and the hypothesis above was because I made the assumption that aerobic respiration would parallel the results found with anaerobic respiration studies, but perhaps they don't. Mathew (2014) found that bread rose best in neutral pH compared to a basic or acidic pH. This endorses my findings, since it shows a neutral pH is creating more gas production than any other non-neutral pH, in aerobic conditions.

I predicted that CO2 production would be less in pH 2 than pH 4, because (Liu et al., 2015) revealed that cell growth is stunted at a pH less than 3, and less growth means less respiration. My results were discordant with their findings – mean balloon heights between yeast in pH 2 and pH 4 weren't significantly different from one another ($p > 0.05$). That 2015 study monitored cell growth under anoxic conditions. It's possible that cell growth is stunted in pH less than 3 when in anaerobic conditions. However, my study was conducted with oxygen still in the system. In fact, another study found no statistical effect of pH on yeast growth constants under oxygenated conditions (Baez et al., 2002). If growth is constant regardless of pH when oxygen is around, then respiration rates should be similar between pH 2 and 4.

Pertaining to the effect of time on gas production (or balloon height), I originally expected aerobic respiration in yeast to not change with time in pH 2 and pH 4, increase with time in pH 7, and not change with time in pH 8. This hypothesis came about by the fact that pH drops with time as yeast respire because of the release of lactic and acetic acid (*"The science of fermentation,"* 2015). A previous study found that anaerobic fermentation rates in yeast were highest when the yeast was *currently* in a pH of 4.5 (Liu et al., 2015).

As such, I expected my pH 2 and pH 4 treatments to get too acidic for yeast in the second hour, and cause a pause in respiration. I expected pH in the control (pH 7) treatment to get more favourable for yeast as the pH dropped in the second hour, and cause an increase in respiration in the control group. Then, for pH 8, I presumed the drop in pH in the second hour wouldn't be acidic enough for it to be favourable for yeast, so I anticipated no change in respiration. In this experiment there was no change in yeast aerobic respiration for pH 2, 4 and 8, but aerobic respiration did indeed increase with time in yeast within the control treatment ($p < 0.0001$), so results matched the hypotheses. Nevertheless, I made those hypotheses based on experiments done in anaerobic conditions. However, another study done in the presence of oxygen also supported my results. Holmes & Hoseney (1987) found that yeast within dough released the most gas (resulting in shorter proof times and greater bread volumes) when the *final* pH (as in the pH after proofing) is at a pH range of 6-7, but released less gas when the final pH was more acidic or basic than this range. My control started at pH 7, which means after two hours it was still within the optimal range for gas release found in the Holmes & Hoseney (1987) study. The yeast in pH 2 and 4 already surpassed that range before it became more acidic with time, and the yeast in pH 8 didn't drop in pH enough to reach this range, so respiration rates remained the same. This explains why only the control's respiration increased with time.

Conclusions:

Divergent from what I originally hypothesized – that pH 4 would show the most gas production – my results showed that yeast in the control (pH 7) produced significantly more CO_2 (as measured by balloon heights) when compared to any non-control pH group. This was true in both the first and second hour. The neutral pH (control) showed a significant increase in balloon height with time, while the non-control groups didn't, and this matched my prediction.

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CITATIONS:

Baez, J. C., Francis, N., Hsu, E., & Sardesai, N. (2002). *Effect of pH on anaerobic growth of yeast*. Retrieved from https://www.seas.upenn.edu/~belab/LabProjects/2002/be210s02r1.doc#:~:text=Since%20respiration%20produces%2019%20times,values%20of%204%20and%207

Frazer, J. (2013). Yeast: making food great for 5,000 years. But what exactly is it? Retrieved from https://blogs.scientificamerican.com/artful-amoeba/yeast-making-food-great-for-5000-years-but-what-exactly-is-it/

Holmes, J. T., & Hoseney, R. C. (1987). Chemical leavening: effect of pH and certain ions on breadmaking properties. *Cereal Chemistry*, *64*(4), 343–348. Retrieved from http://online.cerealsgrains.org/publications/cc/backissues/1987/Documents/64_343.pdf

LaMarco, N. (2019). Risks of a bakery. Retrieved from https://smallbusiness.chron.com/risks-bakery-61962.html

Lawandi, J. (2019). The science behind yeast and how it makes bread rise. Retrieved from https://www.thekitchn.com/the-science-behind-yeast-and-how-it-makes-bread-rise-226483

Liu, X., B. Jia, X. Sun, J. Ai, L. Wang, C. Wang, F. Zhao, J. Zhan, and W. Huang. (2015). Effect of initial PH on growth characteristics and fermentation properties of *Saccharomyces cerevisiae*. *Journal of Food Science*. *80*(4), 800–808. doi:10.1111/1750-3841.12813.

Mathew, S. (2014). The effect of pH on the rising of bread. *Prezi*. Retrieved from https://prezi.com/6_wfryowvxjg/the-effect-of-ph-on-the-rising-of-bread/

Reddy, P. K., Vijay, M., Kusuma, M., & Ramesh, K. V. (2020). Optimum parameters for production of ethanol from synthetic molasses by *Saccharomyces cerevisiae*. *MaterialsToday: Proceedings*, 1–3. https://doi.org/10.1016/j.matpr.2020.07.100

Sitwell, W. (2015). *A history of food in 100 recipes*. London: William Collins.

The Science of Fermentation. (2015). *Bake Magazine RSS*. Retrieved from https://www.bakemag.com/articles/836-the-science-of-fermentation

APPENDIX:

Raw Data:

pH 2	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
t = 1 hr	10.02	10.04	10.07	8.99	10.08	9.89	9.98	9.4
t = 2 hr	10.18	11.1	9.9	8.75	11.5	10.37	9.75	11.25

pH 4	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
t = 1 hr	9.95	10.64	10.1	8.6	10.06	10.02	9.43	10.18
t = 2 hr	10.22	8.9	10.14	9.9	10.16	8.94	11.06	10.35

pH 7 Control	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
t = 1 hr	13.13	13.65	13.07	12.79	14.1	12.74	12.87	11.1
t = 2 hr	15.03	14.56	13.9	14.9	14.3	14.87	16.2	14.38

pH 8	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
t = 1 hr	9.1	9.66	8.5	10	9.41	9.5	8.65	11.2
t = 2 hr	10.54	10.78	9.4	10.62	11.01	8.95	10.47	10.85

Two – Way ANOVA Results:

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	6.595	3	2.198	F (3, 56) = 3.995	P = 0.0120
Time	10.82	1	10.82	F (1, 56) = 3.996	P < 0.0001
pH	180.9	3	60.29	F (3, 56) = 3.997	P < 0.0001
Residual	30.82	56	0.5504		

Effect of pH on Balloon Height (CO_2 Gas Production):

Tukey's multiple comparisons test	Adjusted P Value	Summary
t = 1 hr		
pH 2 vs. pH 4	0.9982	Not significant
pH 2 vs. pH 7 (control)	< 0.0001	Significant
pH 2 vs. pH 8	0.8421	Not significant
pH 4 vs. pH 7 (control)	< 0.0001	Significant
pH 4 vs. pH 8	0.7514	Not significant
pH 7 (control) vs. pH 8	< 0.0001	Significant
t = 2 hr		
pH 2 vs. pH 4	0.7181	Not significant
pH 2 vs. pH 7 (control)	< 0.0001	Significant
pH 2 vs. pH 8	> 0.9999	Not significant
pH 4 vs. pH 7 (control)	< 0.0001	Significant
pH 4 vs. pH 8	0.7534	Not significant
pH 7 (control) vs. pH 8	< 0.0001	Significant

Effect of Time on Balloon Height (CO_2 Gas Production):

Šidák's multiple comparisons test	Adjusted P Value	Summary
(t = 1 hr) – (t = 2 hr)	0.4783	Not significant
pH 2	0.9989	Not significant
pH 7 (control)	< 0.0001	Significant
pH 4	0.1154	Not significant
pH 8	0.4783	Not significant