Exploring the use of redox potential to predict fermentation outcomes in relation to initial juice conditions

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Abstract

Each year in the wine industry, economic loss occurs due to stuck or sluggish fermentations and the corresponding off-flavors produced. While monitoring using standard methods such as °Brix levels may reveal a problem, often the indication is after the quality of the wine has already been impacted and remediation techniques are less effective or intrusive and costly. The use of redox potential, also called Oxidation Reduction Potential (ORP), as a process parameter is being explored in order to predict fermentation outcomes early in fermentation, theoretically before measurable changes in °Brix levels occurs. ORP reports on the tendency for molecules or ions to gain or lose electrons in relation to the chemical makeup of a solution being measured. Consequently, ORP values are sensitive to the fermentative activity of the yeast as metabolic products are released and alter the chemical conditions of the solution. This makes ORP a sensitive tool in understanding the state of the fermenting yeast in a must, even before sugar consumption can be measured. This study aimed to monitor ORP under varying nutrient conditions or with different yeast strains to better understand the relationship between ORP and fermentation outcomes. Wine strains of *Saccharomyces cerevisiae* – EC1118, Elixir, CY3079, Montrachet, and RC212 – were observed, as well as varying pH and nutrient conditions used. ORP values showed repeatable patterns based on fermentation conditions which could be used to assist winemakers in monitoring and decision-making.

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1 Introduction

Winemaking is a practice that has been around for thousands of years and has evolved into a complex series of decisions, from vine to bottle. All choices that go into modern day winemaking affect the final product, including the choice to have a "hands-off" approach and abstain from all or most additives, or the opposite, heavy amelioration. At both ends of the spectrum, winemakers are challenged with a series of choices in how and when to modify their fermentation. Winemakers will base their decisions on the information they have about their fermentation using the different tools at their disposal.

1.1 Winemaking Overview

Wine fermentation procedures will vary based on variety and intended style. In order to create a white wine, grapes will be harvested and immediately pressed off the skins in order to minimize color and tannin pick-up that will occur if the skins are left in contact, as would occur with a red wine fermentation. The result will be a white grape juice, regardless of the initial skin color, which is then either inoculated with cultured or commercial yeast, or left to begin fermentation spontaneously through the yeasts already present in the juice or winery. Processing grapes for a red wine outcome is similar, but instead, the red grapes will be crushed, breaking open the berries upon intake of the fruit, after which, this juice/solids mixture called "must" is fermented together. Tannin compounds will be extracted from both the skins and seeds which contribute to mouthfeel, bitterness, and astringency. Anthocyanins and polymeric pigments will also be extracted which give color to the wine (Boulton et al. 1996, Bisson and Walker 2015). Pump-overs, where juice is pumped from the racking valve, or through a screen and the bottom value over the must, or punch-downs, where the skins and seeds are pushed down to be submerged in the liquid, are ways to promote extraction of these tannins (Bisson and Walker 2015). These

processes also incorporate oxygen, which aids the yeast in building strong cell membranes (Fornairon-Bonneford et al. 2002). As in the white juice process, fermentation may begin through inoculation of yeast, or through spontaneous fermentation from yeast already present. Grape juice provides the necessary nutrients and carbohydrates for the yeast in solution to proliferate, which creates ethanol as the predominate product. Once fermentation is complete for the red wine process, it will be pressed off the skins and seeds. Red wine fermentations contain the skins and seeds throughout the fermentation, unlike white fermentations where these components are removed. Because white wine fermentations lack these solids, they tend to contain fewer nutrients than red wine fermentations, particularly nitrogen. Post fermentation processing, such as malolactic conversion or aging in oak barrels will also change the composition of the wine, but over a longer period of time than fermentation (Boulton et al. 1996).

Prior to harvest, winemakers and viticulturalists can analyze the grape juice in terms of pH, sugar content (brix), total acidity, malic acid, yeast assimilable nitrogen (YAN), and color. During fermentation, the medium's contents change quickly. The rate of sugar consumption (change in °Brix) and the temperature of the fermentation are the metrics often used by winemakers to determine whether the fermentation will become stuck or sluggish, as these two parameters can often indicate the general health of the yeast population. Fast sugar consumption with heat evolution can indicate that the yeast are metabolizing easily, despite the challenges that may be associated with the environment. A fermentation is considered complete when all or most of the sugar in solution is consumed by the microbes, to less than 4 g/L and often lower. Stuck fermentations are those that are left with a higher than desirable residual sugar content. A sluggish fermentation is one that completes after a typical wine fermentation, which is generally between 7 and 10 days, depending on the variety and yeast strain (Bisson 1999).

1.2 Wine Microbiology

As the microbes in the fermentation metabolize sugars in the media, heat will be released from the exergonic processes occurring, thus increasing the overall temperature of the fermentation (Williams 1982). As the yeast become less active with low sugar in the environment and increased levels of inhibitory compounds such as ethanol, the overall heat evolution of the media will be much lower. Heat can speed reactions and lead to a quicker fermentation, however, excessive heat will be harmful to the fermentation, as it will cause stress responses in the microbes, leading to undesirable compounds, or even denaturation of proteins (Boulton et. al 1996, Sener et al. 2006). Temperature is also important to other factors in yeast fermentations such as membrane composition and ethanol tolerance (Beltran et al. 2008; D'amore et al. 1989). For these reasons, winemakers often try to modulate the temperature through heating and cooling the fermentation vessel to achieve a desirable fermentation speed with low stress, but quick population growth (Walker et al. 2021). The longer a fermentation takes, the longer a fermentation vessel is occupied, meaning it cannot be used for additional fermentations, which creates an economical issue for the wine industry if additional fermentation tanks are needed (Ough and Amerine 1961), or cooling costs are increased. Ferrmentations which are slow to consume all of the sugar in the media, or "complete," can also lead to unwanted outcomes (Coleman et al. 2007). If the *Saccharomyces cerevisiae* population is too weak to metabolize all the sugars, it could lead to spoilage microbe populations taking over this niche and contributing unwanted compounds. Slower fermentations will also have extended contact with inhibitory compounds toward the end of fermentation which could decrease overall viability and therefore the chances that the population will be viable enough to complete the fermentation (Bisson 1999, Cramer et al. 2002).

One of the most important elements for cellular metabolism is nitrogen. In addition to being an important component of amino acids, it is also vital to creating nucleic acids. For alcoholic fermentation by *Saccharomyces Cerevisiae*, both the quantity and form of nitrogen compounds are important to the vigor of the fermentation. These yeast can only metabolize certain forms of nitrogenous compounds known as yeast assimilable nitrogen (YAN). Low YAN in solution can lead to stuck or sluggish fermentations due to lack of metabolic building blocks(Coleman et al. 2007). Low amounts of nitrogen can also lead to stress responses which cause production of unwanted sulfur-containing compounds, among other effects. High YAN can increase the speed and heat evolution of the fermentation, which may be good to an extent for a winemaker; but, it can also cause increases in undesirable compounds like ethyl carbamate, biogenic amines, protein build up, and greater than usual amounts of ethyl acetate and volatile acidity. High YAN in solution could also be a source of nutrition for other, undesired microbes in solution, leading to unwanted flavors or aromas (Bell and Henschke 2005, Gutierrez et al. 2012, Boulton et al 1996).

It is important to address stuck and sluggish fermentations to prevent reduced quality in the final wine product. As fermentations progress, the media becomes more challenging with more alcohol present, more competition, and fewer nutrients (Bisson 1999). With these challenges present in their environment, the microbes will respond by altering their metabolism to a stress-response based function. This could include actions like the production of compounds to reduce oxidative stress, for example, H_2S (Li et al. 2018, Matallanda and Aranda 2016). H_2S is among many other compounds in various classes which can be considered undesirable in certain quantities or styles of wine. Many of the off-characters that are produced by *Saccharomyces cerevisiae*, the strain which drives fermentation, are related to stressful conditions like inhibitory compounds, low levels of nitrogen or other nutrients or vitamins, genetic predisposition, or non-ideal temperatures

or pH values (Lopes et al. 2001; Alexandre and Charpentier 1998; Bindon et al. 2019; Coleman et al. 2007; Ough 1966). Other microbes will have similar responses to challenging conditions, potentially releasing various compounds which could contribute to off-characters in wine. The threat of off-characters being contributed by unwanted non-*Saccharomyces* microbes becomes particularly threatening in stuck or sluggish fermentations, due to the population of *Saccharomyces* yeast being too weak to dominate the niche. This could lead to any unused nutrients being consumed by other, potentially detrimental microbes, which contribute compounds to the wine which reduce its quality (Bisson 1999).

1.3 Monitoring Fermentations Based on Oxidation-Reduction Potential

Temperature and Brix are the most commonly used metrics used to track fermentations in modern-day winemaking, and can be used to make informed decisions surrounding the health and progression of the fermentation (Walker et al. 2021, Boulton et al. 1996). With these current parameters, changes in the fermentation and potential problems can be predicted. Yet often, there is a significant delay in when the problem in the fermentation began and when it became obvious enough to observe it with these metrics. Increasing response time to stuck and sluggish fermentations in the wine industry could prevent great economic losses by increasing effectiveness of remediation techniques through early interception (Bisson 1999). Exploration of new monitoring techniques to complement existing parameters could help to increase response time to problem fermentations.

One parameter which exists in wine but is not widely monitored in wine fermentations is oxidation reduction potential (ORP), or redox potential. ORP is the quantifiable measurement of the tendency of a molecule to gain or lose electrons. In a given solution, the ORP will determine the favorability of different half reactions to proceed (Liu et al. 2017). Other industries such as

biofuel production, dairy processing, wastewater treatment, and food safety currently utilize ORP as a metric to understand the status of their respective processes (Walker et al. 2021; Goncharuk et al. 2010; Wareham et al. 1993; Liu et al. 2011; Martin et al. 2011; Reichart et al. 2007). Previous studies involving redox potential in winemaking involve using air to control the redox values (Killeen et al. 2018), exploring the relationship of volatile compound formation and the redox potential of the solution (Fariña et al. 2012), and observing the behavior of the redox potential over the course of the fermentation in Sauvignon Blanc juice (Kuckec et al. 2002). In the biofuel industry, experiments surrounding the control of redox potential in solution for more efficient fermentations has been explored because the ORP of a solution is correlated with stress responses in the yeast. It was found that extracellular ORP can inform on the intracellular ORP, but also that intracellular ORP can be affected by the extracellular ORP. Because of this relationship, controlling the extracellular redox potential of a fermentation through aeration or H_2O_2, which will both increase the ORP, was of interest to create a desired outcome. The result of these experiments showed that ORP could be a viable tool to control fermentations for a favorable result (Liu et al. 2011; Grimalt-Alemany et al. 2021). Before redox potential may be used as widely in the wine industry as it is used in others, more studies which establish typical values or describe its effects at different points during the fermentation on the final product must be conducted.

Redox has been an area of interest for the wine industry for decades now (Rankine 1963; Shultz and Kunkee 1977; Costa 1958), as oxidation-reduction potential is a general chemistry concept that can be widely applied and has been recognized as an important factor to measure in biological systems (Michaelis 1930; Needham and Needham 1926). Controlling redox, namely through delivering precise amounts of air during specified times in the bioprocess, to affect the metabolic responses of microorganisms was of interest both inside and outside of the wine

industry. The goal of controlling redox in this way was to speed fermentations, change the compound contributions of the microorganisms, or reduce the occurrence of undesirable outcomes (Kjaergaard 1977; Liu et al. 2011; Killeen et al. 2018). For winemakers, production of hydrogen sulfide through elemental sulfur reactions or yeast stress responses is a concern due to the off-character it produces. Reduced formation of this compound has been found when the redox values of the fermentation are held at higher levels (Killeen et al. 2018). The effect that redox has on other volatile compounds has also been explored in one study which found that less reductive conditions led to greater amounts of higher alcohols, isoacids, and acetaldehyde, while more reductive conditions favored formation of fatty acids and esters (Fariña et al. 2012). In addition to the effects of ORP on compound formation, another study showed that temperature had an effect on the minimum ORP values a given fermentation was able to reach, with higher temperatures relating to lower ORP minimums (Kukec et al. 2002). As dissolved oxygen in the solution decreases through yeast consumption, so will the ORP; aeration, and more specifically with oxygen, will increase the ORP levels (Liu et al. 2011). These studies show that redox affects various detectable compounds in wine, as well as that commonly controlled variables, such as temperature, have an effect on the ORP values of a fermentation. This leads to two areas of interest surrounding redox which are: how does controlling ORP at different levels affect the outcome of a fermentation and how do different variables that exist in a fermentation affect ORP? These main questions can result in trying to utilize the answers to produce more desirable fermentations through controlling ORP or using ORP as a tool to understand the progression of the fermentation based on various variables in solution.

Wine and grape juice have many components interacting in solution which participate in electron exchange, in addition to compounds which are contributed by microbes present in the

solution. The redox potential of a solution can be measured using reference probes which give readings in millivolts. Common reference electrodes used in literature include the standard hydrogen (SHE) and the Silver-Silver Chloride electrode (Park 2009). These probes are able to provide real-time data about the current ORP in solution. Just as winemakers observe temperatures and Brix specific to different fermentations to understand the progression, ORP is another variable which already exists in the media which could give additional, useful data. In the lifetime of a wine, from juice to finished product, fermentation is the stage in which the most dramatic change in the in the composition occurs in the shortest amount of time. Small differences in the environment can lead to dramatic differences in the end wine. With more data to understand the conditions in the fermentation, winemakers may be able to make more informed decisions and detect potentially problematic fermentations.

1.4 Changes in ORP during Fermentation

During a wine fermentation, yeast modify their environment in terms of redox potential in two major ways. One way is through depletion of oxygen from the environment. Yeast use oxygen to synthesize sterols and unsaturated fatty acids, and also use the molecule as a final electron acceptor in aerobic respiration. Oxygen in solution will raise the overall ORP, so when it is used up by the yeast, ORP will decrease. Yeast can also modify the redox of the solution through production of compounds that are excreted which changes the chemical matrix and therefore, the ORP (Fornairon-Bonneford et al. 2002).

Because yeast need to control their intracellular pH and redox potential in order to maintain the correct environment for certain reactions to occur, compounds will be pumped across their cell membrane into the environment, which is in this case, fermenting juice. As the yeast population grows larger, more cells are pumping out compounds into the solution which causes a decrease in

the solution's redox potential as the cells maintain their intracellular potential in a desirable range for metabolism (Killeen et al. 2018). The NAD+/NADH couple is a cofactor which acts as an electron carrier and is crucial to metabolic processes. For *Saccharomyces cerevisiae* to continue to metabolize and produce ATP, the NAD+ factor must be regenerated through the production of ethanol and glycerol (Killeen et al. 2018). Figure 1.1 from Liu et al. 2013, shows the typical trends of biomass, redox potential, and dissolved oxygen over the course of fermentation. This figure shows that as the biomass increases, the dissolved oxygen and redox potential of the solution decrease. As the microbes use oxygen in solution in order to build sterols, as well as use it as a final electron acceptor, the total dissolved oxygen decreases. Redox potential decreases due to this depletion of dissolved oxygen, as well as from the microbes pumping out reducing compounds in order to maintain intercellular redox potential. In this figure, it is also shown that the dissolved oxygen in solution reaches zero, meaning this metric is no longer useful at showing changes in the fermentation environment. This is not true for ORP, as the curve continues to change over time, and can attain negative values. In a wine fermentation, as the yeast consume oxygen, sugar, and other various compounds, and release reducing compounds and CO_2, the environment will become more reductive until it reaches a minimum ORP for the fermentation overall. Once this minimum is reached, the ORP will begin to rise as yeast metabolism changes and their impact on ORP and wine chemistry is reduced, which may involve increased oxygen in the system. Understanding the implications of ORP values at different points in the fermentation, as well as how different fermentation components change these values could add another point of control for winemakers.

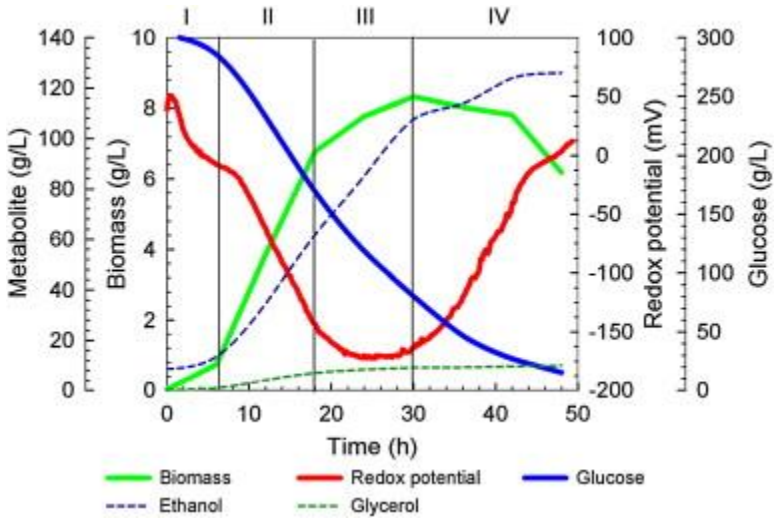

Figure 1.1: Figure 3 from Liu et al. 2013 with following caption: Fig. 3. Time-course of VHG ethanol fermentation by S. cerevisiae *(Data adapted from Lin et al., 2010). Four regions were divided to indicate correlation between ORP and yeast growth: lag phase (I), log phase (II), stationary phase (III), and death phase (IV).*

1.5 ORP as a Wine Fermentation Process Parameter

Oxidation Reduction Potential (ORP) informs on the electron status of different compounds in solution, which, in a wine fermentation, are changing largely due to metabolic reactions in the yeast population. Because of this relationship, ORP is a viable candidate to use as a process parameter in monitoring fermentations to determine progression. Understanding how redox changes due to various fermentation variables may allow winemakers to understand why a fermentation progresses the way it does. This may aid in early intervention of problem fermentations, ultimately leading to better success of remedial techniques. The goals of this study were to observe the changes in ORP over the course of a prototypical wine fermentation, establish typical ORP curves for yeast fermentations, determine how temperature, pH, nitrogen content, and sugar content affect the shape of ORP curves, and to determine whether ORP can be used to predict the outcome of fermentation.

2 Materials and Methods

2.1 Experimental Design. Fermentations using Minimal Must Media (MMM or synthetic must) were carried out in autoclaved 1-L bioreactors from Applikon Biotechnology (Applikon Biotechnology, Schiedam, Netherlands, Figure 2.1.1, 2.1.2). Ten rounds of fermentations were completed using up to 6 bioreactor vessels, with varying conditions (Table A.1 of the appendix).

MMM was generally prepared as described in Spiropoulos et al. 2000, with glucose and fructose concentrations set to 110 g/L, L-arginine to 0.2 mg/L, and diammonium phosphate to 0.5 mg/L. Specific changes to this formulation of MMM are noted below. During fermentation, constant mixing was enabled by an impeller with a set point of 100 rpm (Figures 2.1.3 and 2.1.5). Temperature was controlled through use of a water bath and silicone tubing that ran between each bioreactor to metal tubing that was manufactured to run next to the impeller for temperature control. In each experiment the temperature was controlled within 1 °C of the target setpoint for most experiments, with experiment 7 and 8 being exceptions (Table A.1 of the appendix, Figures A.2-A.10). Temperature was continuously monitored through the ORP probes for experiments 3-10. The water bath in experiments 1 and 2 was set to 23 °C, but was not monitored with the ORP probes. Bioreactors were exposed to minimal amounts of oxygen by keeping ports on the top of the reactors closed, aside from the largest which was opened for about a minute or less in order to take brix measurements. Note that reactors were not completely sealed against air entry and this may have allowed for some oxygen entry into the system.

Yeast strains used were EC1118, CY3079, RC212, Elixir, and Montrachet, which were obtained from the UC Davis Culture Collection. All fermentations were inoculated with only a single strain. Yeast were grown on a yeast extract peptone dextrose (YPD) plate and incubated at 30 °C for two days, and then inoculated into 25 mL of MMM with the same composition used for fermentation and incubated for two days at 25 °C. MMM was then inoculated using these starter cultures by calculating the necessary volume to reach a final optical density (OD) of 0.05 by using a spectrophotometer at 600 nm (Shimadzu Scientific Instruments Inc., Pleasanton, CA). If different vessels in an experiment run required the same yeast strain,

each vessel had its own liquid culture, which originated from the same YPD mother plate. Hamilton ORP probes (EasyFerm Plus ORP Sensors – Arc, Hamilton Company, Reno, NV, Figure 2.1.4) monitored ORP values and temperature every minute throughout fermentation. °Brix were taken manually using a densitometer (Anton Paar, Ashland, VA) one or two times per day through the largest port on the bioreactor (Figure 2.1.2). Between each vessel, the densitometer was rinsed and sprayed with 70% ethanol. Cell count and viability were taken on day 1 (inoculation = day 0), day 3, and when each fermentation reached 0 °Brix (see detail below). Optical density was also taken every day, or every other day depending on the trial to monitor total cell populations.

The experimental design included variations in yeast strain (Section 3.3), pH (Section 3.4), sugar content (Section 3.5), temperature (Section 3.5), and nitrogen content (Section 3.6). Most condition variations were run in triplicate, aside from experiments with nitrogen limitation and when there were technical issues that impacted one of the replicates. In these cases, there were duplicate fermentations. Iterations of the same variable were intentionally done across different runs in order to reduce batch effects. Bioreactor experiments 8–10 were enclosed by cardboard to keep them in the dark after discovering that exposure of the fermentation to sunlight influenced the redox potential and resulted in a diurnal increase and decrease in measured values for reasons that are not known. Covering the bioreactors from sunlight exposure created more reproducibility in the ORP curves.

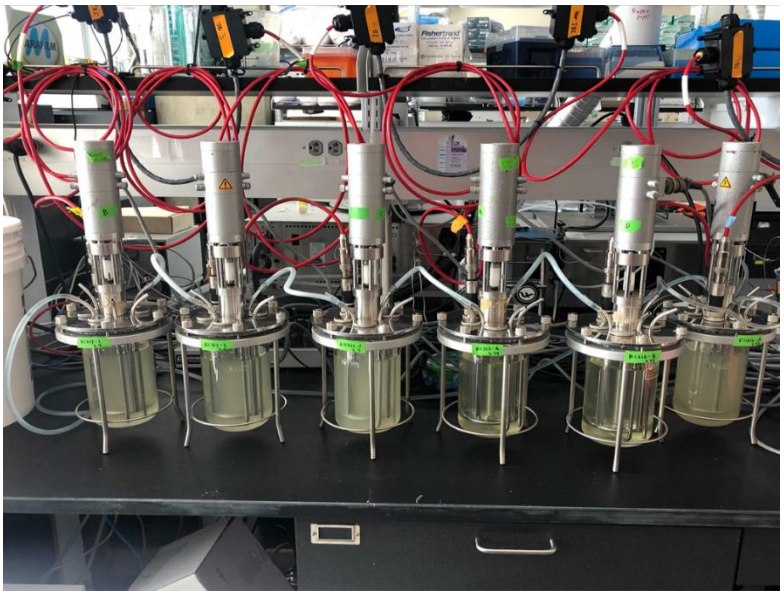

Figure 2.1.1: Image of bioreactor set-up. Water circulation through light blue lines and probes connected to red wires. Sampling occurred through ports in the top.

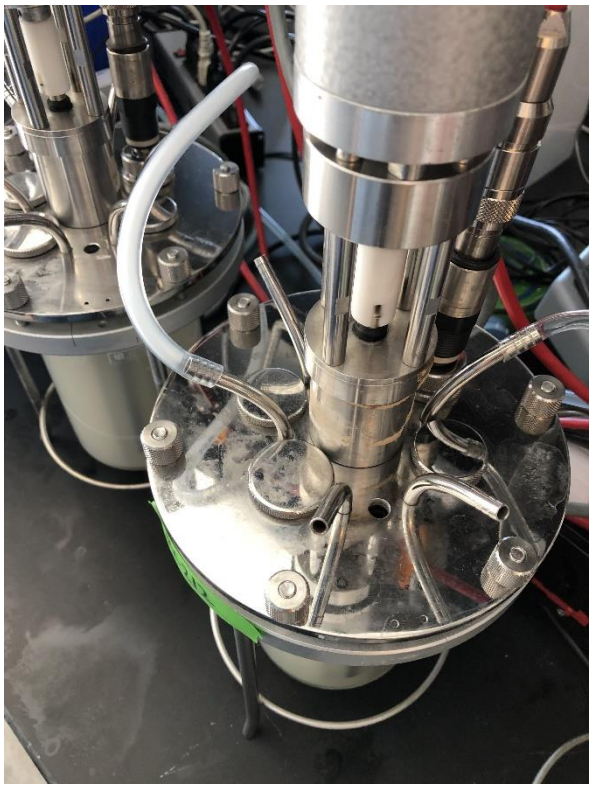

Figure 2.1.2: Top of bioreactor. Water flow for cooling through blue and clear tube. Probe inserted behind motor. Sampling port through largest silver disc at bottom left.

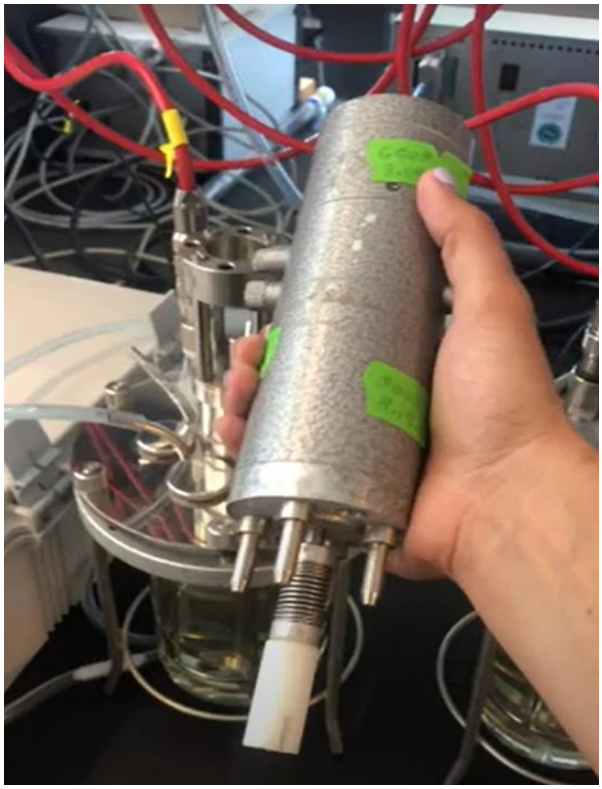

Figure 2.1.3: Motor removed from bioreactor. Bioreactor pictured in the background.

Figure 2.1.4: Image of Hamilton Company EasyFerm Plus ORP Sensors – Arc (Hamilton Company, Reno, NV).

Figure 2.1.5: Image of bioreactor motor control box.

2.2 Alterations to Synthetic Media. MMM was altered in experiments 6–10 (Table A.1 of the appendix) to investigate changes in ORP related to the media. In experiment 6 and 7, the initial pH was raised to 3.5 and 3.75 by using potassium hydroxide while monitoring with a pH meter (Thermo Fisher scientific, Waltham, MA). In experiment 8, the nitrogen content was decreased by reducing the amount of ammonium phosphate and L-arginine HCl. For 52 mg/L nitrogen, 0.125 mg/L ammonium phosphate was added and 0.05 mg/L L-arginine HCl was added. For 26 mg/L nitrogen, 0.0625 mg/L ammonium phosphate was added and 0.025 mg/L L-arginine HCl was added. For 13 mg/L nitrogen, 0.03125 mg/L ammonium phosphate was added and 0.0125 mg/L L-arginine HCl was added. These quantities were chosen in order to keep the proportion of ammonium phosphate to L-arginine the same in the limited media versus the normal media. In experiments 9 and 10, high brix synthetic must was created by adding 145 g/L of both D-fructose and D-glucose, which resulted in a 27 °B must. In experiment 9, fermentation temperature was increased to 27 °C (Figures A.2-A.10 of the appendix) for both the high and normal °Brix fermentations.

2.3 ORP Probes. ORP was measured through Hamilton platinum electrode 120 mm ARC probes (Hamilton Company, Reno, NV). These probes connected to a 120 Ω terminated RS-485 bus and a Modbus gateway (Stride, SGW-MB1511-T) was used to sample and store probe data into internal memory. The probes have a default Modbus address of 1 and use a 19200 baud rate, with eight data bits, one stop bit, and no parity bit. Each probe was programmed to have a unique Modbus address to avoid conflicts on the bus. The gateway's internal memory was read through a Modbus TCP/IP interface using a time-series database (PI, OSIsoft, San Leandro, CA). ORP and temperature were recorded as a function of time. Data was then processed in MATLAB and the probes were retimed to one minute.

The probes were cleaned before use by placing them in a solution of 1% sodium hydroxide for 10 minutes, then rinsing with distilled water. They were then placed in 5% citric acid for 10 minutes, rinsed with distilled water, and then placed back in 3M KCL storage solution for 20 minutes. The probes were calibrated in an ORP standard solution (+272 mV, Hamilton Company, Reno, NV) before placing them in the bioreactors.

The Hamilton Company probes used to measure both ORP and temperature were Silver/Silver Chloride based with a platinum band at the tip. The electronic component of this study is also a potential source of error. Resistance in readings, from tartrate buildup, microbial deposits, or other compound interference could contribute to inaccurate readings. Issues in calibration could also lead to inaccurate readings. Other sources of error could include temporary outages in power, issues in the wiring or series of probes, or manufacturing defects. A future experiment could compare different probes and wiring set-ups to confirm reliability.

2.4 Yeast Cell Monitoring. In order to monitor yeast cell populations, optical density was taken 1) every day for the first four days, and then every other day, or 2) every other day and when the fermentation reached or just surpassed 0 °Brix, or 3) every day, depending on the trial. In bioreactor experiment 1, only a final OD was taken on day 19 when it was clear that CY3079 was stuck. Experiments 2 and 3 followed sampling pattern 1. Experiments 4, 5, 6, and 7 followed sampling pattern 2. Experiments 8, 9, and 10 followed sampling pattern 3.

Starting with experiment 4, cell counts for the fermentations were done on day 1, day 3, and when the fermentation reached or just surpassed 0 °B (Table A.1 of the appendix) using a Levi-Hausser counting chamber under a microscope at 40x magnification. Viability was assessed on the same days as cell counting using methylene blue dye to determine the ratio of live and dead cells. This percentage was combined with the total cell count to determine the total viable cell count (Gilliland et al 1959).

With respect to yeast cell population monitoring, optical density (OD), total cell count, and total viable cell count were taken during a large portion of this study. OD measurements involved the use of a spectrophotometer and dilutions when appropriate to ensure the sample was measurable. OD was taken at 600 nm and diluted with DI water if the OD was above 1.0. Interferences with this method could include scratched or malformed cuvettes, errors in dilution, errors in pipette calibration, or non-homogenous samples. Between replicates, it is important to note that the final OD was taken on the day that the fermentation reached or surpassed zero brix. If the fermentation became stuck, the OD was taken on the

final day of the experiment (Table A.1 of the appendix). Errors in cell counting could include irregularities in the counting chamber glass, pipetted volume, or non-uniform distribution of cells in the sample. Viability staining could include errors such as pulling a non-representative sample from the solution, operator bias, or too long an exposure time to the dye which would lead to an increased number of dead cells. In this study, the operator bias might be reduced because viability measurements were performed by the same person each time.

3 Results

The goal of this research was to investigate the use of oxidation reduction potential (ORP) in monitoring fermentations. Using ORP as an additional metric to understand the progression of a given fermentation could inform decision making. In order to eventually use ORP as a monitoring and control tool, it is important to establish the patterns that are expected for fermentations under different conditions. Understanding how ORP changes over the course of a successful, healthy fermentation is an important initial step so that deviations from this pattern can be found in unsuccessful fermentations. After initial "baseline" ORP curves have been established, variables affecting fermentation can be adjusted in order to observe the resulting differences in ORP measurements. All fermentations in this research were carried out in Minimal Must Media (MMM), or synthetic must, for consistency and control over must components. In this section, baseline ORP curves were established using EC1118 yeast at pH 3.25, and then subsequent experiments altered the fermentation by changing factors such as yeast strain, pH, sugar content, temperature, and nitrogen content.

3.1 Summary of Experiment Runs

Table A.1 of the appendix shows the result of all ten bioreactor experiments and the parameters associated with each reactor. Tables 3.3.0–3.7.0 detail cell count, viability, and OD for each experimental condition. Initial runs in experiments 1–5 (Table A.1 of the appendix) aimed to establish baseline redox curves for various yeast strains chosen based on their use in the industry or known characteristics they present. Comparison of the same strain was done across separate runs, especially initially, in order to prevent effects from using the same synthetic media or initial yeast culture. Once it was found that the ORP curves had good reproducibility, replicates were then run in the same experiment number (Table A.1 of the appendix). Variables adjusted in this work include yeast strain, pH, sugar content, temperature, and nitrogen content.

3.2 Establishment of Baseline ORP curves

In order to eventually differentiate curves in various fermentation environments, it is important to determine what a "standard" ORP curve looks like, and its general shape throughout the course of fermentation. To date, it appears that redox potential has not been measured in synthetic media using ORP probes. Because literature was not available, initial research aimed to establish a general standard.

Figure 3.2.1 depicts a fermentation using yeast strain EC1118 at pH 3.25 and 23 °C. This strain is used as an example as it is a common research strain, and has reliably completed fermentations. The temperature was chosen as a moderate environment, and the pH had been used in previous experiments using synthetic media. Figure 3.2.1 shows the initial ORP on day 0 starts at 300 mV, and quickly drops to a minimum value of -200 mV on day 3. Shortly after this point, when the fermentation had reached about 10°Brix, the ORP begins to increase from the minimum value, and continues to increase until the fermentation has completed. It was determined that the cyclical increase and decrease in the shape of the ORP measurements from day to day was due to diurnal sunlight exposure, which occurs for an unknown reason, but it did not affect the average shape reported. Generally, this figure shows what an ORP curve would look like for a healthy fermentation.

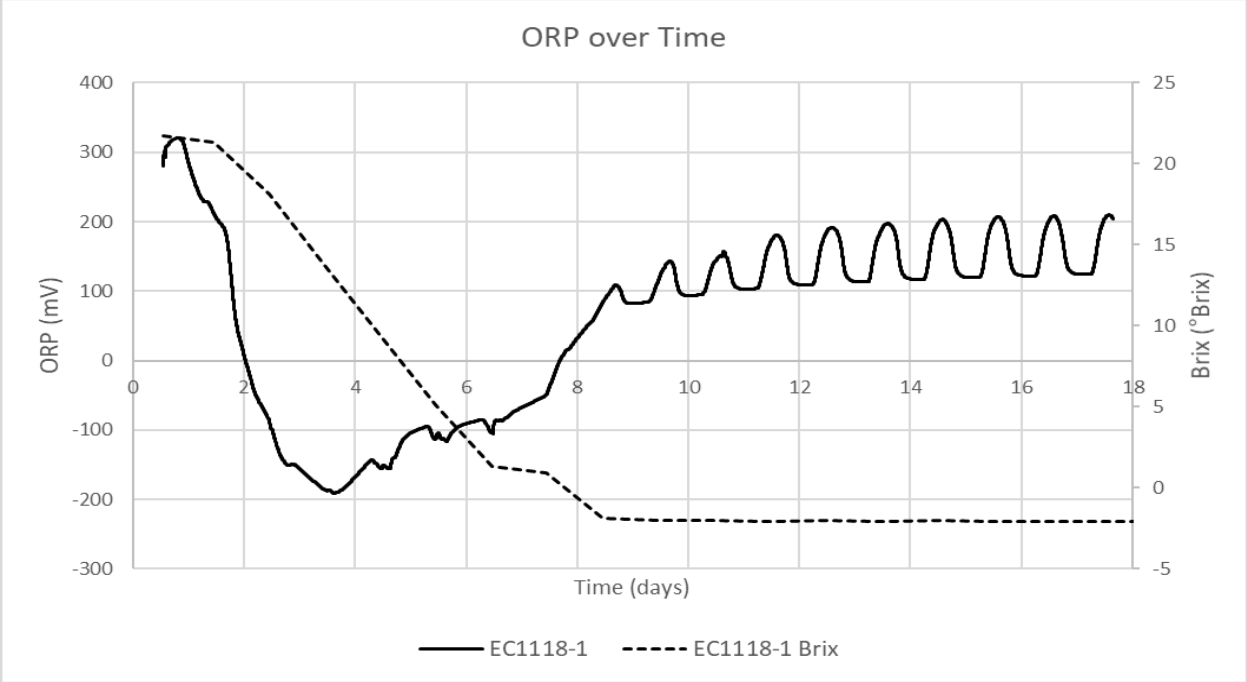

Figure 3.2.1: ORP over time for EC1118 yeast at pH 3.25 and 23 ◦C.

EC1118 is one of many yeast strains that can be used for fermentation. A variety of strains are used in the wine industry based on the characteristics they impart on the wine from their metabolic differences. Because these strains interact with the fermentation environment in different ways, it is reasonable to posit that their effects on the ORP of the solution will also be different.

Figure 3.2.2 shows four graphs of the initial EC1118 ORP curve from Figure 3.2.1, individually overlayed with a successful fermentation from RC212, Montrachet, Elixir, and CY3079 yeast strains. These ORP curves from the different strains were chosen as curves that were representative of how the strain behaved in general in a successful fermentation. CY3079 shows a delay in the decrease to the redox minimum until about day 3, and its lowest value in redox is higher than EC1118. CY3079 also lingered around the low point until about day 8 when the rate of increase in ORP became greater. This is in contrast to EC1118 where the redox begins to rise at a moderate rate soon after it reaches the lowest point in redox potential. In the graph of RC212 versus EC1118, RC212 begins its decrease in redox sooner than that of EC1118. RC212 also does not reach as low of a minimum redox value as EC1118, and had a small period

of hovering around the low point before it gradually began to rise. The ORP curve for Elixir shows a slightly later drop in redox than the representative EC1118 curve, and does not drop as low of an ORP value; the ORP also rises more gradually after reaching its minimum value. Montrachet is in contrast to the other strains in that its minimum is slightly above -200 mV which is lower than the minimum for EC1118. It also shows a similar rate of descent at the onset of fermentation, and has a comparable rate of rise at the end of fermentation, though it maintains its minimum ORP for a slightly longer length of time.

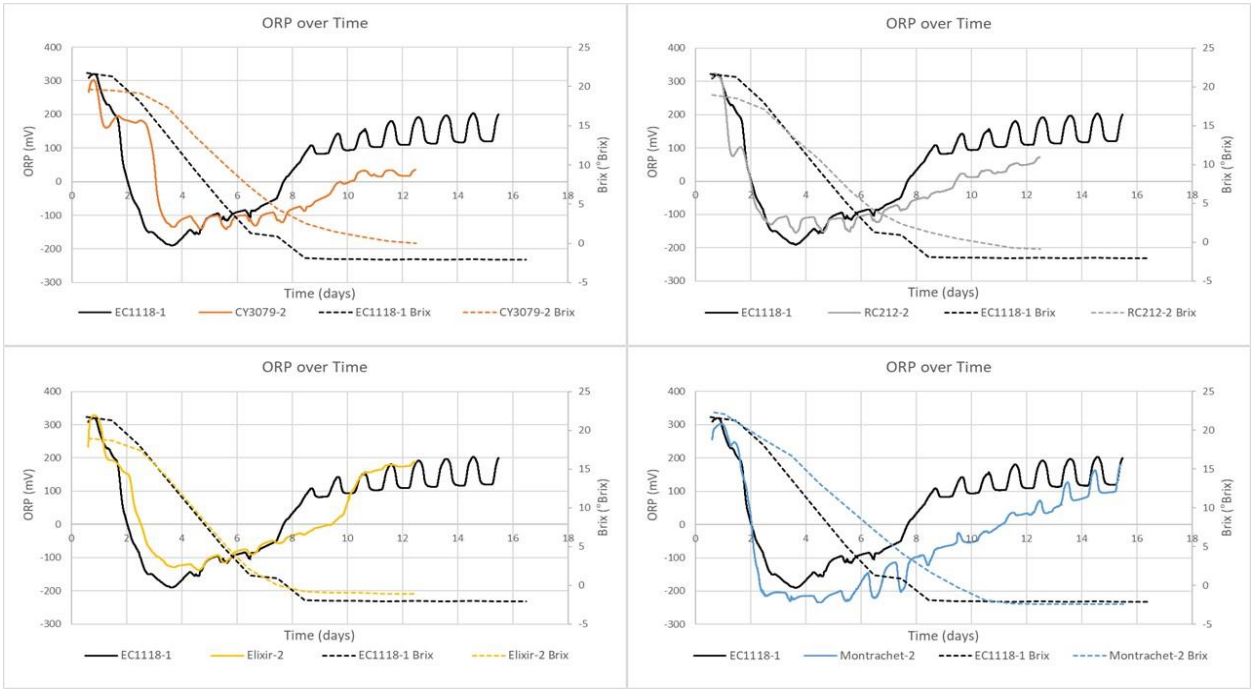

Figure 3.2.2: Four ORP over time graphs. Each graph has an EC1118 curve (Figure 3.2.1) against each experiment using CY3079, RC212, Elixir, or Montrachet yeast. All fermentations were performed at 3.25 pH and 23 ◦C.

Figure 3.2.3 shows all curves from Figure 3.2.1 and 3.2.2 overlayed for ease of comparison between strains. Most curves show similarities at time points throughout the fermentation. It is interesting to note that CY3079-2 and Montrachet-2 had high similarity in their rate of sugar consumption, though their ORP curves were different in numerical value. While this difference is true, the general shape of both, where there is a plateau at the bottom, which transitions into a gradual increase in ORP, is very similar. RC212-2

and Elixir-2 show very similar rates of sugar consumption, with differences in ORP values, up to about day 4, when their ORP values become more similar and the rate of sugar consumption more different. The cause of these differences is not known, but seem to show how the different yeast strains affect the ORP of the solution.

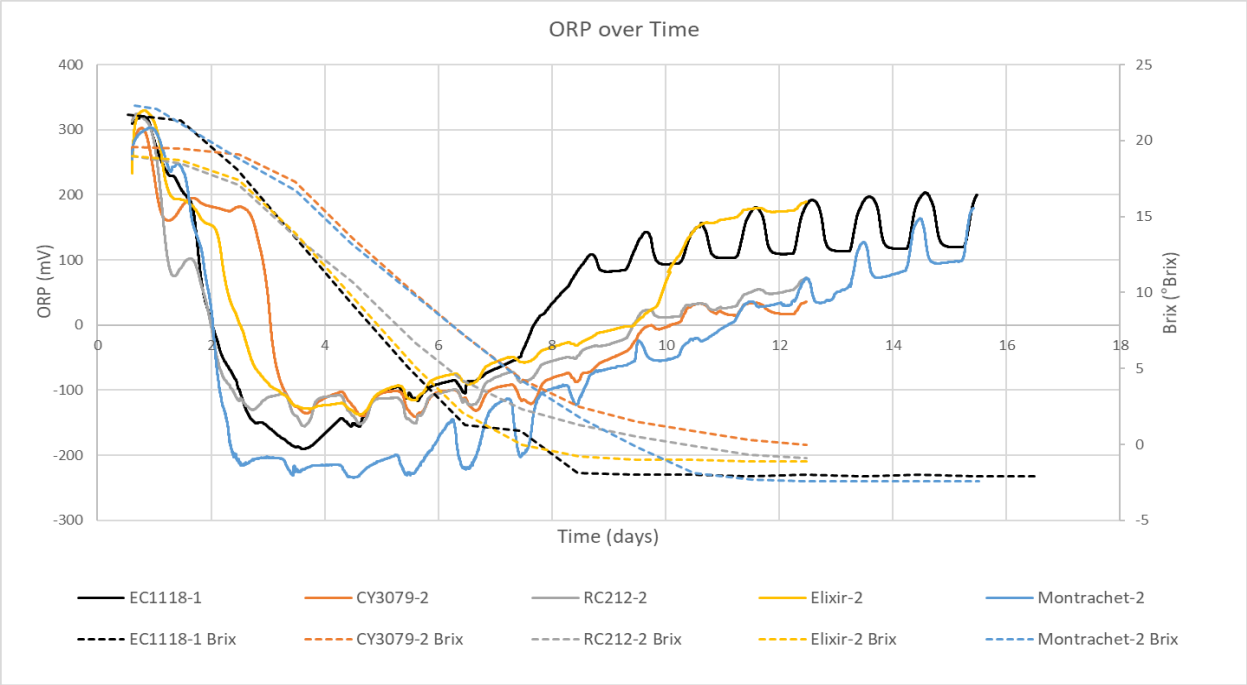

Figure 3.2.3: ORP over time for five yeast strains: EC1118, CY3079, RC212, Elixir, Montrachet. All replicates completed fermentation and were conducted at 3.25 pH and 23 °C.

Throughout the course of executing these fermentations and monitoring each by using ORP, it was found that at pH 3.25, RC212 was unreliable at completing fermentations. In different replicates, there were instances of success, sluggishness, and failure (becoming "stuck"). Figure 3.2.4 shows the successful fermentation of RC212 shown in Figure 3.2.1 along with an additional replicate of RC212 which was also successful. As the figure shows, the differences in the ORP between the two fermentations is very minimal over time. Some differences shown in the graph are that RC212-1 has a small plateau that is slightly higher than that of RC212-2, and on day 2, its decrease in redox is slightly steeper. From there, the ORP is similar between the two replicates, with RC212-1 being just slightly higher than RC212-2 from day 3–6. These differences in ORP could explain the small differences shown in the Brix curve, though more fermentations

would have to be conducted to confirm this. ORP does seem to correlate with the outcome of the fermentation, however.

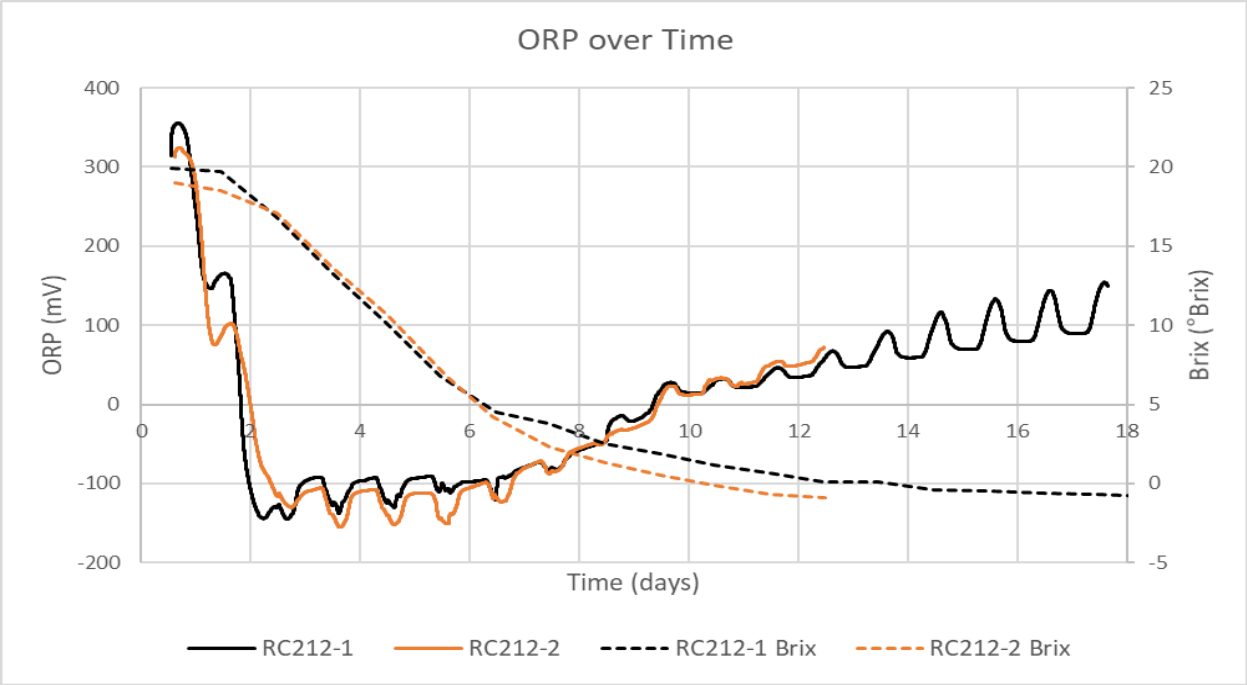

Figure 3.2.4: Two successful fermentations of RC212 and their ORP during fermentation. Both replicates were performed at 3.25 pH and 23 ◦C.

Figure 3.2.5 shows the successful fermentation of RC212 depicted in Figure 3.2.1 against a fermentation that became stuck at about 9°Brix. The rate of change in brix between day 0 and 5 are comparable, but the redox curves show much more obvious differences. With RC212-3, the initial drop in redox is delayed until about day 2 and passing the 0 mV level on day 3. The replicate that stuck had a faster rate of ORP rise toward the end of fermentation than the replicate that completed. This steeper rise in redox comes just before the brix measurement starts to level off. RC212-3 has a starting °Brix value that is about 15% greater than RC212-2 which is a factor to consider in comparing these curves, and shows the need to perform additional replicates under different variables. In the future, final ethanol concentrations, and ethanol concentrations during the fermentation should be measured in order to compare these values and determine its relation to ORP at given time points.

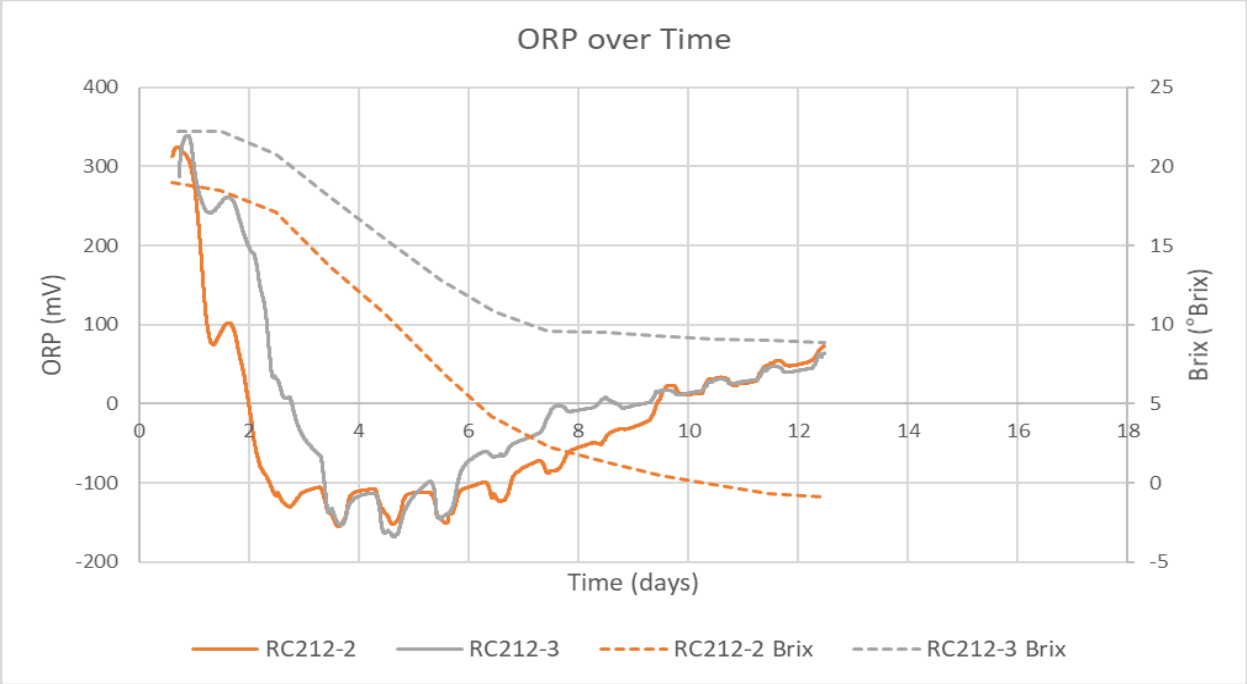

Figure 3.2.5: One successful and one unsuccessful (stuck) fermentation of RC212 yeast and their ORP during fermentation. Both replicates were performed at 3.25 pH and 23 ◦C.

Similar patterns seem to occur with both stuck and sluggish fermentations. Figure 3.2.6 shows a fermentation that was considered sluggish, completing after 16 days, against the initial RC212 curve from Figure 3.2.1, which completed after 10 days. Similar to the fermentation that stuck, RC212-3 (Figure 3.2.5), RC212-4 shows a delay in its initial drop in redox, with the drop intersecting 0 mV around day 3. Rates in sugar consumption between RC212-2 and RC212-4 are similar, with more variability in RC212-4. Unlike the stuck or completed fermentation, RC212-4 hits its ORP minimum, and stays around that value for over 6 days. The brix curve starts to flatten out around day 8, before the ORP has risen at all. This pattern may be an indication for RC212 fermentations that are prone to sluggishness. Note that there is an anomaly in the redox data on day 10 for RC212-4 where the ORP spikes and returns to its original point. The cause of this is unknown, but suspected to be related to the electronics of the system.

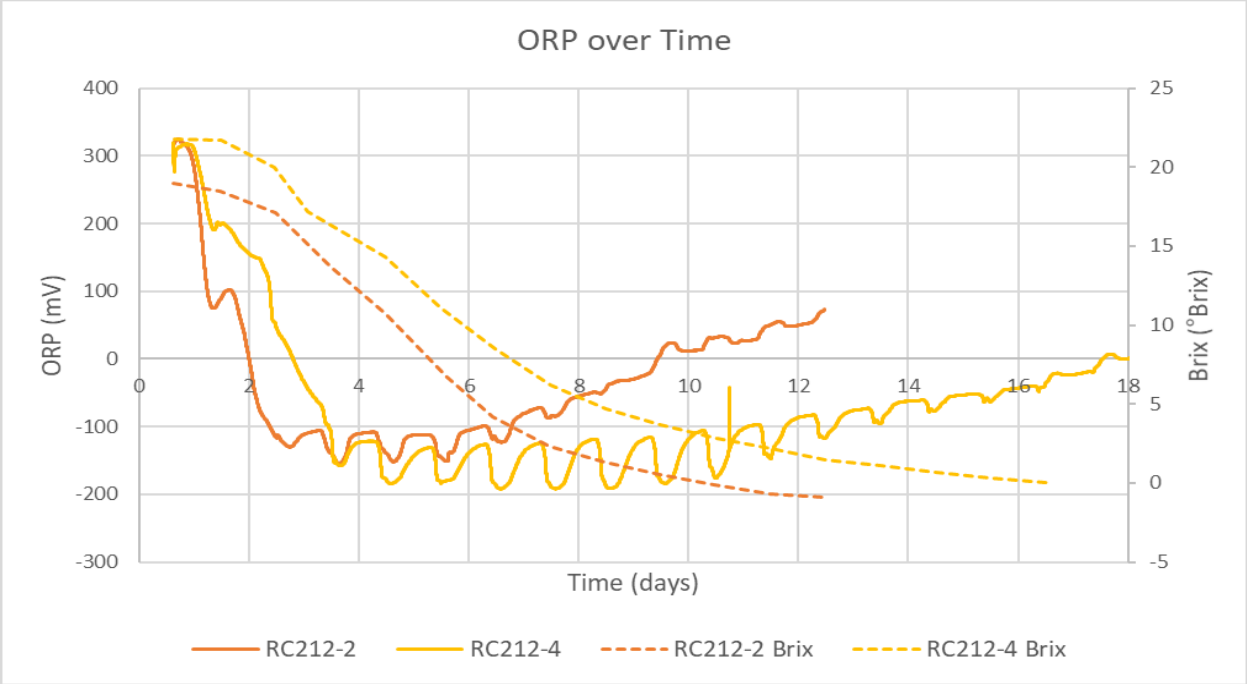

Figure 3.2.6: One successful and one sluggish fermentation by RC212 yeast and their ORP during fermentation. Both replicates were performed at 3.25 pH and 23 ◦C.

After making these initial observations across strains at pH 3.25 and 23 °C, and seeing instances of both success and failure, this prompted questioning on how these ORP curves would change with different parameters. In a following section, *"Investigation of pH,"* (Section 3.4), the result of a trial including RC212 and Elixir yeast under different pH conditions is discussed. RC212 was chosen to be part of this trial because it had already shown all outcomes at pH 3.25, which prompted looking at whether it showed similar patterns at other pH values. Elixir was chosen as well because it showed great reliability in fermentation at the low pH, in contrast to RC212. It was unknown whether this would be true for higher pH values as well, or how the resulting ORP curves would differ, if at all. Once pH had been addressed to some extent, other factors that are important to winemakers, such as sugar content, temperature, and nitrogen content were all explored as well (*"Investigation of sugar content and temperature,"* (Section 3.5), *"Investigation of Nitrogen content"* (Section 3.6)).

3.3 Investigation of Yeast Strain

In order to explore the effects of yeast strain on the ORP throughout fermentation, five strains were used including EC1118, CY3079, RC212, Elixir, and Montrachet. These yeasts were chosen based on use in the industry or known characteristics they present. These fermentations were carried out at pH 3.25, a temperature of 23 °C, and nitrogen level (YAN) of 208 mg/L. The pH was chosen based on use in previous experiments. This pH may be seen by some strains as a challenging environment for fermentation. Because different yeast strains show varying characteristics in terms of flavor contribution, time to completion, ethanol tolerance, and more, it may be expected that the ORP curves would vary between strains. Metabolic differences in terms of compounds made or used, and sensitivities to pH and different compounds in solution could affect the overall redox potential. Strains used, fermentation conditions, brix, and OD values throughout fermentation can be found in Table A.1 of the appendix.

Final OD values were taken for all replicates. The values did not show consistency in replicates for any of the strains. Montrachet's OD values for the three replicates showed the most similarity, with the range from the greatest value to smallest value being 1.66. All other experiments showed OD ranges of 2.42 up to 6.36. There was also similarity seen between different strains in the same run in some cases (Table 3.3.1). For example, fermentations run in the same bioreactor trial number have a range from 1.22 up to 4.14 (Table A.1 of the appendix). More replicate fermentations would have to be run to determine whether the bioreactor trial number affected the final OD of the fermentations.

Cell count and viability was not measured until bioreactor experiment 4; Data is only available, therefore, for two replicates of CY3079, two replicates of RC212, one replicate of Elixir, and all three replicates of Montrachet (Table 3.3.1). For these replicates on day 1, the viability ranged from 71–95%. Elixir-3 had the lowest viability at this time point at 71%, but reached 94% viability by day 3. On day 3 all strain replicates had a viability of at least 90%, aside from RC212-4 which had a viability of 84%. By the zero brix timepoint for these replicates, the range of viability was between 82–90%, with RC212-5 as an

exception at 59% at zero brix. RC212-5 was deemed stuck on day 15, at which point the final OD, cell count, and viability were taken.

In Figure 3.3.4, the Elixir-3 fermentation shows an increase in the redox potential on day two from about -200 mV to -50 mV. This is the point in which the lid to the sampling port was left open overnight and exposure to air resulted in an increase in redox potential. Because of this event early in the fermentation, the effects on the remaining ORP curve are unknown and this aberration should be noted with any comparison against this curve. Graphs created to compare between strain were created using ORP curves that were considered representative of the replicates and strain.

For yeast strain EC1118, the replicates were fairly similar especially in the first four days (Figure 3.3.1). Replicates 1 and 2 showed similar rates of sugar consumption, as well as being the most similar in ORP curve shape. On day 6, replicate 1 showed a slight decrease in rate of sugar consumption, which corresponded to a delay in ORP increase. This instance is the major difference between replicates 1 and 2. Replicate 3 shows great similarity to the other two replicates especially up to day 5, though the rate in brix decrease is slightly less for replicate 3. After day 5, the redox remains near its low point. All replicates rise from their minimum ORP values when the °brix value reached about 10°B; the ORP measurements begging to increase more rapidly at 2°B.

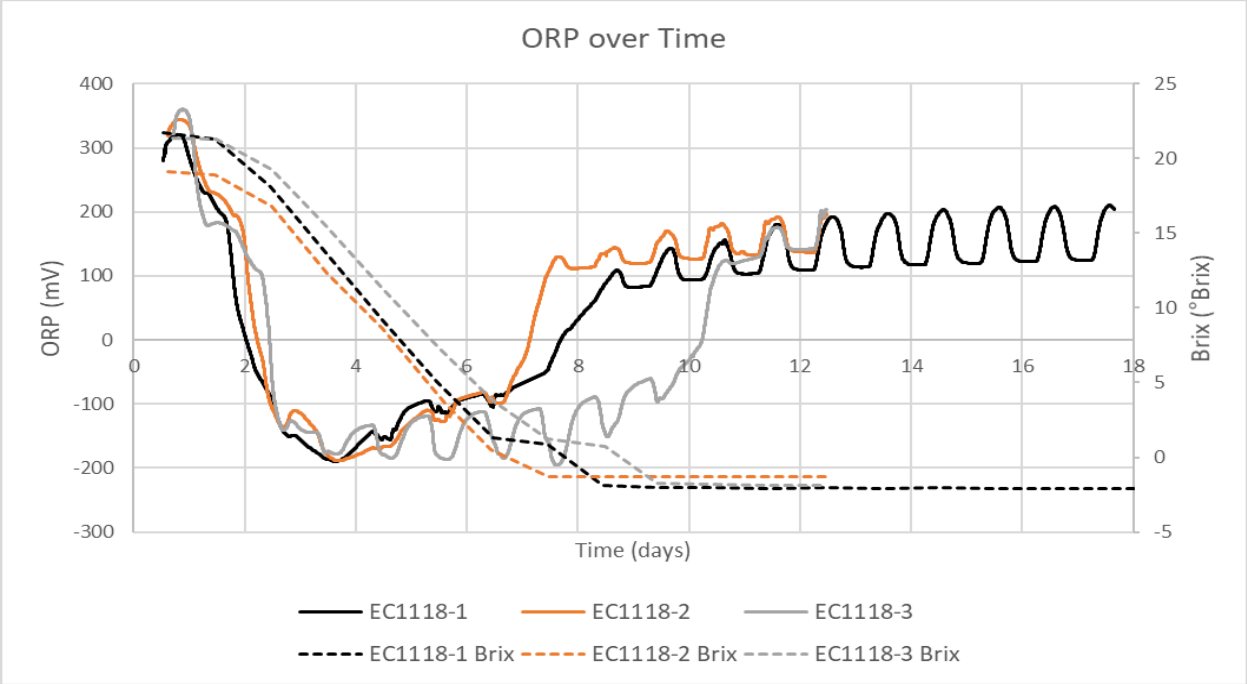

Figure 3.3.1: ORP curves over time for three replicates of EC1118 strain. Fermentations were conducted at pH 3.25 and 23 ◦C. Bioreactor trial number for each replicate can be found in Table 3.3.1.

CY3079 as a strain showed the most variability in both the redox and brix curves when comparing strain replicates (Figure 3.3.2). Replicates 1, 3, and 4 showed the greatest similarities in both redox and brix data, with replicate 2 following, and replicate 5 being the most dissimilar. By day 5, all but replicate 3 had very similar redox potential values. By day 8, replicate 3 was similar to the others. Replicate 1 did not complete fermentation and the final redox values do not rise as high as those that completed fermentation (among the replicates recorded after the fermentations finished). The rate in brix decrease was much steeper for replicate 5 than the others. It was able to reach a lower minimum redox value and the redox curve dropped continuously to its minimum value, unlike the other replicates which showed a small plateau before day 2. All replicates start to gradually increase in redox potential shortly after the redox minimum was reached.

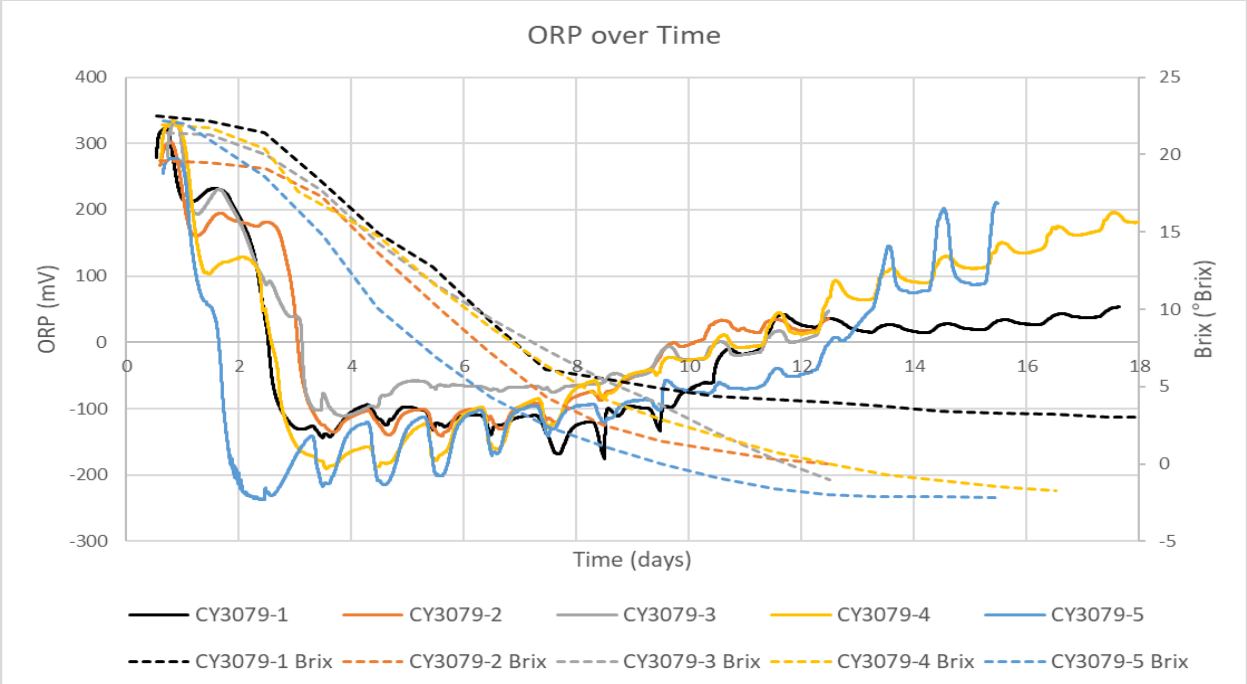

Figure 3.3.2: ORP curves over time for five replicates of CY3079 strain. Fermentations were conducted at pH 3.25 and 23 ◦C. Bioreactor trial number for each replicate can be found in Table 3.3.1.

ORP curves for RC212 replicates showed similarities in those that had similar outcomes (Figure 3.3.3). RC212-1 and RC212-2 had almost identical redox curves, aside from where they initially plateaued on day 1, and a variation in slope on day 2. These two replicates both reached zero brix, however, at different times after inoculation. Replicate 1 reached zero brix on day 14, though it hovered at 0.1 for day 12 and 13, while replicate 2 reached zero brix on day 10. Replicates 3 and 4 showed great similarity especially up to day 6. After this time, the ORP of replicate 3 began to increase, and 4 remained around the minimum ORP value of -200 mV (excluding the diurnal shifts which caused daily ORP increases and decreases). Replicate 5 was very similar to 1 and 2, but differed in that it reached a lower minimum redox value and though it started to rise on day 6, the rise was at a much slower rate than 1, 2, or 3. Replicates 3, 4, and 5 were the fermentations that showed issues, with 4 being sluggish, and the other two did not complete fermentation of glucose/fructose. These three fermentations also showed the greatest differences in ORP curves. The ORP of Replicate 3 increased on day 6 at a greater rate than those that completed fermentation and showed a delay in decreasing to its redox minimum. Replicate 4 also showed this initial delay in ORP decrease and

remained near its ORP minimum for longer time. The ORP of replicate 5 initially behaved similarly to those that completed fermentation, however on day 8, the redox remained much lower. The brix curves for those that completed (RC212-1 and RC212-2 in Figure 3.3.3) are nearly the same, and the other three fermentations show more similarity to each other.

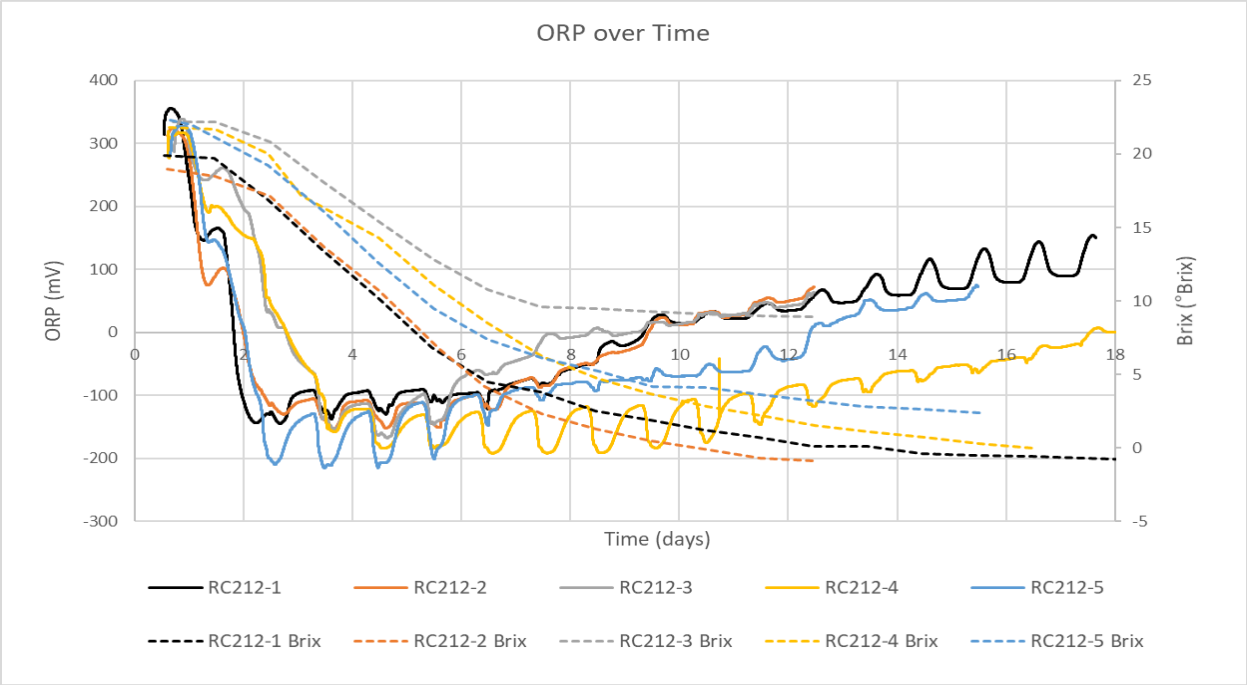

Figure 3.3.3: ORP curves over time for five replicates of RC212 strain. Fermentations conducted at pH 3.25 and 23 °C. Bioreactor trial number for each replicate can be found in Table 3.3.1.

For Elixir, replicates 1 and 2 are very similar initially. In comparison, replicate 3 shows a more rapid decrease in redox to its minimum point and a shorter plateau before day 2 (Figure 3.3.4). By day 6, replicates 2 and 3 become more similar, and replicate 1 increased in redox at a greater rate. All three of these fermentations completed. Replicates 1 and 2 had nearly identical rates of sugar consumption, though their redox curves were the most dissimilar. Replicate 3 can only be compared without scrutiny until day 2 when the redox shows an increase over several hours from the technical issue where it was exposed to oxygen. While it is not known whether this incident affected the remainder of the fermentation, the redox quickly realigns with replicate 2 after the issue was corrected. Because there were complications with Elixir-3, it was not used in the three graphs which compare between strains.

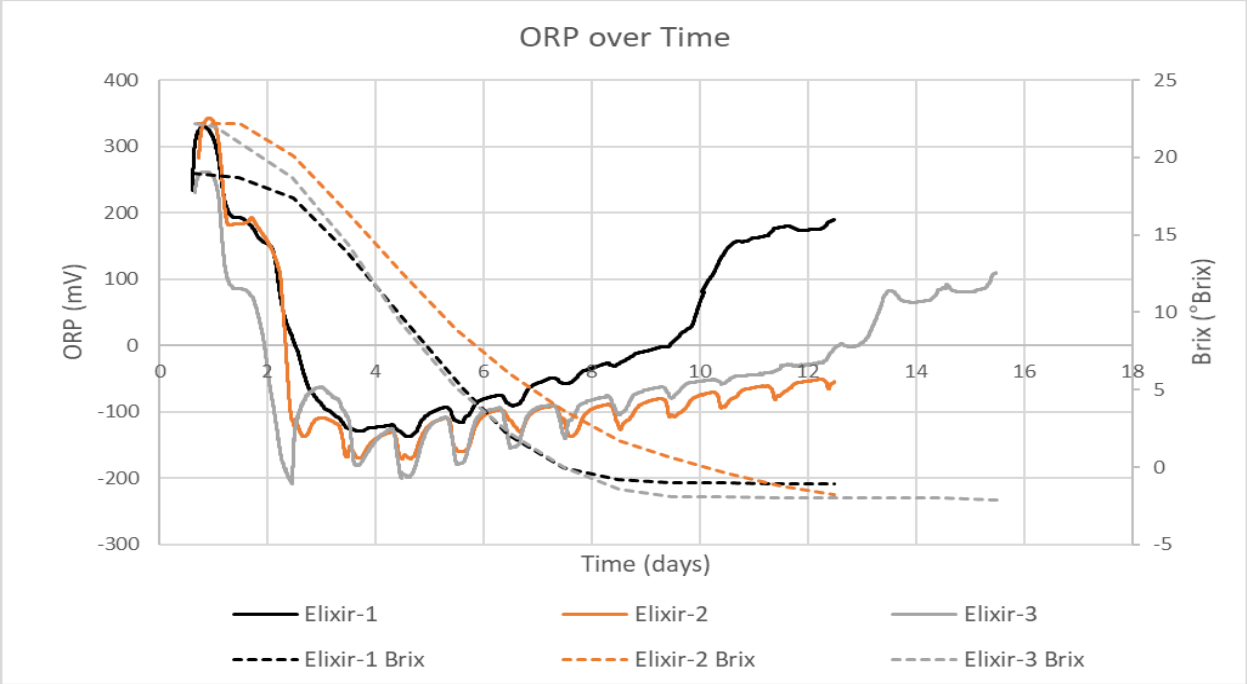

Figure 3.3.4: ORP curves over time for three replicates of Elixir strain. Fermentations conducted at pH 3.25 and 23 °C. Bioreactor trial number for each replicate can be found in Table 3.3.1. Replicate 3 shows an increase in ORP over several hours on day 2 from the technical issue where it was exposed to oxygen.

All three Montrachet replicates had nearly identical redox curves and even more similar brix curves (Figure 3.3.5). Replicates 2 and 3 started slightly higher than replicate 1, but had the same general shape and all aligned by day 2. On day 5, replicate 3 deviated from the other two and maintained a slightly higher redox potential through the remainder of fermentation, though the fermentation outcome, on the basis of brix, was the same. Probes were compared and calibrated during each experimental run and were found to report similar values. There was great reproducibility between redox and brix curves for the replicates in this trial. It is important to consider that these replicates were run in the same trial.

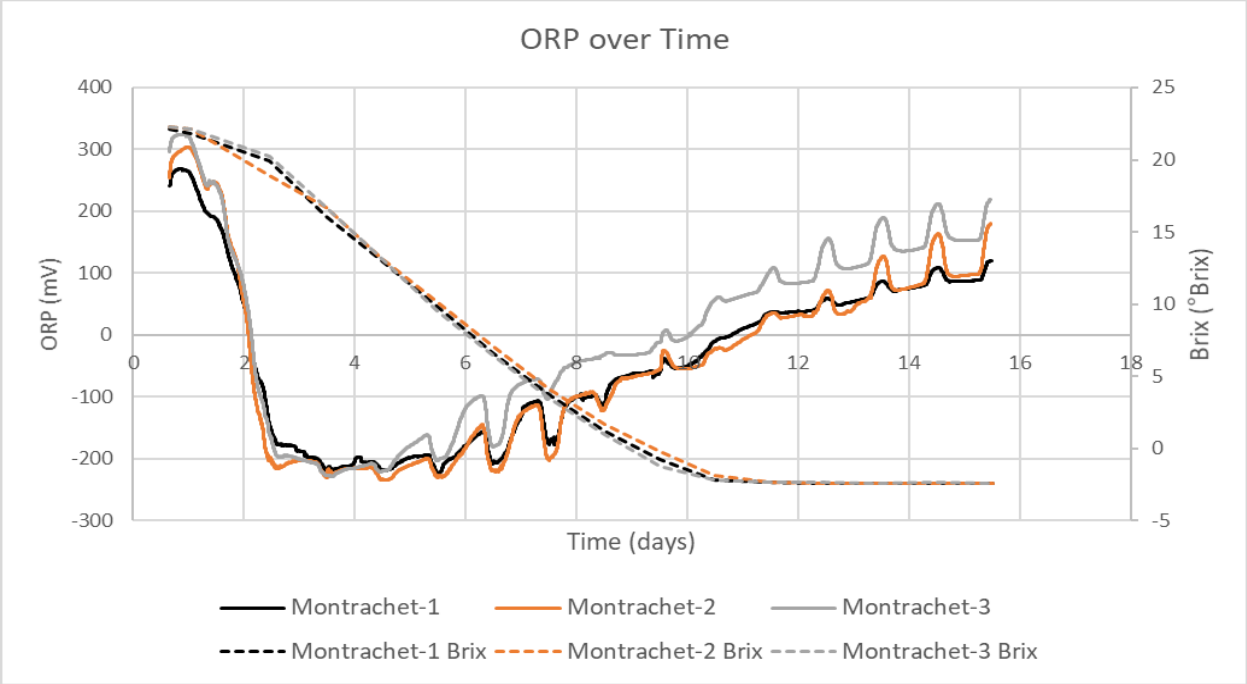

Figure 3.3.5: ORP curves over time for three replicates of Montrachet strain. Fermentations conducted at pH 3.25 and 23 ◦C. Bioreactor trial number for each replicate can be found in Table 3.3.1.

Figure 3.2.3 displays one representative ORP curve from each strain used in these experiments. Between strains, CY3079 tends to take the longest to initially decrease to its ORP minimum and have the largest plateau before dropping to its redox minimum. Montrachet reached the lowest redox minimum values, followed by EC1118. RC212, Elixir, and CY3079 drop to around the same minimum value. Strains show differences in rate of rise after reaching a redox minimum during fermentation. EC1118 generally shows a steep rise in redox at one time point, gradually increasing on either side of this rise as seen in Figure 3.3.1. All other strains seem to show a much more gradual, constant rate of ORP rise, aside from Elixir-2 (Figures 3.3.1–3.3.5). Across these graphs, the time it takes for the fermentation to finish is similar across replicates, with the exception of RC212 which was unreliable for completion in general.

The results of the investigation into how different yeast strains respond to the same environment in terms of ORP showed that ORP is a viable monitoring tool in the context of all strains. ORP shows repeatable patterns both within strains and more general patterns across strains. Differences within strains

may reveal issues with the fermentation environment, and differences across strains may be due to variance in metabolism and compound contribution.

*Table 3.3.1: Cell count, percent viability, and total viable cell count for various yeast strains at pH 3.25 and 23 °C. This data was not collected for bioreactor experiments 1–3. The OD values correspond to when the fermentations reached or just surpassed zero brix. Experimental conditions for each bioreactor trial can be found in Table A.1 of the appendix. *Fermentation stuck*

		Day 1			Day 3			0°B			
	Run in Trial #	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	OD
EC1118-1	1	-	-	-	-	-	-	-	-	-	9.84
EC1118-2	2	-	-	-	-	-	-	-	-	-	6.20
EC1118-3	3	-	-	-	-	-	-	-	-	-	9.66
CY3079-1*	1	-	-	-	-	-	-	-	-	-	7.20
CY3079-2	2	-	-	-	-	-	-	-	-	-	5.08
CY3079-3	3	-	-	-	-	-	-	-	-	-	8.40
CY3079-4	4	8.25E+06	95.42	7.87E+06	4.98E+07	92.59	4.61E+07	9.70E+07	89.65	8.70E+07	9.22
CY3079-5	5	7.75E+06	91.04	7.06E+06	1.19E+08	90.89	1.08E+08	1.05E+08	86.10	9.04E+07	11.44
RC212-1	1	-	-	-	-	-	-	-	-	-	7.36
RC212-2	2	-	-	-	-	-	-	-	-	-	6.94
RC212-3*	3	-	-	-	-	-	-	-	-	-	5.98
RC212-4	4	1.03E+07	88.20	9.08E+06	1.08E+08	84.67	9.14E+07	1.09E+08	83.82	9.14E+07	8.00
RC212-5*	5	6.75E+06	92.17	6.22E+06	1.28E+08	92.09	1.18E+08	1.10E+08	59.13	6.50E+07	8.40
Elixir-1	2	-	-	-	-	-	-	-	-	-	6.56
Elixir-2	3	-	-	-	-	-	-	-	-	-	9.36
Elixir-3	5	8.50E+06	71.27	6.06E+06	1.55E+08	94.33	1.46E+08	1.66E+08	90.96	1.51E+08	12.72
Montrachet-1	5	1.60E+07	95.58	1.53E+07	1.14E+08	98.58	1.12E+08	1.17E+08	83.38	9.75E+07	9.16
Montrachet-2	5	1.35E+07	93.59	1.26E+07	8.00E+07	97.78	7.82E+07	1.09E+08	84.85	9.25E+07	8.58
Montrachet-3	5	1.10E+07	83.00	9.13E+06	1.27E+08	97.49	1.24E+08	1.43E+08	82.04	1.17E+08	10.24

3.4 Investigation of pH

RC212 and Elixir were chosen to be part of this trial based on their previously shown differences in ability to complete fermentations. RC212 was able to complete at pH 3.25 on occasion, but was often sluggish or would become stuck. Elixir had no issues completing at pH 3.25. Because these strains showed different capabilities at pH 3.25, determining their performance at different pH values was of interest.

These experiments were conducted at pH values of 3.25, 3.5, and 3.75 with a temperature of 23 °C and starting °Brix values of 21 (Tables A.1 and figures A.2-A.7 of the appendix). Because pH 3.25 is considered low for wine and challenging for yeast growth, it was expected that as the pH was increased, the rate of sugar consumption would also go increased. It was also expected that because the environment is more favorable with a slightly higher pH, the yeast would be more metabolically active and be able to drive the redox lower. These experiments were carried out at a moderate fermentation temperature of 23 °C, which should be able to facilitate growth. It was found that RC212 was able to reliably complete at pH 3.5 and 3.75 and showed little difference between these levels. Elixir was also able to reach 0°B at the higher pH values, and completed faster than at pH 3.25. The difference in ORP curve shape between pH 3.5 and 3.75 for Elixir was also minimal.

Cell count and viability were not taken until the 4th run of bioreactors, so replicates 1 and 2 for both Elixir and RC212 only have a final OD value. For RC212-1 pH 3.25, the final OD was taken 4 days after the fermentation reached 0°B when the experiment ended, as a protocol had not been established yet. Overall, the cell count and total viable cell count for RC212 across all pH values were fairly similar (Table 3.4.1). The percent viability on day 1 for RC212-1 pH 3.75 and RC212-2 pH 3.75 were lower than all other RC212 replicates at any pH with values of 81.4, and 77.8%. However, all replicates had at least a 96% viability by day 3, aside from RC212-4 pH 3.25 which had a percent viability of 84.7%. The first three replicates at pH 3.25 did not have this data taken, so it is unknown whether low viability was an issue in those fermentations, though the final OD's taken suggested a lower cell density than all other replicates at the other pH values. The average OD for the replicates at pH 3.25 was 7.13, while the combined average

for replicates at both pH 3.5 and pH 3.75 was 11.60. RC212-3 pH 3.25 stuck, so the final OD was taken on day 12 when the experiment concluded. The highest OD recorded for this replicate was 9.08 taken on day 8.

As for Elixir across different pH values, all total cell and viable cell counts were within the same order of magnitude and were very similar in general (Table 3.4.2). This data was not taken for the first two replicates of Elixir at pH 3.25. Elixir-3 pH 3.25 had the lowest viability on day 1 with 84.7%, while all others had a viability of at least 98%. By day 3, the viability for Elixir-3 pH 3.25 had risen to 96.9%, with the next lowest being 99.6%. By the final cell counts, RC212-4 pH 3.25 actually had the second highest value at 94.9%, and most others being in the low 90's. As far as final OD values go, the lowest by far were the first three replicates at pH 3.25. The other values were similar with RC212-4 pH 3.25 and RC212-1 pH 3.75 being the lowest.

In general, fermentations at a higher pH reached a lower redox value. The difference in the minimum ORP value is slight between fermentations at 3.5 and 3.75 with both reaching values of around -220 mV. The difference is more pronounced between pH 3.25 and 3.75, with the fermentations at pH 3.25 only reaching a redox value of about -150 mV (Figures 3.4.1, 3.4.2). At a pH of 3.25 for both yeast strains, the redox value initially drops on day 0, but plateaus before it begins dropping again, which is not a characteristic seen at the two higher pH values.

In Figure 3.4.1, RC212-1 pH 3.25 was considered sluggish, with the fermentation surpassing 0°B by day 14, and RC212-3 pH 3.25 was considered stuck by about day 7 with a final brix value of 8.9°B. In each of these fermentations, the redox value starts to increase from its minimum at a higher °brix value than those that complete. For RC212-1 at pH 3.25, the ORP begins to rise at around 5°B, while for RC212-3 at pH 3.25 begins to rise at around 12°B; and those fermentations that complete do not begin to increase in ORP until the fermentation has reached around 3°B. This pattern is true for Elixir fermentations as well (Figure 3.4.2). For RC212 fermentations, this increase coincides with day 6 or 7 of the fermentation, and for Elixir, the increase begins on day 5 or 6.

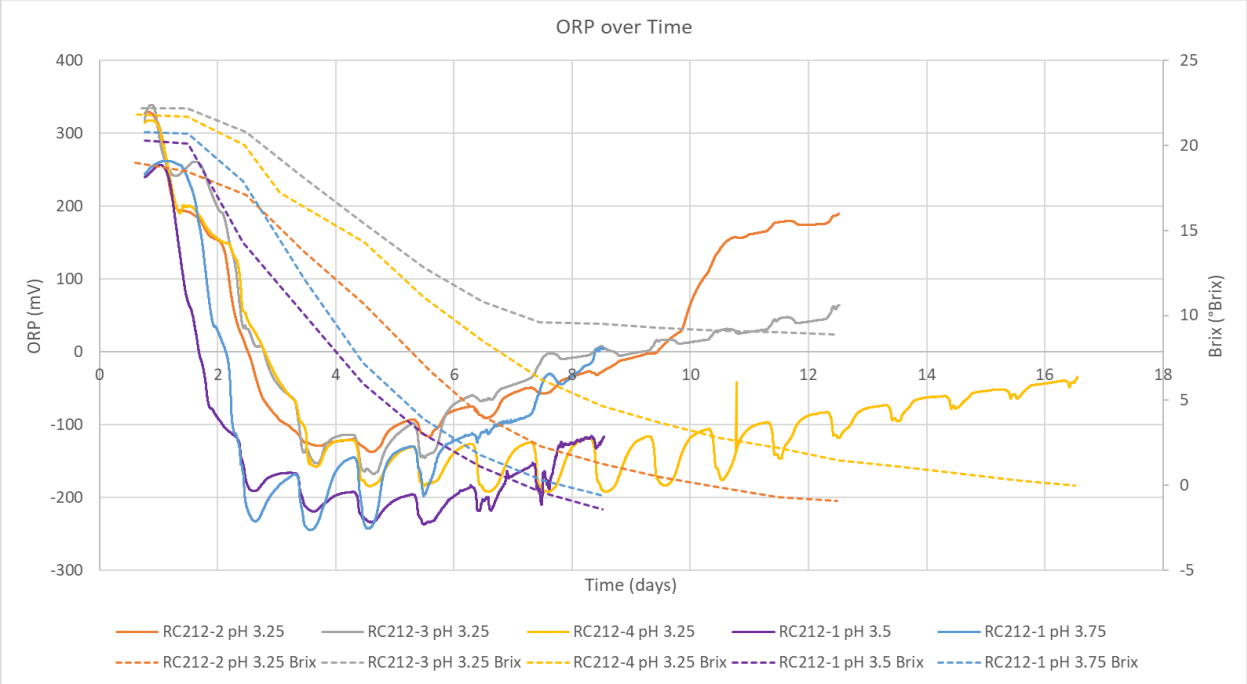

Figure 3.4.1: *ORP and Brix curves for RC212 yeast at pH values 3.25 (three replicates show one stuck, one sluggish, and one complete), 3.5, and 3.75. Initial conditions start at about 21 °B and a temperature of 23 °C. Bioreactor trial number for each replicate found in Table 3.4.1.*

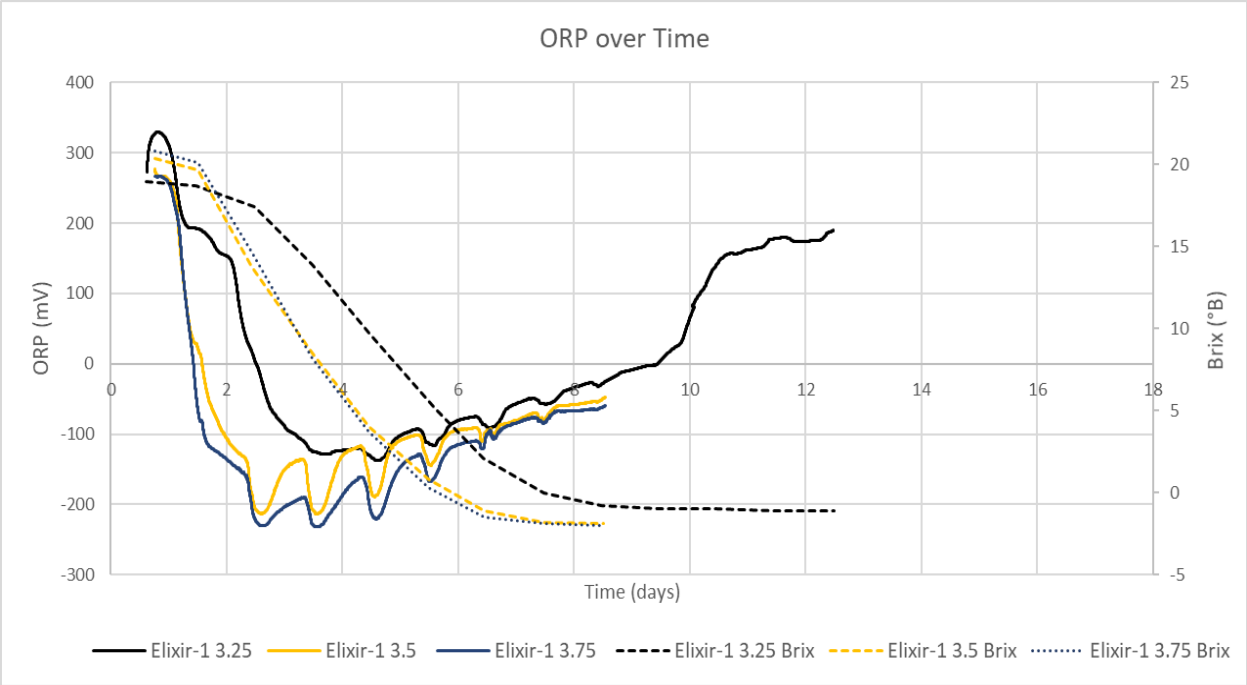

Figure 3.4.2: ORP and Brix curves for Elixir yeast at varying pH values. Initial conditions start at about 21°B at a temperature of 23 °C. Bioreactor trial number for each replicate found in Table 3.4.1.

In the set of replicate fermentations at pH 3.25, the ORP curves show more variation (Figure 3.4.3). Replicate 4 at pH 3.25, which finished on day 16 and was considered sluggish, showed the most similarity in the first five days to replicate 2, which became stuck around 9 °brix. The curves begin to diverge just before day 6 where the second replicate's redox begins to rise while at about 12°B, whereas the third replicate stays at its redox low point until day 10 and 3°B. Replicate 1 dropped much more quickly initially in terms of redox. The ORP began to rise on day 6 at around 4°B, at which point the rate of sugar consumption appeared to slow, though it did complete eventually on day 14. For all replicates, the minimum redox was similar.

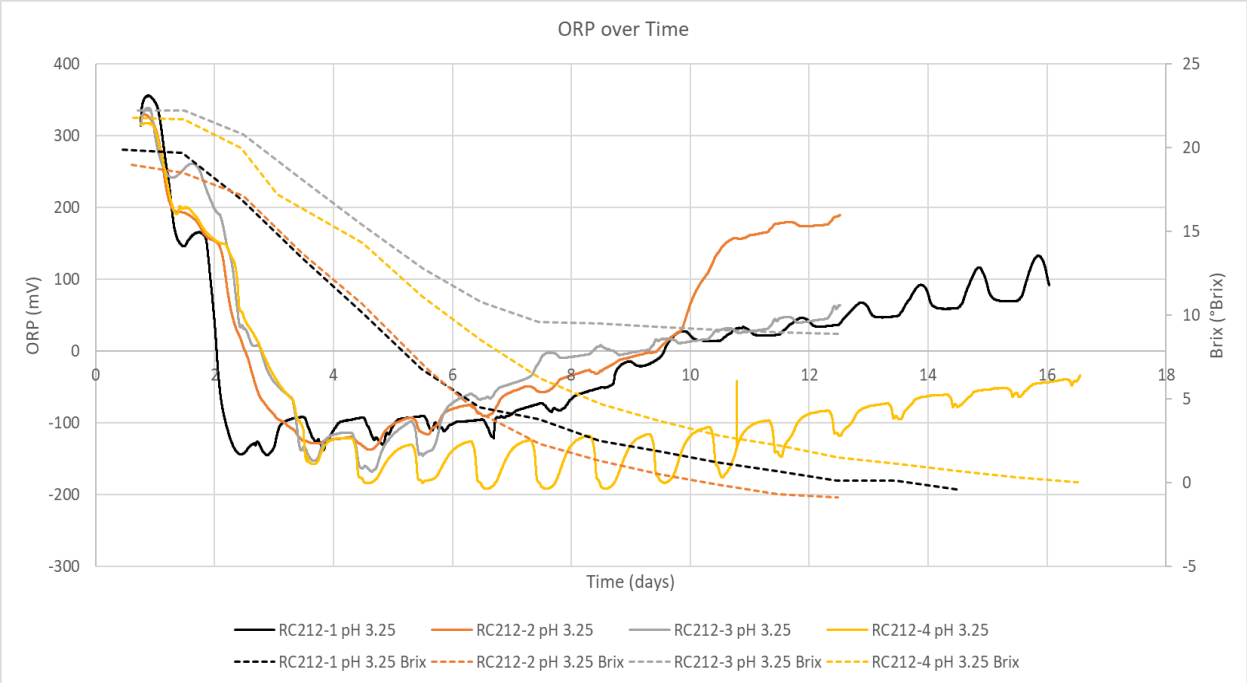

Figure 3.4.3: ORP and Brix curves for RC212 yeast at pH value 3.25*. Initial conditions start at about 21◦B and a temperature of 23 ◦C. Bioreactor trial number for each replicate found in Table 3.4.1. The aberration in ORP for RC212-3 pH 3.25 on day 10 is unexplained but thought to be an electronic error.*

As shown in Figures 3.4.4 and 3.4.5, the redox curves for RC212 at pH 3.5 and 3.75 are highly reproducible. At pH 3.75, the ORP of three replicates for RC212 increased when the fermentations were between 5 and 7 brix, whereas at pH 3.5, the redox would increase around 5°B. While this difference was observed, the time to completion was similar between the two pH values. In Figures 3.4.4 and 3.4.5, one

replicate in each shows a delay in increasing ORP from the minimum value toward the end of fermentation. The brix data for these replicates trended most closely with the replicate which shared similar ORP data on day 2 and 3.

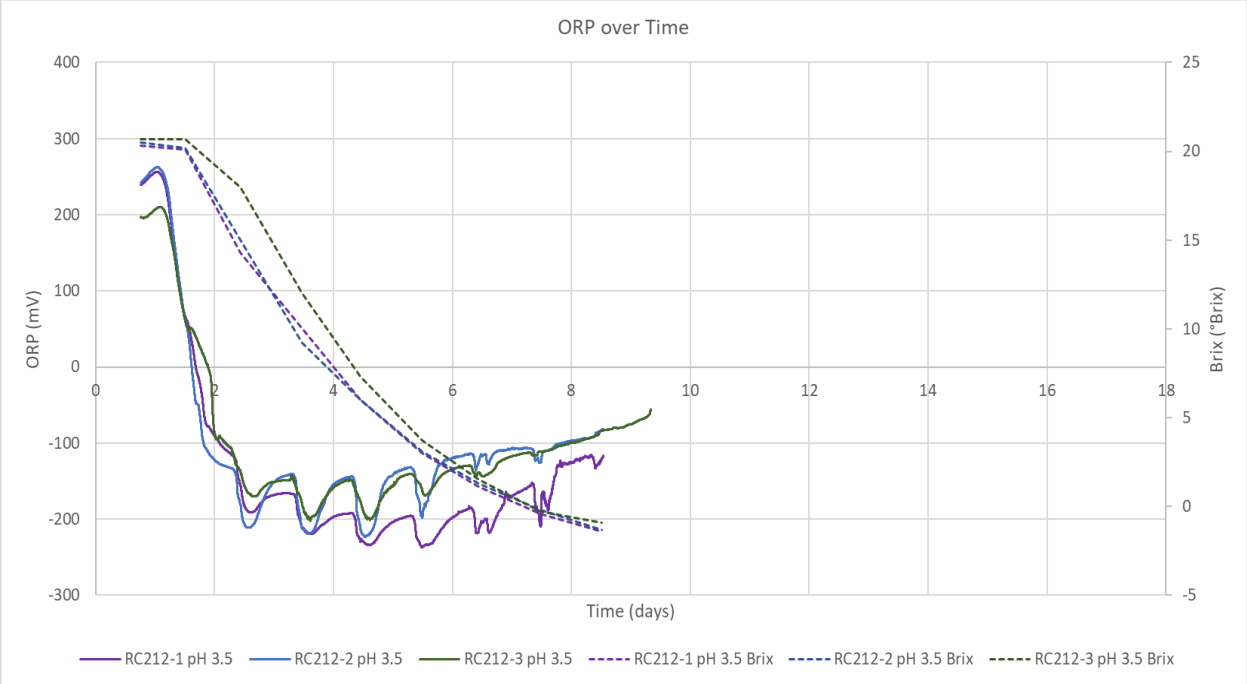

Figure 3.4.4: ORP and Brix curves for RC212 yeast at pH value 3.5. Initial conditions start at about 21◦B and a temperature of 23 ◦C. Bioreactor trial number for each replicate found in Table 3.4.1.

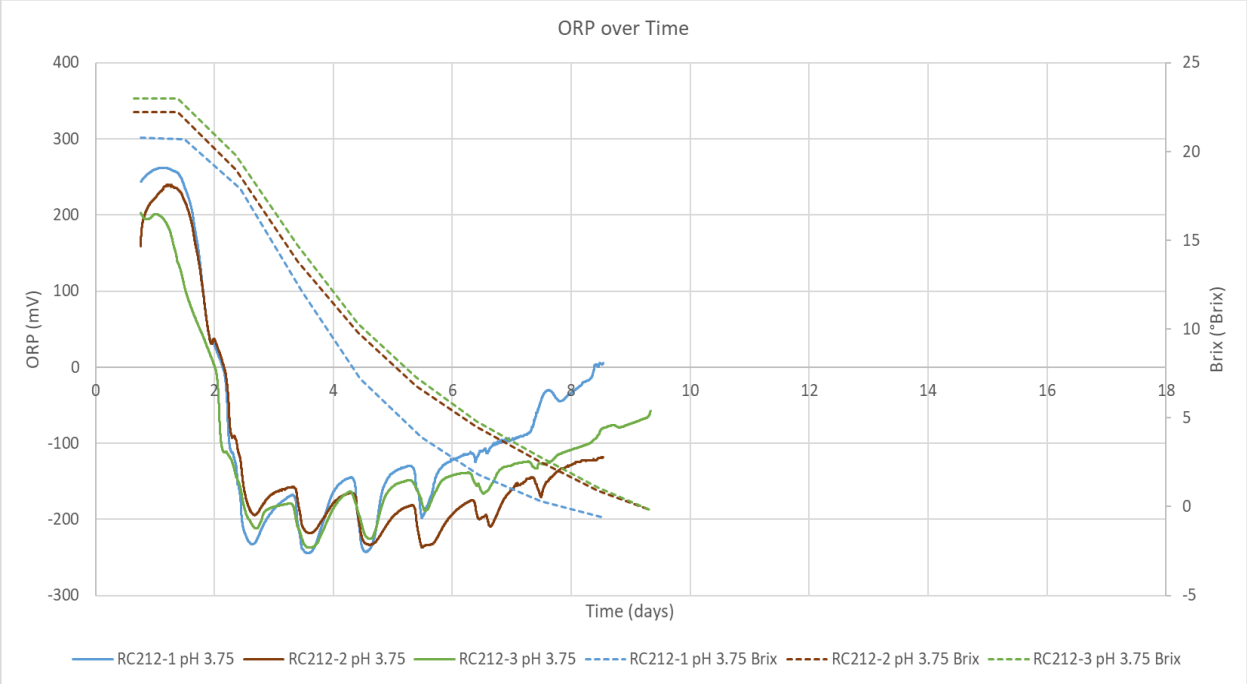

Figure 3.4.5: ORP and Brix curves for RC212 yeast at pH value 3.75. *Initial conditions start at about 21◦B and a temperature of 23 ◦C. Bioreactor trial number for each replicate found in Table 3.4.1.*

The redox curves for Elixir showed similar trends. At pH 3.25 there was more variability in the shape of the curves between replicates (Figure 3.4.6), whereas at pH 3.5 and 3.75 the curves were very reproducible, and even similar between the two pH values (Figure 3.4.7). The rate of sugar consumption for pH 3.5 and 3.75 also showed minimal difference. The third replicate of Elixir pH 3.25 was exposed to oxygen overnight on day 2 when the sampling port was left open, which coincides with an increase in ORP from -200 mV to about -50 mV. After this occurrence, the ORP greatly resembles the second replicate, though it is not known how this oxygen exposure affected the outcome of the curve.

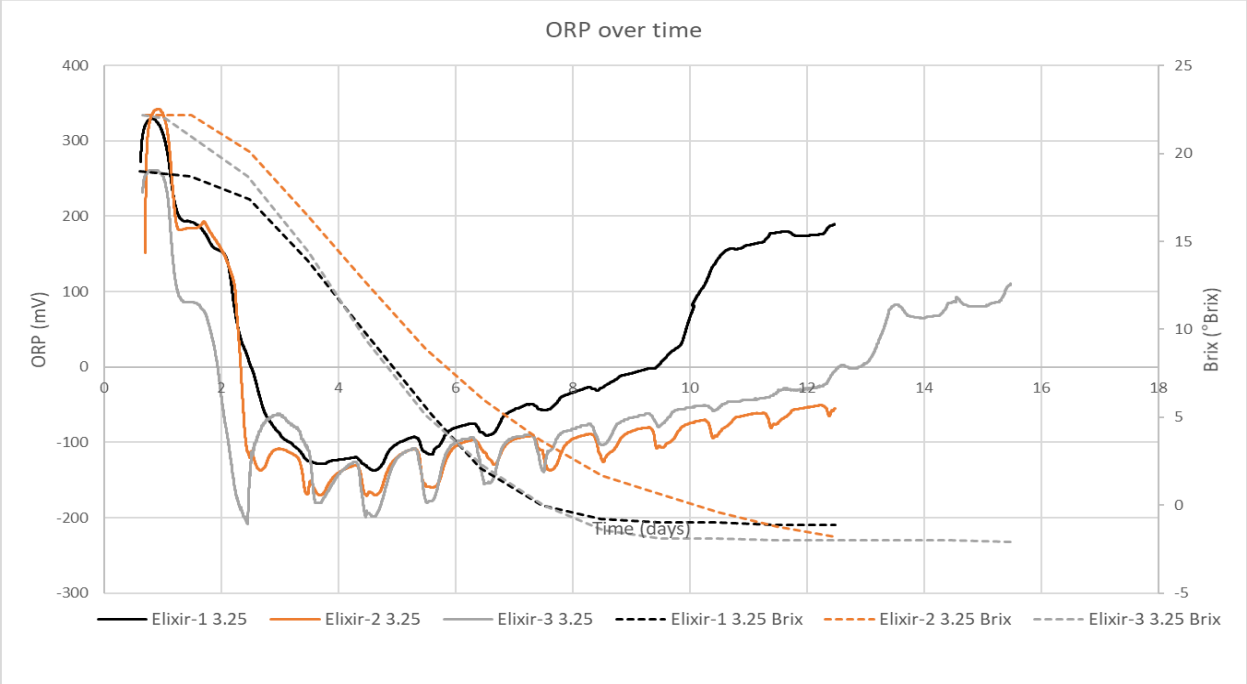

Figure 3.4.6: ORP curves for Elixir yeast at pH 3.25. Initial conditions start at about 21°B at a temperature of 23 °C. At the point where Elixir-3 pH 3.25 rises from -200 mV to about -50 mV near day 2.25, the sampling port was left open overnight which caused oxygen ingress and a rise in ORP. Bioreactor trial number for each replicate found in Table 3.4.2.

Figure 3.4.7: ORP curves for Elixir yeast at pH 3.5 and 3.75. Initial conditions start at about 21°B at a temperature of 23 °C. Bioreactor trial number for each replicate found in Table 3.4.2.

The result of the pH trial shows that the difference in yeast strain performance is much less between pH 3.5 and 3.75 than between pH 3.25 and 3.5. Overall, the OD values were comparable between 3.5 and 3.75, with the values being slightly lower at pH 3.25. Viability did not show as clear a correlation in this set of experiments, however. It was observed that there was greater variability in ORP within the 3.25 pH experiments, and 3.5 and 3.75 curves were more repeatable across both strains. In general, it was also observed that the replicates at pH 3.5 and 3.75 were also able to reach a lower redox minimum than those at pH 3.25. More replicates would have to be conducted to confirm this trend, and also that the replicates at 3.75 were also just slightly lower than those at 3.5.

*Table 3.4.1: Cell count, percent viability, and total viable cell count for RC212 yeast at pH 3.25, 3.5, and 3.75. This data was not collected for bioreactor experiments 1–3. OD values are listed for when the fermentations reached or just surpassed zero brix. For RC212-1 3.25, the final OD was taken four days after it reached the zero value, when the experiment ended. MMM fermented by RC212 yeast at 23 °C. Experimental conditions for each bioreactor trial can be found in Table A.1 of the appendix. *Fermentation stuck. OD was taken on day 12 at experiment end.*

		Day 1			Day 3			0°B			
	Run in Trial #	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	OD
RC212-1 3.25	1	-	-	-	-	-	-	-	-	-	7.36
RC212-2 3.25	2	-	-	-	-	-	-	-	-	-	5.96
RC212-3 3.25*	3	-	-	-	-	-	-	-	-	-	5.98
RC212-4 3.25	4	1.03E+07	88.20	9.08E+06	1.08E+08	84.67	9.14E+07	1.09E+08	83.82	9.14E+07	9.22
RC212-1 3.5	6	1.90E+07	97.45	1.85E+07	1.10E+08	98.23	1.08E+08	1.45E+08	88.17	1.28E+08	11.9
RC212-2 3.5	6	2.13E+07	97.59	2.08E+07	1.31E+08	96.72	1.27E+08	1.23E+08	80.78	9.94E+07	12.46
RC212-3 3.5	7	9.25E+06	100.00	9.25E+06	1.17E+08	98.83	1.16E+08	1.28E+08	75.61	9.68E+07	12.94
RC212-1 3.75	6	3.00E+06	81.42	2.44E+06	1.14E+08	97.03	1.11E+08	8.25E+07	40.64	3.35E+07	9.24
RC212-2 3.75	6	3.50E+06	77.80	2.72E+06	5.95E+07	98.06	5.83E+07	7.63E+07	66.33	5.06E+07	10.76
RC212-3 3.75	7	6.25E+06	98.45	6.15E+06	1.39E+08	98.76	1.37E+08	1.34E+08	84.01	1.13E+08	12.32

Table 3.4.2: Cell count, percent viability, and total viable cell count for Elixir yeast at pH 3.25, 3.5, and 3.75. This data was not collected for bioreactor experiments 1–3. OD values are listed for when the fermentations reached or just surpassed zero brix. MMM fermented by Elixir yeast at 23 °C Experimental conditions for each bioreactor trial can be found in Table A.1 of the appendix.

		Day 1			Day 3			0°B			
Strain and pH	Run in Trial #	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	OD
Elixir-1 3.25	2	-	-	-	-	-	-	-	-	-	6.56
Elixir-2 3.25	3	-	-	-	-	-	-	-	-	-	9.36
Elixir-3 3.25	5	1.53E+07	84.69	1.30E+07	1.58E+08	96.86	1.53E+08	1.20E+08	94.93	1.14E+08	12.72
Elixir-1 3.5	6	4.90E+07	98.54	4.83E+07	1.12E+08	99.58	1.12E+08	1.36E+08	95.80	1.30E+08	14.42
Elixir-2 3.5	7	1.13E+07	100.00	1.13E+07	1.21E+08	100.00	1.21E+08	1.82E+08	89.84	1.63E+08	15.04
Elixir-3 3.5	7	1.03E+07	98.77	1.02E+07	1.42E+08	100.00	1.42E+08	1.70E+08	93.88	1.60E+08	15.84
Elixir-1 3.75	6	4.25E+07	99.00	4.21E+07	1.10E+08	99.63	1.10E+08	1.15E+08	89.65	1.03E+08	13.54
Elixir-2 3.75	7	1.00E+07	100.00	1.00E+07	1.40E+08	100.00	1.40E+08	1.39E+08	90.83	1.26E+08	14.90
Elixir-3 3.75	7	1.20E+07	100.00	1.20E+07	1.01E+08	100.00	1.01E+08	1.80E+08	92.14	1.66E+08	14.74

3.5 Investigation of Sugar Content and Temperature

RC212 was used to investigate the effects of temperature and sugar content on the outcome of fermentation and the corresponding ORP curves. These factors are important to winemakers and are often monitored daily throughout the course of fermentation. For this reason, the contribution of this factor to changes in ORP at different values is of interest.

RC212 was chosen to be part of this trial because it had previous runs with both successful and unsuccessful fermentations which enabled either outcome to be compared to previous runs. All sugar and temperature conditions were performed at a pH value of 3.25 as had been chosen for previous experiments, which would allow for comparison. All fermentations had a nitrogen level of 208 mg/L. Experimental design was based on a 2x2 factorial that included conditions of brix at 21°B or 27°B at 23 °C and 28 °C. Both high sugar content and high temperature will create a more stressful fermentation environment. High sugar content has an inhibitory effect at the onset of fermentation, and causes a higher final ethanol concentration (Boulton et al. 1996). It was expected that as temperature increases, the time to complete fermentation would decrease, as would the likelihood of the fermentation becoming stuck or sluggish. Increase in initial sugar content was anticipated to increase the fermentation time and the likelihood of becoming stuck or sluggish. Higher initial sugar content was also expected to cause a delay in fermentation onset as well as viability challenges later in fermentation due to higher alcohol content.

The first three replicates of the 21B 23 °C conditions had yeast cell monitoring through OD only. RC212-1 21B 23 °C and RC212-3 21B 23 °C had their final OD measured when the experiment concluded, on days 19 and 12, respectively. For future experiments, a protocol to measure the OD once the fermentation had reached or just surpassed zero brix, which would have applied to RC212-1 21B 23 °C. RC212-3 21B 23 °C was measured on day 12 because it had stuck, and this was the final day of the experiment. The lowest final ODs were replicate 2 and 3 in the 21B 23 °C trial, followed by all three replicates of the 27B 28 °C trial (Table 3.5.1). All five of these low OD replicates had OD values lower than 7 and were all

considered stuck fermentations except for replicate RC212-2 21B 23 °C. The RC212-2 21B 23 °C replicate completed on day 14. RC212-1 21B 23 °C also had one of the lower final OD values at 7.36, and this replicate completed fermentation on day 10. The highest OD values were achieved by three replicates at 21B 28 °C, which all finished fermentation in 6 days with values between 9.48–9.58. RC212 in all replicates at 27B and 23 °C had OD values between 8.18–8.46, which was higher than average combined OD across all conditions, but all replicates in this condition became stuck after 11 days of fermentation. The OD values in this condition were similar to the values for RC212-4 and -5 under the 21°B and 23 °C conditions. These two replicates, with initial fermentation conditions of 21°B and 23 °C, were sluggish and stuck.

Percent viability and cell count were measured for at least two replicates in each condition (Table 3.5.1). RC212-4 and -5 21B 23 °C had the lowest viabilities on day 1 and day 3, ranging from 84–92%, as well as the lowest populations. All other conditions had between 94 and 100% viability, with viable cell counts all one order of magnitude higher than the replicates at 21B 23 °C. By the final cell count at the conclusion of the experiments, the lowest percent viability by far were the three replicates at 27°B and 28 °C which were all at about 1%, and had the lowest viable cell populations at one to two orders of magnitude lower than other condition replicates. All other fermentations had a final viability of between 49 and 64%, aside from RC212-4 21B 23 °C which had a final viability of 83.8%. While this replicate had the highest percent viability, RC212-1 and -2 21B 28 °C had similar viable cell populations. RC212-4 21B 23 °C had a viable cell population of $9.4x10^7$ cells/mL, while RC212-1 and -2 21B 28 °C had populations of $1.12x10^8$ and $1.28x10^8$ cells/mL, respectively.

The first condition tested was RC212 at 21°B and 23 °C (Figure 3.5.1). These curves were produced in bioreactor experiments 1 through 5 and were also used in comparisons of strain and pH. Replicates 1, 2 and 3 completed on days 14, 10, and 16, respectively. Replicate 3 did not complete and was considered stuck at around 9°B by day 12. Replicate 5 did not complete either and was considered stuck at 2.4°B on day 15. Discussion of differences in curve shape can be found in the results section under *Investigation of Yeast Strain* (Section 3.3). The replicates in this condition show undulations in the ORP curve because they

were conducted prior to the protocol change where the bioreactors were shielded from sunlight. Because the other three sugar and temperature conditions occurred after this protocol change, the ORP curves appear more continuous.

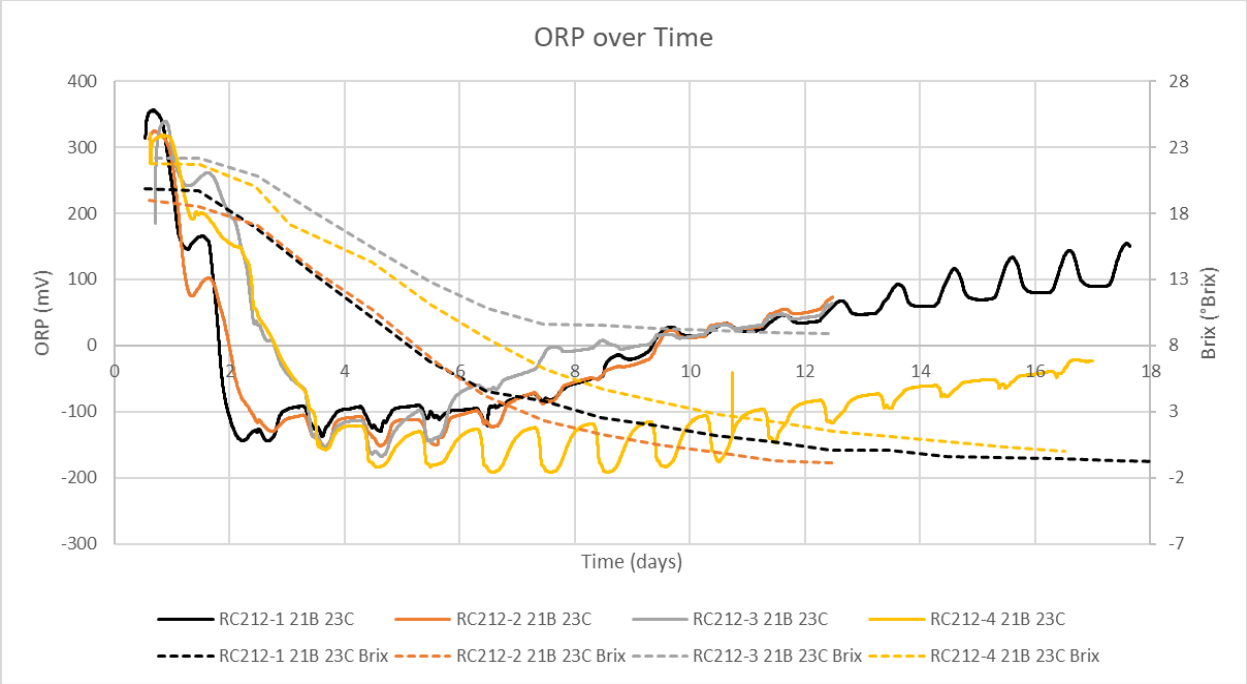

Figure 3.5.1: ORP curves for replicates 1 through 4 of the 21°B sugar conditions at 23°C. RC212 yeast was used at a pH of 3.25 for all replicates. Bioreactor trial number for each replicate can be found in Table 3.5.1.

RC212 was also tested at the same temperature of 23 °C in a high brix condition of 27°B. Each replicate was conducted in bioreactor experiment number 10. The resulting curves show little differences between replicates (Figure 3.5.2). The only notable visual difference between replicates was that the ORP curve for RC212-3 27B 23 °C showed a slight plateau on day 1 which was higher than the plateaus shown on the other replicates. This third replicate also showed a slight aberration in the brix data on day 3 to 4 where the line was not as continuous as the others. Aside from these observations, the curves for each replicate were nearly identical. In these curves, there are slight spikes in the lines which are especially noticeable from day 6 to 8. This is thought to be an electronic error due to interferences, though the general shape of the curve at those points is thought to be accurate.

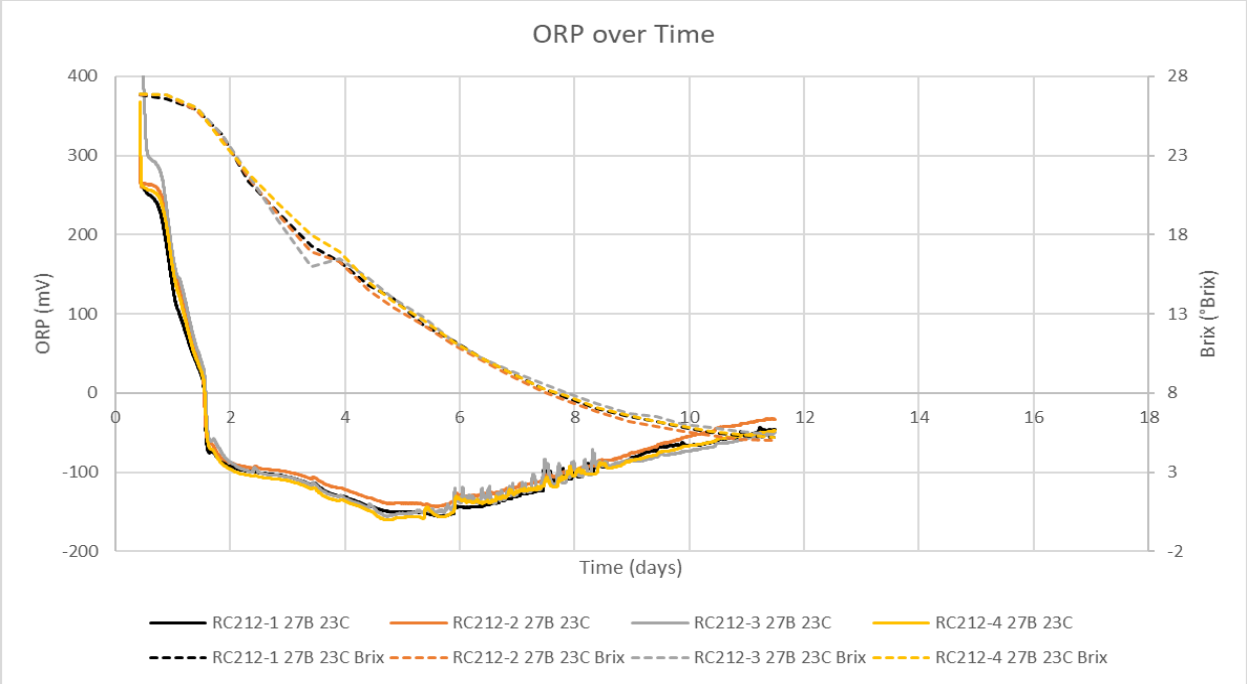

Figure 3.5.2: ORP curves for replicates 1 through 4 of the 27°B sugar conditions at 23 °C. RC212 yeast was used at a pH of 3.25 for all replicates. Bioreactor trial number for each replicate can be found in Table 3.5.1.

Another condition tested was 21°B at a higher temperature of 28 °C. Each of the three replicates were conducted in bioreactor experiment 9 and showed extremely similar curve shapes (Figure 3.5.3). The increase in ORP for RC212-3 21B 28 °C on day 7 and 8 was due to an error where the motor which controlled the continuous mixing was shut off overnight. Once mixing was restored, the ORP curve showed similar values to the other two replicates. The first replicate shows a slightly slower rate of sugar consumption from day 1 to 3, and also had a slightly lower rate of rise in ORP from day 5 to 8.

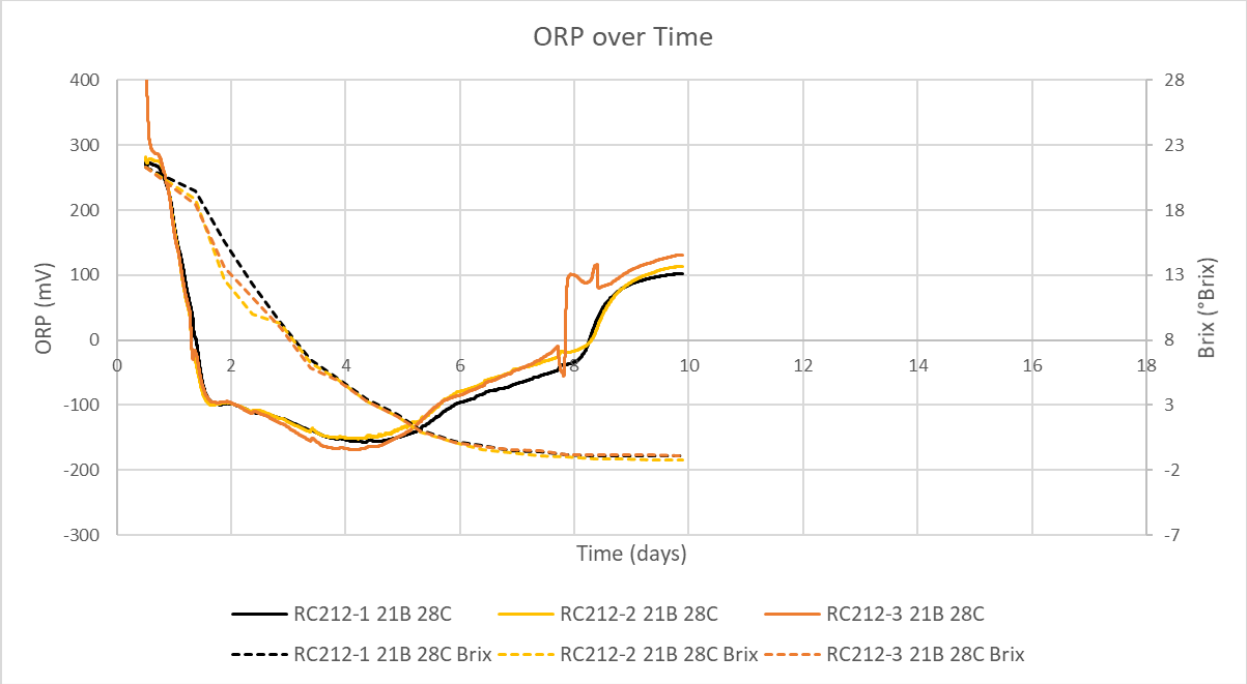

Figure 3.5.3: ORP curves for replicates 1 through 3 of the 21°B sugar conditions at 28°C. RC212 yeast was used at a pH of 3.25 for all replicates. Bioreactor trial number for each replicate can be found in Table 3.5.1.

The final sugar and temperature condition tested was 27°B at 28 °C. These replicates were also all run in bioreactor experiment 9. Curves between replicates were again, nearly identical and the only major difference was that the ORP of replicate 3 did not increase as quickly as the other two replicates on day 7, though the brix curves showed no practical difference (Figure 3.5.4). Replicate 3 had its brix curve trend visually higher than the other two replicates but the difference is essentially negligible.

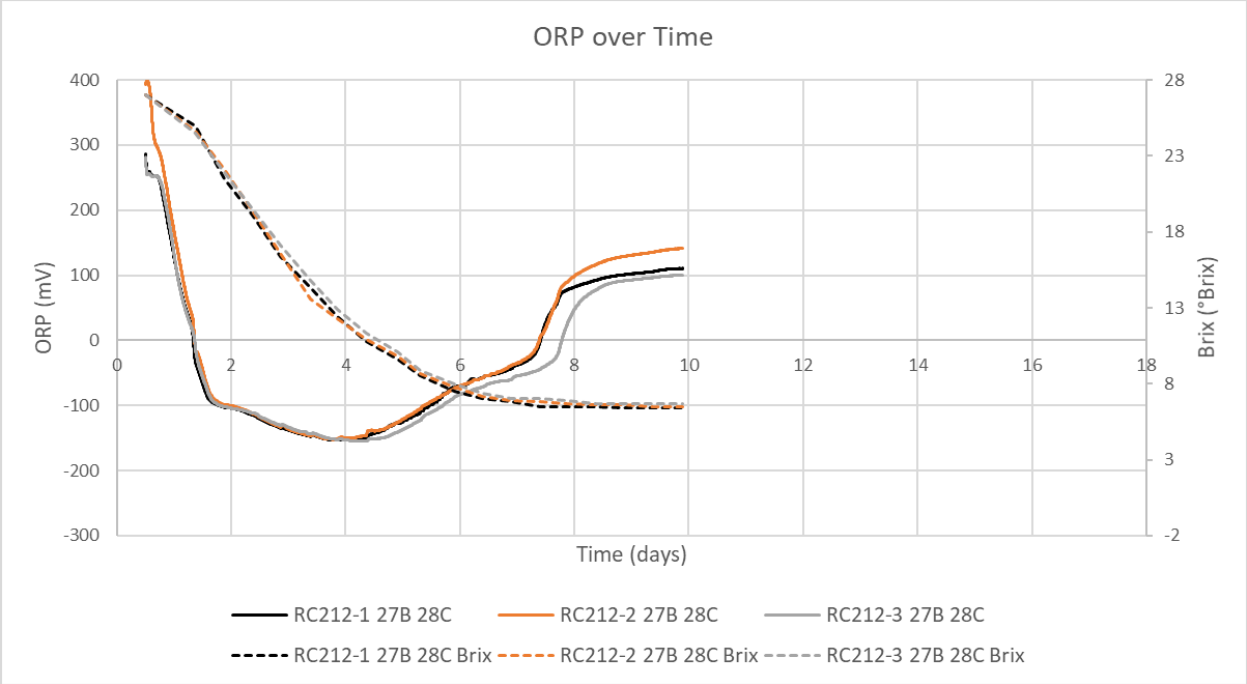

Figure 3.5.4: ORP curves for replicates 1 through 3 of the 27°B sugar conditions at 28 °C. RC212 yeast was used at a pH of 3.25 for all replicates. Bioreactor trial number for each replicate can be found in Table 3.5.1.

Figure 3.5.5 shows one representative replicate for the four conditions. These conditions were combinations of 21°B or 27°B at either 23 °C or 28 °C. As mentioned previously, the 21B-23 °C replicates are less continuous due to sun exposure which affected the measured value of the ORP cyclically. The general shape can still be compared. All sugar and temperature conditions reach about the same ORP minimum of -150 mV. In Figure 3.5.5, all replicates' ORP curves have a similar initial descent in ORP, except for the replicate at 21B and 23C. The replicate at 21B and 23C passes the 0 mV ORP value on at the start of day 2, while the other replicates reach this point on day one. All replicates reach a similar ORP minimum, though the point at which the ORP begins to increase from this point is different for each. The replicates which stuck show different trends in the ORP increase. For RC212-1 27B 28 °C, the curve follows similar trends to RC212-1 21B 23 °C, which completed, until day 4 where the replicate at 27B 28 °C begins to rise first. RC212-1 21B 23 °C does not begin this rise until about day 5. The brix curves between these replicates look very similar until almost day 6. ORP indications showed that there was a difference in the

fermentations. The other replicate that stuck, RC212-1 27B 23 °C, showed the opposite pattern against the curve that completed by remaining around its ORP minimum for longer. This replicate does not begin its ascent until day 6 and the rate is much more gradual than RC212-1 21B 23 °C. The difference in ORP between the replicates that complete and the replicates that do not, it may be possible to predict an issue with fermentation.

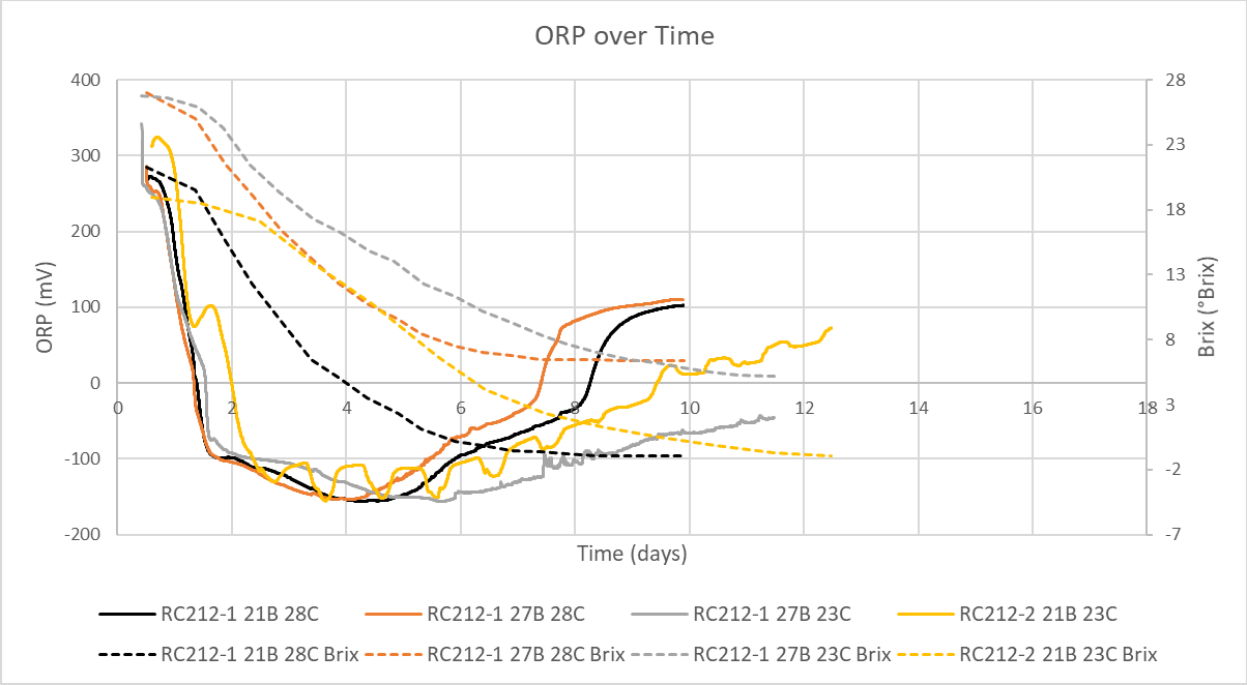

Figure 3.5.5: ORP curves for one replicate each of the 21°B or 27°B sugar conditions at both 28 °C and 23 °C. RC212 yeast was used at a pH of 3.25 for all replicates. Bioreactor trial number for each replicate can be found in Table 3.5.1.

The data resulting from the temperature and sugar content trial suggests that ORP is capable of reporting on problems. This ability is seen in Figure 3.5.5 in the difference between the 21B 28 °C and 27B 28 °C replicates, the latter of which stuck. The shape of these curves is very similar, but the timing of the notable changes in ORP is different, and the sugar content is much different along significant time points on the curve. The 27B 23 °C replicate in this figure can also be expected to have issues due to the lag in increasing from the redox minimum. The 21B 23 °C replicate has an ORP curve which shows the patterns

that a healthy fermentation generally follows, although slightly extended, which may explain the slightly longer fermentation than the other successful fermentation that is shown.

*Table 3.5.1: Cell count, percent viability, and total viable cell count for RC212 yeast at 23°C or 28°C and 21°B or 27°B. This data was not collected for bioreactor experiments 1–3. OD values are listed for when the fermentations reached or just surpassed zero brix. Experimental conditions for each bioreactor trial can be found in Table A.1 of the appendix. *Fermentation stuck*

		Day 1			Day 3			0°B			
Condition	Run in Trial #	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	OD
RC212-1 21B 23C	1	-	-	-	-	-	-	-	-	-	7.36
RC212-2 21B 23C	2	-	-	-	-	-	-	-	-	-	5.96
RC212-3 21B 23C*	3	-	-	-	-	-	-	-	-	-	5.98
RC212-4 21B 23C	4	1.03E+07	88.20	9.08E+06	1.08E+08	84.67	9.14E+07	1.09E+08	83.82	9.14E+07	9.22
RC212-5 21B 23C*	5	6.75E+06	92.17	6.22E+06	1.28E+08	92.09	1.18E+08	1.10E+08	59.13	6.50E+07	8.40
RC212-1 21B 28C	9	8.63E+07	97.90	8.45E+07	1.32E+08	99.09	1.31E+08	1.74E+08	64.09	1.12E+08	9.58
RC212-2 21B 28C	9	6.65E+07	98.51	6.55E+07	1.53E+08	99.53	1.52E+08	2.02E+08	63.57	1.28E+08	9.58
RC212-3 21B 28C	9	8.95E+07	94.83	8.49E+07	1.06E+08	99.54	1.06E+08	1.58E+08	50.40	7.96E+07	9.48
RC212-1 27B 28C*	9	1.02E+08	98.20	1.00E+08	1.27E+08	96.81	1.23E+08	1.30E+08	1.33	1.73E+06	6.38
RC212-2 27B 28C*	9	6.73E+07	95.69	6.44E+07	1.03E+08	98.62	1.02E+08	7.60E+07	1.39	1.05E+06	6.20
RC212-3 27B 28C*	9	5.85E+07	96.86	5.67E+07	1.35E+08	99.05	1.34E+08	1.10E+08	0.92	1.01E+06	6.64
RC212-1 27B 23C*	10	3.08E+07	99.53	3.07E+07	1.16E+08	99.53	1.15E+08	1.33E+08	49.82	6.63E+07	8.18
RC212-2 27B 23C*	10	4.65E+07	99.04	4.61E+07	1.33E+08	98.19	1.31E+08	1.04E+08	52.20	5.43E+07	8.46
RC212-3 27B 23C*	10	4.28E+07	99.50	4.26E+07	1.26E+08	99.02	1.25E+08	8.88E+07	57.75	5.13E+07	8.38
RC212-4 27B 23C*	10	2.98E+07	100.00	2.98E+07	1.27E+08	99.07	1.26E+08	8.93E+07	53.20	4.75E+07	8.26

3.6 Investigation of Nitrogen Limitation

Another fermentation condition commonly scrutinized by winemakers is nitrogen content (i.e. YAN). Both overly abundant or insufficient nutrients in the environment can affect yeast metabolism, which subsequently affects the ORP of the solution. Because of this relationship, limitation of nitrogen was a factor of interest to investigate.

In order to explore the effect of nitrogen limitation on the ORP curves, Elixir yeast was chosen for fermentation at a pH of 3.5 and target temperature of 23 °C (Table A.1 of the appendix). In previous experiments, Elixir was shown to be a robust fermenter, which suggested that if this strain were sluggish or failed, the others would as well. Because this strain generally had little trouble fermenting MMM, Elixir was a good initial choice to observe the effect of nitrogen limitation on the fermentation and redox profiles. The pH and temperature for this trial were chosen to be in a favorable range so that nitrogen limitation was the only clear challenge for fermentation. The control level of nitrogen for other experiments was 208 mg/L through the use of ammonium phosphate and L-arginine.

In this experiment, the levels of nitrogen were reduced to 52 mg/L, 26 mg/L, and 13 mg/L, as these quantities are much lower than what is considered the minimal amount for fermentation: 140 mg/L (Tahim and Mansfield 2019). This range was chosen to try to find the range in which Elixir could no longer complete the fermentation due to low nitrogen, and to observe the associated differences in ORP profiles from those that complete. This range was not found, however, because all three nitrogen levels performed in duplicate reached 0°B within 12 days.

The OD values for each of the replicates were similar throughout the fermentation (Table 3.6.1). The 13 mg/L condition had the lowest values at the final reading, with the first replicate of the 52 mg/L condition being the third lowest value. OD, cell count, and viable cell count for 26 mg/L and 52 mg/L of nitrogen were more similar than the values for 13 mg/L. This is in contrast to the brix data which suggests

that 26 mg/L and 13 mg/L were more similar in sugar consumption and completion time. The OD values show good correlation with the cell count values.

As nitrogen levels increased in the initial media, the total viable cell count did as well for the day 3 and final day values; day 1 cell counts did not show this trend (Table 3.6.1). This was true across replicates, although only two were performed at each nitrogen level. Because the redox curves were nearly identical across levels of nitrogen limitation, cell density is likely not a driving factor in determining the fermentation's redox minimum. All of the fermentations showed good viability throughout the entirety of the fermentation. All reactors reached at least 99% viability by day 3. The number of dead cells present in the day 1 data was likely similar to the day 3 number, but the proportion became less due to growth in population. Had the fermentation and monitoring continued, the viability would have likely gone down due to exposure to ethanol and reduced nutrients, which resulted in an increase in ORP as has been observed in previous experiments.

In this experiment, the target temperature was 23 °C in order to stay consistent with control redox runs that were performed previously. Due to a technical issue with the water bath and cooling lines, the temperature tended around 21.5 °C with one downward spike to 19 °C on one day (Table A.8 of the appendix). Neither the redox nor brix data show obvious effects from the lower temperature or spike. As 21 °C is still a reasonable temperature for fermentation, this data should still elucidate ORP trends based on Nitrogen limitation. In Figure 3.6.2, two curves from bioreactor experiment 7 were added in order to compare the nitrogen limitation at different concentrations relative to typical values. For this experiment, all parameters besides nitrogen content were constant with pH at 3.5, Elixir yeast strain, and temperature at an average of 21 °C (Table A.1 of the appendix). Bioreactor 7 and 8, the two experiments used to create this graph, also happened to be the two that had technical issues with temperature, with both around 21 °C instead of the target temperature of 23 °C (Table A.7 and A.8 of the Appendix). This similarity in temperature makes comparison between these experiments reasonable, however.

Figure 3.6.1 shows the result of the nitrogen limitation runs at the three concentrations. Figure 3.6.2 shows the same data with the 208 mg/L control level of nitrogen. It is important to note that the two replicates at 208 mg/L were recorded in bioreactor runs that were unprotected from sun exposure, whereas the other replicates were not. The general trend of the curve is not expected to have been affected by light exposure, though the line undulates from day to day. The covered reactors do not have these waves in the ORP curves. As shown by the Brix curve in Figure 3.6.2, the 208 mg/L condition completed about two days sooner than those that were nitrogen limited, and reached a lower minimum ORP value of about -200 mV. Those that were limited all had a minimum ORP value of -100 mV and showed almost identical curves throughout fermentation. The two lowest levels, 13 mg/L and 26 mg/L had similar rate of sugar consumption, and both replicates for each finished on days 12 and 11, respectively. The two replicates at 52 mg/L finished on day 10 and 11. This suggests that as nitrogen levels decrease, the time to achieve 0°B increases. In Figure 3.6.1, it is interesting to note that all levels of limitation reach -100 mV, and it was not a stepwise change of ORP based on nitrogen levels. ORP does not rise at the end of fermentation as with other experiments, however, it is expected that if the fermentation had been monitored longer, it would eventually increase. This assumption should be addressed in future work with additional replicates. Because the ORP did not rise at the end of fermentation it suggests that, in this case at least, ethanol production is not the driving factor in increasing the ORP toward the end of fermentation. This idea may be explored more in future work by tracking alcohol production throughout the fermentation and comparing that to the ORP, as well as comparing the rate in alcohol production with the rate of rise of ORP.

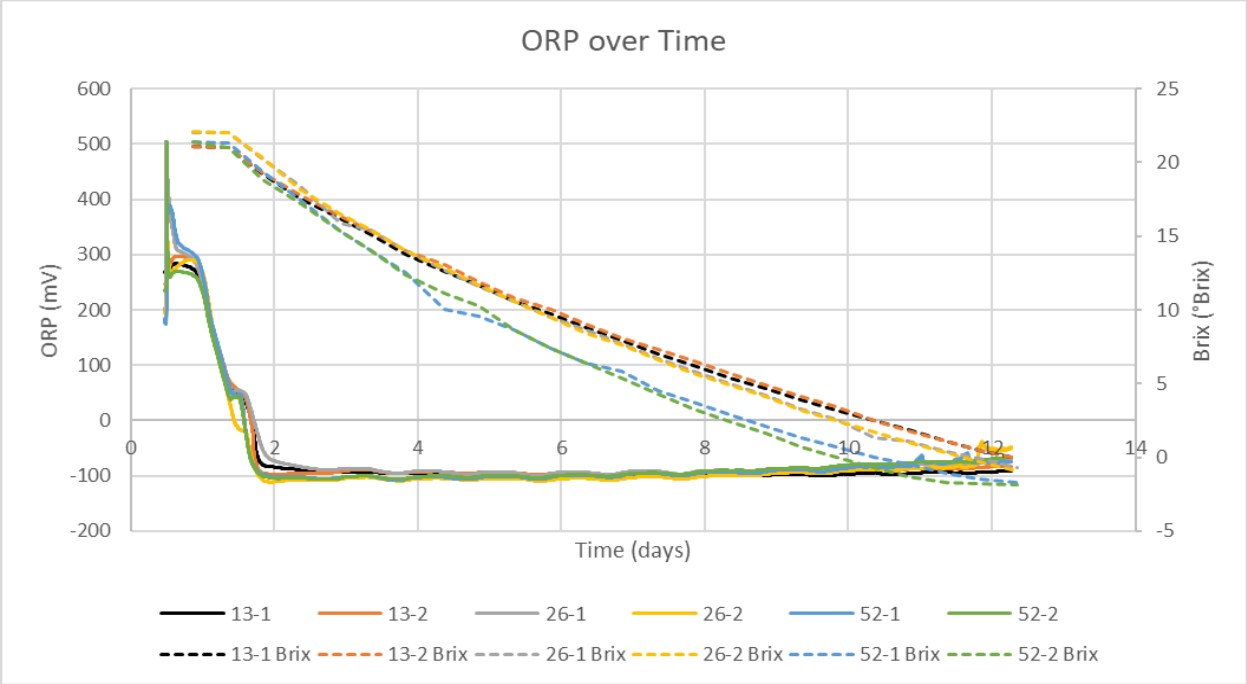

Figure 3.6.1: The impact of nitrogen limitation on ORP during fermentation. The yeast strain used was Elixir at a pH of 3.5 and temperature of 21 ◦C. Concentrations of nitrogen tested included 13, 26, and 52 mg/L (bioreactor experiment 8, Table A.1 of the appendix).

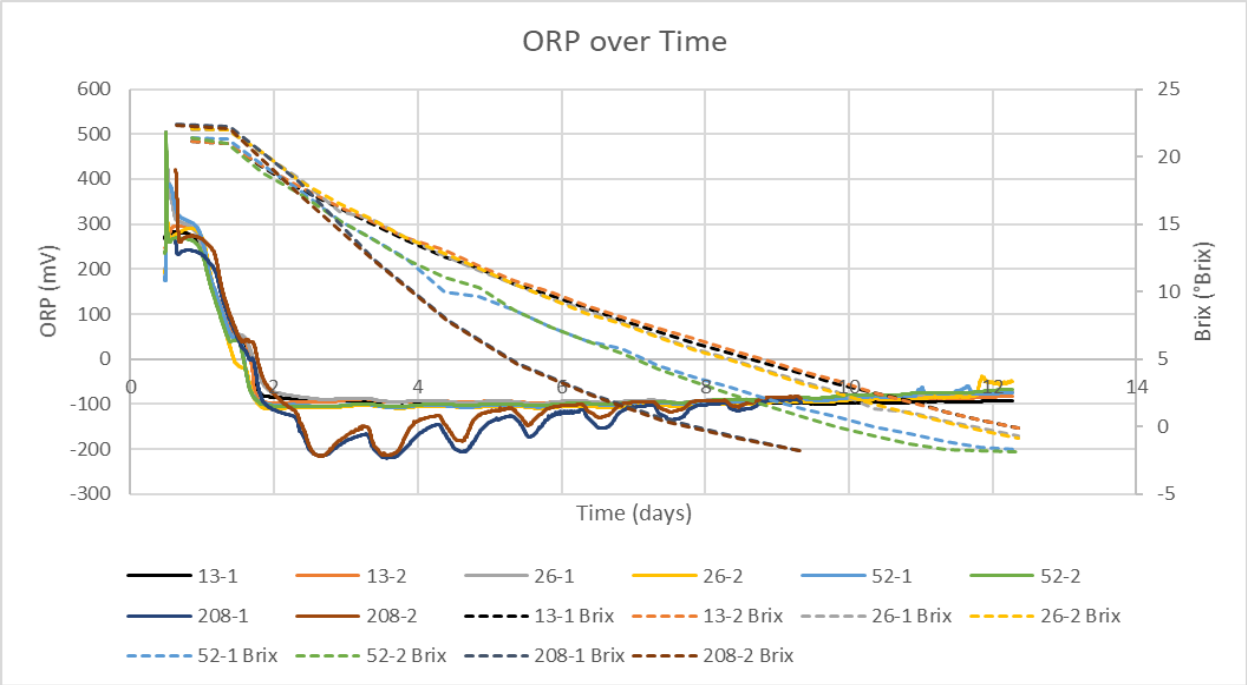

Figure 3.6.2: The impact of nitrogen limitation on ORP during fermentation with the addition of data from a fermentation with a typical level of nitrogen with the same parameters. The yeast strain used was Elixir

at a pH of 3.5 and temperature of 21 ◦C. Concentrations of nitrogen tested included 13, 26, and 52 mg/L (bioreactor experiment 8, Table A.1 of the appendix), as well as 208 mg/L (bioreactor experiment 7, Table A.1 of the appendix).

The nitrogen trial shows that nutritional deficiencies affect the fermentation environment in a way that can be reported through ORP. The ORP curve can also indicate that the fermentation is performing adequately despite differences in other monitoring metrics. The ORP for the 208 mg/L nitrogen content replicates go to a much lower redox potential (Figure 3.6.2), which may indicate that the nitrogen limited fermentations are in fact limited, though all replicates are able to complete the fermentation. The replicates at and below 26 mg/L show a difference in sugar consumption throughout the fermentation versus the replicates at 52 mg/L. If monitoring was only occurring at the brix level, this difference in brix data may cause concern for the completion of the fermentation, but the ORP data shows that the yeast cells are actually performing at the same level despite this difference. If the redox curve is showing that fermentations are identical for a fermentation which has perceived limitations versus one that is expected to complete, this might assuage fears.

Table 3.6.1: Cell count, viability, and viable cell count for nitrogen limitation trial including concentrations of 13, 26, and 52 mg/L nitrogen (bioreactor experiment 8, appendix table 2.1) as well as two control nitrogen levels of 208 mg/L (bioreactor experiment 7, appendix table 2.1). All fermentations were carried out at a pH of 3.5 and temperature of 21 ◦C. Experimental conditions for each bioreactor trial can be found in Table A.1 of the appendix.

	Day 1			Day 3			0°B			
	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	Cell count (cells/mL)	% Viability	Total Viable Cell count (cells/mL)	OD
13-1	5.33E+07	96.90	5.16E+07	9.10E+07	99.15	9.02E+07	9.60E+07	99.07	9.51E+07	8.00
13-2	2.68E+07	99.12	2.66E+07	8.70E+07	99.55	8.66E+07	8.85E+07	99.55	8.81E+07	8.14
26-1	5.13E+07	98.62	5.06E+07	1.05E+08	99.53	1.04E+08	1.16E+08	99.55	1.15E+08	13.08
26-2	4.18E+07	98.97	4.14E+07	1.26E+08	99.57	1.25E+08	1.14E+08	99.17	1.13E+08	13.24
52-1	6.73E+07	99.07	6.67E+07	1.36E+08	99.59	1.35E+08	1.26E+08	99.54	1.25E+08	10.90
52-2	5.60E+07	99.49	5.57E+07	1.23E+08	99.52	1.22E+08	1.36E+08	99.53	1.35E+08	13.22
208-1	1.13E+07	100.00	1.13E+07	1.21E+08	100.00	1.21E+08	1.82E+08	89.84	1.63E+08	15.04
208-2	1.03E+07	98.77	1.02E+07	1.42E+08	100.00	1.42E+08	1.70E+08	93.88	1.60E+08	15.84

3.7 Combined Results

The overall results of these trials show that ORP is a viable tool for monitoring fermentations due to distinctions between successful and less successful fermentations and has the potential to report on problems. The health of the fermentation is tied to yeast metabolic activity which can be correlated with ORP. ORP curves show repeatable patterns in different fermentation environments.

4 Discussion

In this work, investigation into the use of oxidation reduction potential (ORP) to monitor synthetic media fermentations involved experiments that manipulated yeast strain, pH, temperature, sugar content, and nitrogen content. Generally, it was found that ORP can show differences in various fermentation environments which lead to different outcomes. For example, differences in ORP across successful and less successful fermentations were observable, and varying patterns emerged with the different causes of fermentation failure. Based on these findings, it is expected that ORP curves contain usable information which may allow for winemakers to use this metric as an additional monitoring tool. Uses could include assisting in the early detection of problems or confirmation of predicted behavior based on other metrics like °Brix and temperature once more research has been done to establish clear, repeatable patterns in different scenarios. This research has suggested that the timing of the initial drop in redox, the depth of the drop, and when the ORP begins to rise from the minimum value are all potential points at which ORP may be able to offer information on fermentation health.

ORP has been used as a metric for monitoring different solutions in industries such as dairy, food technology, biofuel, and wastewater treatment (Walker et al. 2021). Because redox potential reports on the ratio of oxidative to reductive components in a medium, and is not specific to a special composition, it has the capability of being used in many different fields. This ability is much like pH, which is nonspecific to one environment, but is informative for understanding subjects like intracellular environments, cleaning agents, soil conditions, water quality, and more. Redox as a parameter considers pH, as well as dissolved oxygen, temperature, and chemical half reactions that are occurring. The combination of these inputs results in an ORP value which reports on the electron status of the components in solution. With this in mind, ORP is an additional value that winemakers may be able to utilize for greater understanding and control over the fermentation.

When executing a wine fermentation, there are many different variables that contribute to the outcome, and winemakers try to understand and control these variables to create the most favorable product possible out of the starting inputs. Examples of different factors include brix, YAN, phenolic compounds, pH, acid content, temperature, and more. By understanding these variables and the ranges that they are most favorable to the yeast, winemakers can guide the fermentation to a desirable outcome. To track if fermentations are proceeding as expected in the wine industry, the metrics most used to monitor progress are brix and temperature. Based on this work, it is possible that with the establishment of more data with these repeatable patterns in less successful fermentations, ORP may be able to offer information about the health of a fermentation. Moreover, this value exists in the solution regardless of whether it is monitored, which makes it a good candidate to be investigated for the advancement of wine fermentation practices. Notably, ORP is a measurable value throughout a wine fermentation, in juice, and in aging wine, which may make ORP a valuable parameter for winemakers to track across wine production.

While Brix has been, and will continue to be, an important metric to the wine industry, there are advantages to using ORP in monitoring fermentations. ORP is very sensitive to changes in the media, which causes the values to change rapidly as reactions are occurring. This means that observable differences between fast and sluggish fermentations may be more obvious due to larger discrepancies in the ORP values than would be seen in the Brix values. Figures 3.2.3, 3.2.5, and 3.2.6 show that ORP shows more obviously different values for ORP versus Brix at a given time point. Because of this, comparison of values can be done more readily over the course of hours, rather than days, as is commonly done with Brix. Additionally, the figures mentioned show that the ORP data can reveal issues in the fermentation before a change in Brix would. With each of these figures, the standard rate fermentations and sluggish fermentations tend to have similar rates in decrease of sugar content in the first few days, with decreased rates in the slower fermentation afterward. This is compared to the ORP data which shows almost a full 24 hours sooner than the brix data that there are differences in yeast performance in the media. Furthermore, ORP data shows changes in the fermentation that do not involve changes in sugar. By considering more than just one compound type, a more wholistic picture of the changes occurring in solution can be seen. ORP probes are

already commercially available, which makes this parameter easily accessible to winemakers who are interested in experimenting with using ORP to track fermentations. As more winemakers adopt this parameter as a tracking tool, more data will be available to understand the nuances between ORP data and fermentation outcome.

4.1 ORP, Synthetic Media, and Yeast Strains

Because this study was the first, to our knowledge, to monitor ORP with the use of probes in synthetic media (MMM), it was necessary to establish baseline ORP curves which would show what a typical, healthy fermentation would look like from a redox perspective. From this point, unsuccessful fermentations could be compared against these baselines to understand what redox looks like in a failure situation, and determine whether monitoring fermentations through redox could enhance current monitoring systems.

Synthetic media was chosen to be fermented because it ensured consistency between the initial contents of each fermentation, and to ensure the conditions could be repeated within the course of this study and in the future. By using synthetic media rather than fresh grape must, juice, or concentrate, this eliminated inconsistencies that might come with storage for replicate experiments, uneven distribution of contents in the solution, and repeatability in general. Grape composition can vary based on their location in the vineyard, which means that creating the exact same concentrations of nutrients, metals, sugars and more, in a fermentation would be near impossible. For the purposes of establishing initial ORP figures over the course of successful and unsuccessful fermentations, synthetic media was deemed the most appropriate choice. While synthetic media was chosen as the best way to provide the same fermentation conditions between replicates, it is important to recognize that while it is a comparable media to grape juice, there could be differences in the responses of yeast to the two media. Future experiments should include using grape-derived media to determine whether ORP patterns are the same as in synthetic media, as they are expected to be (Marinelli, 2022., Killeen et al. 2018, Kukec et al. 2002).

Yeast were chosen in this study based on known characteristics that they present or their use in the wine industry. These strains included hybrid *Saccharomyces cerevisiae* x non-saccharomyces yeast, as well

as strains used for different grape varieties like Pinot noir and Chardonnay, to ensure diversity in the yeast tested. Initial mother plates with pure cultures were obtained from the UC Davis Culture Collection. The strains used included EC1118, RC212, CY3079, Elixir, and Montrachet. EC1118, *Saccharomyces cerevisiae var. bayanus*, is a commonly used research strain, and is also used in white, red, rosé, and sparkling wine. RC212 is a *Saccharomyces cerevisiae cerevisiae* strain used for Burgundian reds, namely Pinot noir. CY3079 is a *Saccharomyces cerevisiae cerevisiae strain* known for its use in Chardonnay fermentations. Elixir is a *Saccharomyces cerevisiae* hybrid yeast from the University of Stellenbosch yeast hybridization program and can be used in white and rosé wines. Montrachet is a *Saccharomyces cerevisiae* strain which is a well-known legacy strain from the UC Davis Culture Collection. The plates obtained for these experiments all originated from pure cultures. If these experiments are repeated in an industrial or scientific setting, it is important to note the possibility that differences could exist due to mutation, and baseline curves should be established with each yeast strain used from a new source. Future experiments could consist of yeast strain sequencing to correlate potential differences in fermentation, metabolism, and redox to genetic variations.

4.2 Baseline Establishment

Baseline ORP curves for what was considered a "standard" fermentation were established at specified pH values, nitrogen (YAN) content, and temperature, using specific yeast strains (Section 3.2, Table A.1 of the appendix). These baseline ORP figures are specific to these conditions and future work should involve a greater number of replicates to ensure that healthy fermentations are accurately represented. With future experiments, it will be important to re-establish baseline curves using the yeast, and fermentation parameters of interest. From this work, it appears that there are general patterns of healthy and unhealthy fermentations, but if more specific comparison of values is desired, a new baseline would allow for the best representation of the conditions. This would be especially true if the volume is different than 1 liter, the volume used in this work, because these experiments have not been conducted in bioreactor vessels larger than this. It is not currently known how scale impacts the ORP curves, especially if the solution is more difficult to keep homogenous, or with the presence of grape solids and phenolics, both of

which are differences in this research and Walker et al. 2021. Oxygen exposure changes the ORP of a solution, which would prompt additional experiments to understand how this would change baseline ORP readings. This would be especially important prior to use in the wine industry due to the common practices of oxygen incorporation such as punch-downs or pump-overs. Other factors such as type of fermentation (e.g., carbonic maceration versus standard fermentation) or type of fermentation vessel (e.g., open top or closed top vessel; stainless steel or wood material) would require additional investigation before ORP can be implemented as a monitoring tool in those situations. Another consideration in baselines for specific, repeated fermentations could include the effect of additions throughout fermentation on the ORP of the solution.

Because the baseline curves were established prior to the discovery that diurnal shifts caused an increase and decrease in the measured ORP values of the fermentations, many of these curves see this pattern over the course of each day. Based on other replicates which were shielded from sunlight, it appears that the ORP curves exposed to sunlight still oscillate around the approximate ORP value at that time. The trough in the daily increases and decreases of a sunlight-exposed curve seems to approximate the value that would be read had the fermentation been covered. This effect can be seen in figure 3.5.5 when comparing RC212-2 at 21°B and 23 °C against the other three replicates on the graph (Section 3.5). RC212-2 at 21°B and 23 °C was the only replicate exposed to sunlight, and these troughs stop at about where all other replicates are at their minimum. Additional replicates would have to be performed in order to confirm this assumption, especially because all replicates on this graph had different fermentation conditions. When comparing ORP curves of fermentations that had been exposed to sunlight versus those that had not, caution should be applied, especially with plateauing in values on day one and two. Comparisons are still reasonable generally, however.

In terms of the shape of the ORP curves for successful fermentations, each of the strains tested have repeatable differences associated with that strain (Section 3.2 and 3.3, Figure 3.2.3). This concept is discussed further in Section 4.2. Even with subtle differences in overall ORP curve shape due to strain, successful fermentations as a category also share similarities. Successful fermentations start at an ORP

value on day zero of between 250 to 400 mV. The ORP then begins to decrease to a minimum value between -100 mV and -200 mV, reaching this value between day 2 and day 5. For successful fermentations, once this minimum value is reached, the ORP typically begins to increase within the following 24-48 hours, with CY3079 as an exception for some successful fermentations. While CY3079 is potentially the exception to this pattern, it also happens to be one of the strains that takes longer to ferment to zero brix. With this in mind, it would be interesting to see the ORP of CY3079 at pH 3.5 or 3.75 to see if the more favorable fermentation environment allows for CY3079 to follow the pattern that the other successful strains follow. This rate of ORP increase toward the end of fermentation is constant for the most part. Some strains show a gradual increase, and then a point where the ORP increases more rapidly, which will then even out, for example EC1118 and Elixir, on days 7 and 9, respectively (Figure 3.2.3).

Unsuccessful fermentations also show general trends. In some cases, such as RC212-3 (Figure 3.3.3) and RC212-1 at 27B and 28 °C (Figure 3.5.5), the fermentation begins its increase in ORP values toward the end of fermentation sooner than would a healthy fermentation, at a higher °Brix value. In Figure 3.3.3, on day 6, the ORP values for RC212-3 increase much more rapidly than any other replicate in the figure, though it is at a higher °Brix value than all others. This increase in ORP at an earlier stage in fermentation could be an indication that the yeast may not be able to complete it, and that there are differences in ORP between fermentations which are able to complete and those that are not. If, in the future, winemakers learn to interpret these ORP curves in relation to existing metrics, it could mean taking remedial approaches for problem fermentations sooner, which could increase the overall effectiveness of the treatment and potentially save time and money.

Another commonality in the ORP curves of fermentations that struggle and sometimes become stuck, is that they will reach a redox minimum point, and remain around this value for longer than would a healthy fermentation. The increase from this redox minimum is also at a much lower rate than a healthy fermentation shows. This tendency to remain near the minimum point and have a reduced rate in increase can be seen in Figure 3.3.3 with RC212-4 and RC212-5, Figure 3.3.2 with CY3079-1, and Figure 3.5.2 with all replicates. This pattern is also similar to that seen in the nitrogen limitation fermentation replicates in

Figure 3.6.2. With these replicates, the yeast are challenged with fewer nutrients, and the ORP values remain near the minimum point, with virtually no increase toward the end of fermentation. Another commonality that sometimes occurs in fermentations that are sluggish or do not complete is the initial delay in dropping to a redox minimum in the first three days. Instead of decreasing at a constant rate to the redox minimum, there will be a point where the redox plateaus and then continues to decrease. This can be seen in Figure 3.3.3 with replicates RC212-3 and RC212-4. The occurrence of plateaus can also be seen in Figure 3.3.2, however this instance could be a pattern specific to CY3079 as a strain, because four out of five replicates show this trend. The two replicates that have a plateau at the highest ORP value do happen to be replicates that are slower than others to complete fermentation. Because the appearance of these plateaus could be related to diurnal shifts in redox readings from sunlight exposure, it would be important to repeat these experiments; though, plateaus can be seen in Figure 3.6.1, which was a covered experiment. More replicates for each cause of failure would have to be completed to determine whether these general patterns are true across strains, or if specific aberrations can be found within strains or conditions which could aid in prediction.

4.3 Influence of Yeast Strain

As stated in Section 4.1, yeast strains were chosen based on known characteristics or use in the wine industry, as well as to ensure diversity in the strains tested. The strains chosen to be tested in the various experiments were EC1118, RC212, CY3079, Elixir, and Montrachet. Different yeast strains interact with their environment in various ways with genetic differences between strains causing each to differ in overall metabolic outputs, ethanol tolerance, and nutritional needs. Each strain having an optimal range of conditions that enable population growth. The ORP of the solution reports on the electron status of the compounds in the yeast's environment– compounds which the yeast are constantly interacting with in ways like use and production. Because the electron status of these compounds is important to whether certain chemical reactions will proceed, it is reasonable to assume that yeast will have a range of ORP values that facilitate growth as well, and that this range may vary with strain. To determine the patterns in ORP values

with respect to strain, all other factors remained constant in this section of the study, with the pH at 3.25, the temperature at 23 °C, and the nitrogen level (YAN) at 208 mg/L.

Population growth data through OD measurements for the strain experiments were inconclusive due to differences in replicates within the same strain. Some replicates of different strains that were executed during the same set of fermentations even showed more similarity than between the same strain over different fermentation sets. It is unknown whether this observation is significant or by chance, so more replicates would have to be repeated to determine whether there were batch effects. All trials were treated the same in terms of preparation and execution, except for changing the target variables. Errors may have stemmed from incorrect initial OD readings, resulting in too little or too much of an initial inoculation into the bioreactors. Other errors may come from inaccurate OD readings at other time points during the fermentation due to scratched or irregular cuvettes, errors in calibrating the spectrophotometer, non-homogenous samples, among other potential issues.

Cell viability tended to correlate with total cell count. Because total cell count and cell viability are only available for some of the replicates, especially in the strain trial, it is difficult to make claims about trends. More replicates for each strain would have to be completed to observe correlates. The three replicates that were deemed stuck fermentations in the strain experiment were CY3079-1, RC212-3, and RC212-5. Of these replicates, only RC212-5 had cell count and viability data taken, the other two only had optical density data. RC212-5 had the lowest viability across all strains and replicates at 59%, but the highest final OD of all RC212 replicates. RC212-3 also stuck, but had the lowest final OD of the RC212 replicates. One major difference in these values is that RC212-5 had its final OD taken on day 15, whereas RC212-3 had its final OD taken on day 12. While the time allowed to increase in population density was different, these values reflect the population density at which the fermentation halted. In future experiments, cell count and viability should be taken to compare fermentation viability in comparison to the ORP values of the fermentations. In general, the day 1 values for cell count and viability are not indicative of ORP values or day 3 values, likely because these populations are changing so rapidly. Day 3 viability tended to be higher than the final or day 1 viability, which is expected, as day 3 is in the peak of fermentation, and

often when ORP values reach their minimum. Reaching the ORP minimum likely means that the yeast were at their peak metabolic activity with the greatest impact on their environment as measured by the ORP of the solution. In general, the viable cell populations were at their highest on day 3 and slightly less than the day 3 values by the end of the fermentation, which may be due to viable but fairly unactive yeast (Cramer et al. 2002). This is exemplified in Table 3.3.1.

In Figure 3.3.1, three replicate fermentations by yeast strain EC1118 are depicted by their ORP over the course of fermentation. From day 0 to 2, the rate of sugar consumption appears to correlate with the initial rate of decrease in ORP. For replicates 2 and 3, there is a slight plateau on day 1, before each line descends to the minimum. For replicate 1, the rate in decrease to the minimum is more constant, and corresponds to a greater rate in sugar consumption. This difference in both ORP and sugar consumption is slight, however, and requires more investigation. The points at which the rate of ORP increase in replicates 1 and 3 is less than that of replicate 2, both instances occurring on day 7, are also the points that coincide with a decrease in rate of sugar consumption on the Brix curve. This could be due to the yeast being less metabolically active, and therefore affecting the ORP of the media to a lesser extent. With lessened sugar consumption, the alcohol production will be reduced, as will the production of other compounds because of metabolism, which will affect the trends seen in the figure.

Figure 3.3.2 shows the ORP values during fermentation using CY3079 yeast. This figure echoes the same pattern that the RC212 strain figure presented (Figure 3.3.1), in that the ORP of CY3079-5 decreases to its minimum ORP value at a faster rate than all other replicates, which coincides with the greatest rate of decrease in Brix. The shape of the redox curve for CY3079-5 is also the most different from the other replicates, which all have an initial plateau in ORP values, and decrease to a higher average ORP minimum than replicate 5. The difference in the ORP curve for replicate 5 versus the other replicates could be due to natural variations in the range of potential ORP values due to its metabolism. Other explanations might involve contamination by another yeast at either the YPD mother plate or liquid starter culture level. Replicate 1 was considered stuck, and the ORP of this replicate decreased to its minimum value much like the others, but remained around this minimum point for longer without showing an upward trend. It also

began to increase in ORP while the brix was still at greater than 5. This is most obviously in contrast with replicates 4 and 5, where once the ORP values of these fermentations reach a minimum, the ORP soon begins to constantly trend upward, even if at a slow rate.

Figure 3.3.3 depicts the ORP curves of the RC212 yeast strain replicate fermentations. Replicates 1 and 2 show the greatest similarities both in the shape of the ORP curves as well as the brix curves. RC212-5 shows some similarity to RC212-1 and RC212-2 in terms of ORP values from day zero until day 8. On day 8 the rate in ORP increase is more like that of replicate 4, a sluggish fermentation, and the rate in brix decrease slows as well. This decrease in the rate of ORP increase compared to healthy fermentations seems to be an indicator of a struggling fermentation. While this pattern is not true for replicate 3, which was also a stuck fermentation in addition to replicate 5, the ORP begins to increase more rapidly than those that complete on day 6. This increase in ORP sooner than expected may also be an indication that the fermentation will struggle, and coincides with a decrease in sugar consumption. This behavior may be explained by a reduction in yeast metabolism, which would mean less compound contribution and alteration of the environment, causing the changes that are seen on the graph. Replicates 3, 4, and 5 may have reduced yeast activity in comparison to healthy replicates 1 and 2, where 4 and 5 are still able to maintain the redox minimum, while replicate 3 is not. It could also be the case that the healthy fermentations begin their increase in ORP minimum toward the end of fermentation when the alcohol inhibition reaches a certain level. Replicates 4 and 5 may not have reached this percent alcohol which allowed them to continue contributing to the redox potential, though at a reduced rate of sugar consumption.

Figure 3.3.4 shows the ORP over time for the 3 replicate fermentations using the Elixir yeast strain. Each of these replicates completed and were faster than most other fermentations across all strains. Elixir-1 has almost identical ORP values to Elixir-2 up until just before day 3 where the rate in decrease of ORP became less than that of replicate 2. This difference seems to be portrayed in the brix curve, because the rates in sugar consumption up until just before day 3 is similar between the two replicates, at which point the rate becomes slightly faster in replicate 2. Replicate 3 decreased to the ORP minimum the fastest and most continuously out of the three replicates, which correlates with the greatest rate of sugar consumption

as well. This correlation was seen in other strains. On day 2, the ORP begins to rapidly increase for Elixir-3, which was the point where the sampling port had been left open overnight. While the ORP seemed to return to the same value it was at prior to this error, and then closely matched the ORP of replicate 2, it is unknown whether the ORP of the curve was affected over the entire course of the fermentation due to the oxygen exposure early in fermentation. While this error means there should be caution in comparing it with the other replicates, it also shows that changes in the fermentation environment can be reported through ORP, in this case, unwanted oxygen exposure. It is known that the introduction of oxygen will increase the ORP of the solution (Killeen et al. 2018).

The final yeast strain tested in this study was Montrachet, and Figure 3.3.5 depicts the ORP over time for three replicate fermentations. These fermentations showed great reproducibility in both the brix values and ORP. The replicates were run in the same set of fermentations, which could mean that there were batch effects due to either the initial YPD mother plate, or the synthetic media created for the set of fermentations. More replicates would have to be run to confirm these results, however all other strains showed good reproducibility regardless of fermentation set. All three Montrachet replicates were essentially identical in terms of brix decrease over the course of fermentation. Replicate 3 differs form the other two fermentations in terms of ORP starting around day 6, where the ORP values trend slightly higher than the other two replicates. Montrachet-3 also has the largest OD and viable cell population by the end of fermentation, though the percent viability is lower than the other two replicates. This difference may explain the difference in the curve, though it is ultimately unknown, and not reported in the brix curve. All fermentations performed identically in terms of time to completion despite this difference in ORP.

In figure 3.2.3, it is shown that when a successful fermentation by each strain is compared against each other, the general shape of the ORP curves are slightly different, while trends exist more universally across them. Between strains, it can be seen that the minimum redox value the strain is able to reach in the fermentation is not necessarily an indication of performance. For example, Montrachet reaches the lowest redox value, while Elixir has one of the highest minimum values. Elixir is one of the faster fermenters in

this study, as well as EC1118 which has the second lowest redox minimum. This minimum value may say more in the context of the replicate fermentations, or other variables such as pH.

Overall, the ability to track differences in the fermentation environment based on ORP across strain and successful vs. unsuccessful fermentations suggests that ORP could be a viable monitoring tool. Moreover, the variable ORP curves across yeast replicate fermentations, that were otherwise indistinguishable based on Brix, may further indicate that ORP is very sensitive to small changes in metabolism and fermentation health. It is expected that with further research, the range of ORP values at different times during fermentation, and what changes in ORP mean at various points, will become more defined, making the tracking and prediction of fermentation outcomes more feasible in the future. This research also shows that the general patterns in ORP over the course of a fermentation are similar between strains, which may allow for comparison.

4.4 Influence of pH

In the pH section of this study, the yeast chosen were RC212 and Elixir because RC212 was often unreliable at completing fermentation at the initial pH of 3.25, and Elixir was one of the stronger fermenters at pH 3.25. The goal was to see if the yeast would perform similarly to the fermentations at pH 3.25 and how changes in pH would impact ORP.

In terms of OD, RC212 showed no notable difference between pH 3.5 and pH 3.75, though the values for pH 3.25 tended to be lower than these. This is likely due to the fact that pH 3.25 is a challenging environment for this yeast to build up biomass, and is more successful in doing this at higher pH values. RC212-4 at pH 3.25 was the only replicate at this pH value to have the cell count and viability taken in addition to the OD. This replicate was more similar in OD to the replicates at the higher pH values, and the cell count and viability by the end of fermentation was comparable as well. The only major difference in yeast population monitoring with replicate 4 at pH 3.25 was that the viability on day 3 was relatively low at 84%, while all other replicates had a viability of at least 96%. This may be a result of the lower pH, however it would be interesting to perform more replicates at pH 3.25 to determine whether the viability

challenge on day 3 is specific to this fermentation, and to understand why viability was lower in the other replicates at pH 3.25.

Elixir showed similar trends in terms of OD across pH values, where the difference was negligible between 3.5 and 3.75, but 3.25 seemed to have lower values. The pH trials for Elixir also had just one replicate at pH 3.25 where cell count and viability were taken in addition to OD. This means that comparison with this replicate is not definitive and will require more replicates at this pH value with the additional testing measures. Across all replicates within each time point in fermentation these data were recorded, there was no observable difference in total cell count or viable cell count across the pH values tested. Similar to RC212-4 at pH 3.25, Elixir-3 at pH 3.25 had a lower percent viability on day 3 than the replicates at higher pH values, though it is still considered a good percent viability at 96%. This difference is not reflected in the total viable cell count, however. Again, additional replicates across all values and especially pH 3.25 would be necessary to confirm suspected trends for both Elixir and RC212 yeast population data.

Across the ORP data for both RC212 and Elixir, ORP values were lower during fermentation for fermentations at higher pH values. Fermentations at higher pH values also tended to complete faster. The difference in values between 3.25 and 3.5 were greater than the difference in values between 3.5 and 3.75 for both ORP and time to completion. Fermentations at pH 3.25 tended to have ORP curves that were less continuous and more irregular than ORP curves at other pH values. This may show that the yeast are not as easily able to ferment at the lower pH. This idea is exemplified in Figures 3.4.1 and 3.4.2. Because pH is one of the factors considered in reporting ORP, it was expected that there would be a difference in ORP across the different pH values. The figures in this section also show patterns that reflect the general trends found over the course of the study. For example, many of the replicates have ORP curves that initially decrease rapidly to the ORP minimum, and those with the lowest ORP minimum, are also the fermentations with the greatest rate of sugar consumption at that time point. In Figure 3.4.1, it is also shown that RC212 has a much lower potential redox minimum than was shown with fermentations at pH 3.25. It is unclear whether the fermentation speed was increased due to this ability to reach a lower redox potential, or if the lower redox potential was a product of the faster fermentation speed. The fermentation medium at this pH

may also be less able to buffer reactions occurring in solution, leading to greater changes in ORP. Nonetheless, these data again indicate that ORP reflects changes in the fermentation environment that are correlated with overall fermentation completion times.

4.5 Influence of Sugar Content and Temperature

Brix and temperature are currently the most utilized process parameters when it comes to monitoring wine fermentations. These factors are very important to winemakers as they are some of the only tools available to give insight into the health of the fermentation due to their ease of measurement. Because of the importance of these factors in the wine industry, the investigation of how sugar affects ORP and how temperature affects ORP was of interest. This experiment was designed as a 2x2 factorial to test two sugar levels at two different temperatures to understand not only how sugar and temperature affect ORP individually, but also the combined effects of these factors.

In this study it was expected that as initial sugar concentration increased, the fermentation environment would become more challenging for the yeast, and as temperature increased, fermentation would be encouraged. With increased sugar concentrations, the initial osmotic pressure on the yeast cells is greater, which is known to prolong the lag period in cell growth as the population adjusts its metabolism (Nishino et al. 1985, Boutlon et al. 1996). There is also a greater potential alcohol production that comes with higher sugar content, which means that more of the population may be inhibited by alcohol as the fermentation proceeds. With sugar still present at these higher alcohol values, there is the possibility that the yeast will not be viable enough to ferment to zero glucose/fructose concentration. As for the temperature, a reasonable increase in temperature will allow for faster fermentation kinetics. Both 23 °C and 28°C are considered reasonable fermentation temperatures for these wine yeast, which led to the expectation that fermentation would proceed faster at the higher temperature. When fermentation temperature and sugar content are considered together, it was anticipated that the greater temperature might offset the initial increase in the lag phase caused by the greater sugar content, however the effects of alcohol toward the end of fermentation may be exaggerated at a higher temperature. At higher temperatures, the

fluidity of the membrane is affected which can cause increased sensitivity to alcohol concentration (Boulton et al.1996).

The yeast population monitoring data seems to be fairly similar across all replicates in the different conditions aside from the replicates at 27°B and 28 °C (Table 3.5.1). The replicates in this condition showed almost no viability by the end of the fermentation, which may be a result of the higher temperature increasing the effects of alcohol on the cells (Coleman et al. 2007). The final OD for these replicates was also lower, which may have been a result of the population being subjected to osmotic stress. Comparable OD values can be seen in replicates 2 and 3 of the 21°B and 23 °C condition, though viability or population data was not taken. The stress of the pH at a lower temperature may have led to similar difficulties in building up population density.

In this study, RC212 yeast was chosen because both success and failure fermentations had been observed in previous experiments, which allowed for more comparison against either outcome. Because RC212 was found to be a poor fermenter, it may have been interesting to include Elixir or EC1118 in this study to see the effects of these variables on ORP in a stronger fermenter. All fermentations at 27 °B were unable to complete fermentation of glucose/fructose. In the brix curve, it seems that the anticipated initial delay in sugar consumption was not observed, though the rate of sugar consumption in the replicate at 27°B and 23 °C had a reduced rate of sugar consumption across the entire fermentation. Figure 3.5.5 shows that the higher temperature did seem to increase the rate of fermentation, however the higher temperature paired with higher sugar (27°B and 28 °C) matched the rate of the replicate at the lower temperature and lower sugar (21°B and 23 °C) from day 3 to 5. The challenge of the higher sugar content seemed to be offset by the advantage of the higher temperature, at least while the alcohol concentration in solution was not inhibitory. Day 5, the point at which these brix curves diverge, is also the point where the ORP curves become more dissimilar. The ORP of the replicate at 27°B and 28 °C begins to increase at a greater rate than the replicate at 21°B and 23 °C. This premature rise may be an indication of a failure fermentation, as discussed above.

Factors affecting this portion of the study may include the set of fermentations that each replicate was run in (Table A.1 of the appendix), the difference in exposure to sunlight, the motor shut-off event in Figure 3.5.3 with replicate RC212-3, and the assumed probe interferences present in the ORP curves of the 27°B and 23 °C replicates starting around day 6 (Figure 3.5.2). As is the case with replicates run in the same set of fermentations in different sections of this study, it is important to note that the initial cultures of yeast come from the same YPD mother plate, and if irregularities exist in this plate, there is potential for each of the replicates to not be representative of the strain. Because there was good reproducibility in ORP values over fermentation in early trials of this study, it was deemed reasonable to perform the replicates in the same set of fermentations. Additional replicates of these conditions should be performed to confirm conclusions made. As discussed in previous sections, the influence of diurnal shifts on the ORP readings of the fermentations is apparent. Because these trials were conducted in fermentation sets both before and after this discovery was made, it is important to note that the total effect is not known, but generally comparing the curves appears to be reasonable. In Figure 3.5.2, there appears to be a rapid increase in ORP on day 8, followed by the return of the ORP values to the expected trend line. This was found to be a motor shut off event. While the effects on the fermentation and ORP curve after this event are unknown, it again shows that ORP is able to report on changes in the fermentation environment. This is much like the oxygen exposure event depicted in 3.3.4, where this event was not intended, but has a visible effect on the ORP of the solution, in this case due to a less homogenous fermentation. This event also suggests that the redox of the fermentation will be variable if there is not constant mixing, and the existence of a redox gradient, which could be another point of study in the future, as constant mixing is not standard in the wine industry. The last factor to be considered is the appearance of small, continuous spiking in the ORP curves of the 27°B and 23 °C replicates starting around day 6 (Figure 3.5.2). This is assumed to be interference in the electronics, potentially through the wiring of the cables at a point which affected each of them in series. This could also be tartrate or microbial build up on the probes which led to interferences in reading the media. Despite the small spikes in the curve, the average value seems to report the general trend of the ORP in solution.

The results of the experiment involving sugar content and temperature show that ORP can report on the changes in fermentation. ORP changes at notable points in the fermentation can indicate differences in the fermentation environments and that one may begin to decline in health. Early indication of problems can alert winemakers to potential issues which may allow for remediation sooner, which may save resources, time, and improve treatment effectiveness, once clear, repeatable patterns in problem fermentations have been established.

4.6 Influence of Nitrogen Content

Another component of fermentation that is important to the outcome of fermentation, much like pH, sugar concentration, and temperature, is nitrogen content (YAN). Both over- and under-abundance of nitrogen can cause problems for the fermentation. When the YAN is low in a fermentation, yeast do not have the necessary nutrients to build a strong population and have reduced fermenting abilities. It can also lead to the production of undesirable compounds because of stress. Excessive amounts of YAN can lead to overly vigorous fermentations that reach temperatures that begin to affect compounds in the solution, as well as increased risk of microbes other than *Saccharomyces cerevisiae* utilizing the nitrogen and producing faults in the wine. The amount and type of YAN at different points in fermentation are important to monitor to ensure that optimal conditions are met (Bell and Henschke 2002).

In this experimental condition, Elixir was chosen as the test yeast strain because of previous runs which showed that it was a strong fermenter. It is reasonable to assume that as a strong fermenter, if the fermentation struggles at a certain level of nitrogen limitation, other, weaker fermenting strains will struggle as well. This experiment may have benefitted from observing the effects of nitrogen on RC212 yeast, which has been used as an example of a weaker strain in other experimental conditions. This is especially true because the level of nitrogen limitation which causes the Elixir yeast to fail was not found but may have been found with a weaker strain. A pH value of 3.5 was chosen for these fermentations to ensure that Nitrogen limitation was the challenging aspect of the environment. This fermentation, as well as the typical nitrogen level replicates used to compare against the lower levels of nitrogen, were in the two sets of fermentations that experienced a technical issue where the temperature was 21 °C instead of the target

temperature of 23 °C. From the results of the temperature experiment, the effect of this temperature shift likely slowed the time to completion. The nitrogen limitation set of fermentations was notable because the ORP minimum values were all the same at -100 mV, instead of the expected result that there would be stepwise differences. Temperature did not appear to affect the ORP minimum in the temperature set of experiments, and the ORP minimum of the replicates at 208 mg/L and 21 °C were not affected, so the cause is likely the quantity of nitrogen in solution.

In terms of yeast population monitoring, the differences in the four nitrogen concentration levels (13, 26, 52, 208 mg/L) were slight but still visible (Table 3.6.1). Viability across all concentration and time periods these data were taken were essentially the same, aside from the final percent viability for the fermentations at 208 mg/L. At this concentration, the percent viability was lower for unexplained reasons. While this is true, the total viable cell count and OD for these two fermentations were the highest of all replicates. OD was lowest in the 13 mg/L fermentations and comparable for the 26 and 52 mg/L fermentations. Viable cell count followed this trend, though the 52 mg/L fermentations were slightly higher than the 26 mg/L fermentations in this case. These trends may be due to the yeast not having adequate nitrogen to grow the population, and with lower concentrations of nitrogen, lower cell density results. Regardless of this trend, based on the ORP of the solution, the metabolic activity of these fermentation are essentially identical, aside from the fermentations at 208 mg/L which show a more typical ORP curve (Figure 3.6.2).

As stated previously, the results of this experiment in terms of ORP were unexpected due to the ORP minimum's being equivalent across all lower concentrations of nitrogen (Figures 3.6.1 and 3.6.2). This could be due to the absence of compounds with low ORP that require nitrogen as a building block. If these compounds drive the redox potential lower, and are not present at 52 mg/L, then the lower concentrations of nitrogen will also be limited at the same point. Another possible take away from this experiment is that ORP has been shown to potentially report on problems in the fermentation, but in this set, it could show that the fermentation is likely to succeed. The brix curves between the 52 mg/L nitrogen concentration and the 13 and 26 mg/L concentrations are visibly different, with the 13 and 26 mg/L

concentrations decreasing at a slower rate. By the ORP curve, three concentration level replicates have identical ORP curves, and do not diverge at the end of fermentation which could be an indication that the fermentations will be able to complete.

This experiment again shows that ORP can report on nuanced differences in the fermentation and aid in understanding the overall health of the fermentation.

5 Future Direction and Concluding Thoughts

The goal of this research was to explore the use of Oxidation Reduction Potential (ORP) in monitoring fermentations with different initial conditions in terms of yeast strain, pH, temperature, sugar content, and nitrogen content. It was found that ORP was able to report on changes in the fermentation environment especially between different fermentation outcomes. With more data and pattern establishment, ORP in wine fermentations may have the ability to predict when a fermentation may be struggling or when perceived limitations (e.g., nitrogen) are not truly limiting.

Other avenues for research related to the use of ORP in the wine industry arose over the course of this research. Within the variables tested, the experiments involving pH used Elixir and RC212. These were chosen because they were thought to be on opposite ends of the performance scale (i.e., a relatively slow fermenting yeast and a relatively fast fermenting yeast); other strains could be tested in the future, as different strains may have different tolerances for changes in pH. More trials with different strains, and more subdivided pH values between intervals may reveal more about the effect of both strain and pH on ORP. In future experiments, the change in pH over the course of fermentation may be interesting to compare to the overall ORP of the solution. Integrating the pH experiments with the sugar content and temperature experiments may also be a future direction. Investigating the magnitude of the effects the variables have individually and combined on ORP may elucidate more about how the yeast respond metabolically to their environment under different conditions, including multiple stressor conditions. Other questions include: Are changes in ORP at different pH values due to the yeast's ability to buffer change in the environment? Do different environmental challenges change the ability of the yeast to buffer ORP? It is known that different nutrients have more/less availability due to the pH of the solution, and because this is directly related to ORP, there may be a range of availability in ORP values that compliments this. In addition to expanding the nitrogen trials in terms of strain used, ranges of nitrogen from zero to excess levels, and adjusting the other parameters which were constant, other nutrients in solution should be tested for their ability to change the ORP. Limiting different nutrients, altering metal ratios, and other experiments in adjusting the contents of the medium may show unique patterns in ORP that might one day allow

winemakers to see an issue in the fermentation and understand why the ORP is responding in a certain way. Looking at the factors addressed in this study at narrower intervals, such as smaller ranges in levels tested in pH, temperature, sugar, or nitrogen concentration could also be interesting to see how much change is necessary in each of these variables to cause observable differences in ORP.

Other factors in fermentation that were not addressed in this study may also contribute to changes in ORP. Other areas of research surrounding ORP such as using a grape-derived media (such as grape must, juice, or concentrate), altering the nutrient concentration to be in excess or limited, and exploring the use of *non-Saccharomyces* yeasts and bacteria could give additional information on how ORP changes with different variables. In this study, the synthetic media used most closely mimics a white grape juice fermentation, due to the lack of phenolic compounds. Phenolic compounds and their interaction with other compounds in the juice and wine environment are very important, and understanding their role in the ORP of the solution could also be important work before implementation in the industry. Other important topics to research may be making the fermentations more similar to those which occur in the wine industry, potentially by observing the effects on ORP from using a spontaneous fermentation versus a non-spontaneous fermentation, using mixed-species fermentations, or observing the effects of malolactic conversion. Other more process-driven changes could also be important to explore, such as exposing the fermentations to air periodically or constantly, as would happen with punch-downs or an open top fermentor, respectively. Another process change could be limiting the mixing to once or twice a day, as many fermentations in the industry are subjected to, in order to observe both the overall, and gradient effects of leaving the fermentation less homogeneous for periods of time.

Because the ORP curves of both successful and unsuccessful fermentations show repeatable patterns, this may allow for automation through machine learning in the future. If redox ranges can be defined to acceptable or problem ranges over different points in fermentation, this could allow for software to be built that will automate the prediction process, reducing the skill level required of the operator in understanding the redox values.

In Conclusion, redox data is a viable process parameter that has the potential to predict problem fermentations. With this current research, it was shown that patterns in fermentations which complete are similar, and patterns in those that struggle are similar, even across different strains. More work should be done to establish clear relationships in the various factors that influence fermentation outcome and ORP at different time points during the fermentation.

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7 Appendix

Table A.1: Summary table of all 10 bioreactor experiments and variables. The pH, nitrogen content (N), sugar content, temperature, and yeast strain for each vessel is listed, as well as final brix and OD at various points during fermentation.

Bioreactor Experiment	Vessel	1	2	3	4	5	6
1	Yeast Strain	CY3079	EC1118	RC212			
	pH	3.25	3.25	3.25			
	Temp (°C)	23	23	23			
	N (mg/L)	208	208	208			
	Initial °Brix	22.5	21.7	19.9			
	Final °Brix	3	-2.1	-0.8			
	Day 1 OD						
	Day 3 OD						
	OD near 0°B	7.2	9.84	7.36			
2	Yeast Strain	CY3079	EC1118	RC212	Elixir		
	pH	3.25	3.25	3.25	3.25		
	Temp (°C)	23	23	23	23		
	N (mg/L)	208	208	208	208		
	Initial °Brix	19.6	19.1	19	19		
	Final °Brix	0	-1.3	-0.9	-1.1		
	Day 1 OD	0.278	0.398	0.492	0.363		
	Day 3 OD	2.46	5.28	3.7	3.56		
	OD near 0°B	5.08	7.96	5.96	6.56		
3	Yeast Strain	CY3079	EC1118	RC212	Elixir		
	pH	3.25	3.25	3.25	3.25		
	Temp (°C)	23	23	23	23		
	N (mg/L)	208	208	208	208		
	Initial °Brix	21.4	21.4	22.2	22.2		
	Final °Brix	-1	-1.9	8.9	-1.8		
	Day 1 OD	0.34	0.615	0.255	0.446		
	Day 3 OD	3.42	5.06	3.38	4.2		
	OD near 0°B	8.4	9.54	5.98	9.36		

4	Yeast Strain	CY3079	RC212	Elixir	Montrachet-1	Montrachet-2	Montrachet-3
	pH	3.25	3.25	3.25	3.25	3.25	3.25
	Temp (°C)	23	23	23	23	23	23
	N (mg/L)	208	208	208	208	208	208
	Initial °Brix	21.9	21.8	22.1	21.7	21.8	21.9
	Final °Brix	-1.7	0	-1.7	-2	-2	-2
	Day 1 OD	0.448	0.41	0.403	0.342	0.346	0.286
	Day 3 OD	4.24	6.02	7.28	4.84	5.28	4.52
	OD near 0°B	8	9.22	11.48	8.44	9.84	8.18
5	Yeast Strain	Montrachet-1	Montrachet-2	Montrachet-3	CY3079	RC212	Elixir
	pH	3.25	3.25	3.25	3.25	3.25	3.25
	Temp (°C)	23	23	23	23	23	23
	N (mg/L)	208	208	208	208	208	208
	Initial °Brix	22.1	22.3	22.3	22.2	22.3	22.2
	Final °Brix	-2.4	-2.4	-2.4	-2.2	2.4	-2.1
	Day 1 OD	0.505	0.52	0.498	0.431	0.621	0.545
	Day 3 OD	7.84	5.9	6.66	7.74	6.76	9.54
	OD near 0°B	9.16	8.58	10.24	11.44	8.4	12.72
6	Yeast Strain	RC212-1	RC212-2	Elixir-1	RC212-1	RC212-2	Elixir-2
	pH	3.5	3.5	3.5	3.75	3.75	3.75
	Temp (°C)	23	23	23	23	23	23
	N (mg/L)	208	208	208	208	208	208
	Initial °Brix	20.3	20.5	20.4	20.8	20.7	20.8
	Final °Brix	-1.4	-1.3	-1.9	-0.6	-0.9	-2
	Day 1 OD	0.68	0.734	1.135	0.099	0.098	1.088
	Day 3 OD	12.42	13	13.06	10.76	9.66	12.98
	OD near 0°B	11.9	12.46	14.42	9.24	10.76	13.54
7	Yeast Strain	RC212-1	Elixir-1	Elixir-2	RC212-2	Elixir-1	Elixir-2
	pH	3.5	3.5	3.5	3.75	3.75	3.75
	Temp (°C)	21	21	21	21	21	21
	N (mg/L)	208	208	208	208	208	208
	Initial °Brix	22.2	22.4	22.3	23	23.1	23.2
	Final °Brix	-0.2	-1.8	-1.8	-0.2	-1.4	-1.1
	Day 1 OD	0.241	0.409	0.381	0.212	0.282	0.304
	Day 3 OD	10.56	14.66	13.36	11.26	12.08	11.54
	OD near 0°B	12.94	15.04	15.84	12.32	14.9	14.74
8	Yeast Strain	Elixir (13-1)	Elixir (13-2)	Elixir (26-1)	Elixir (26-2)	Elixir (52-1)	Elixir (52-2)
	pH	3.5	3.5	3.5	3.5	3.5	3.5
	Temp (°C)	21.5	21.5	21.5	21.5	21.5	21.5
	N (mg/L)	13	13	26	26	52	52
	Initial °Brix	21.1	21.1	22	22.1	21.4	21.4
	Final °Brix	-0.1	-0.1	-0.7	-0.9	-1.7	-1.9
	Day 1 OD	0.664	0.653	0.633	0.593	0.592	0.643
	Day 3 OD	6.7	7.36	7.22	7.54	7.46	8
	OD near 0°B	8	8.14	13.08	13.24	10.9	13.22

9	Yeast Strain	RC212-1	RC212-2	RC212-3	RC212-1	RC212-2	RC212-3
	pH	3.25	3.25	3.25	3.25	3.25	3.25
	Temp (°C)	28	28	28	28	28	28
	N (mg/L)	208	208	208	208	208	208
	Initial °Brix	21.3	21.3	21.3	27	27	27
	Final °Brix	-0.9	-1.2	-0.9	6.4	6.5	6.7
	Day 1 OD	0.225	0.262	0.218	0.226	0.206	0.214
	Day 3 OD	8.44	8.76	9.2	7.56	8.02	7.4
	OD near 0°B	9.58	9.58	9.48	6.38	6.2	6.64
10	Yeast Strain	RC212-1	RC212-2	RC212-3	RC212-4		
	pH	3.25	3.25	3.25	3.25		
	Temp (°C)	23	23	23	23		
	N (mg/L)	208	208	208	208		
	Initial °Brix	26.8	26.8	26.9	26.9		
	Final °Brix	5.2	5	5.4	5.2		
	Day 1 OD	3.14	3.26	3.04	3.12		
	Day 3 OD	9.56	9.64	9.12	9.18		
	OD at 0°B	8.18	8.46	8.38	8.26		

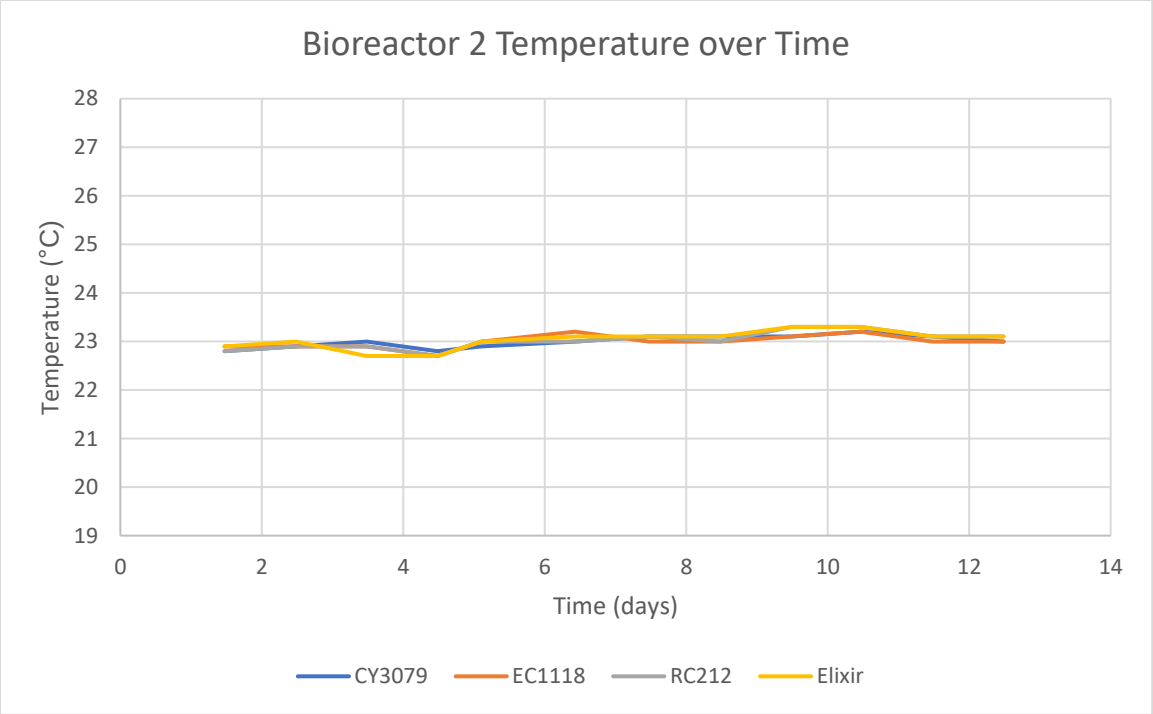

Figure A.2: Temperature (°C) over time for bioreactor experiment 2. See Table A.1 for experiment parameters.

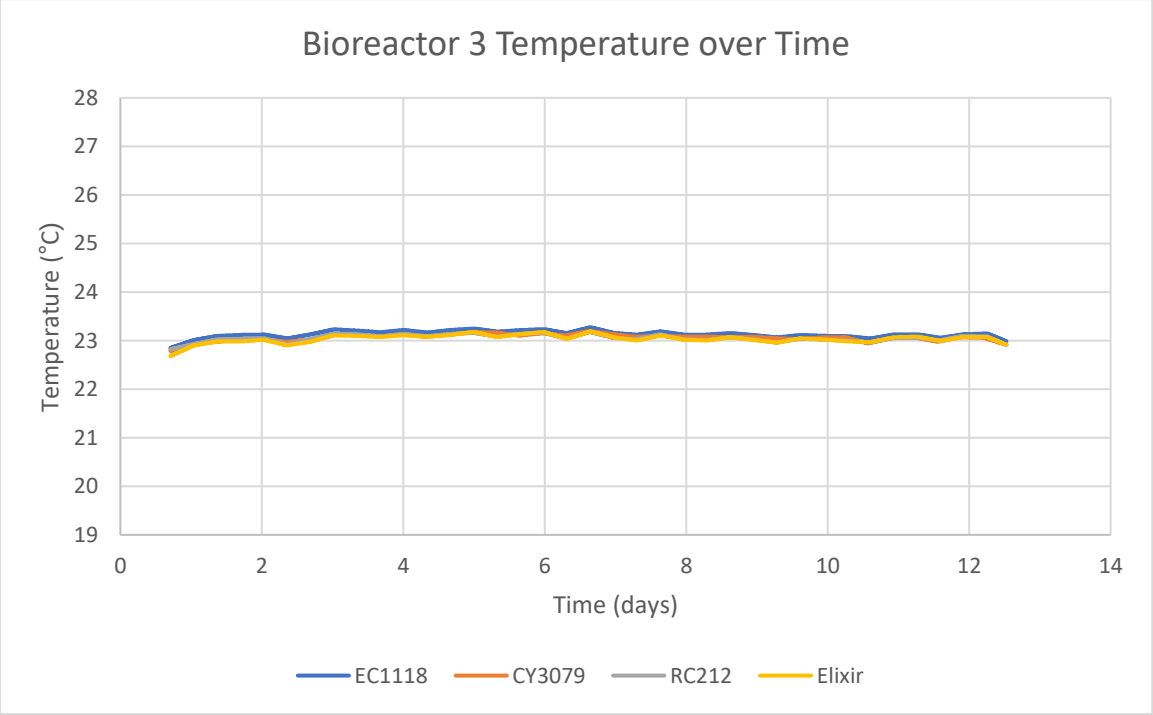

Figure A.3: Temperature (°C) over time for bioreactor experiment 3. See Table A.1 for experiment parameters.

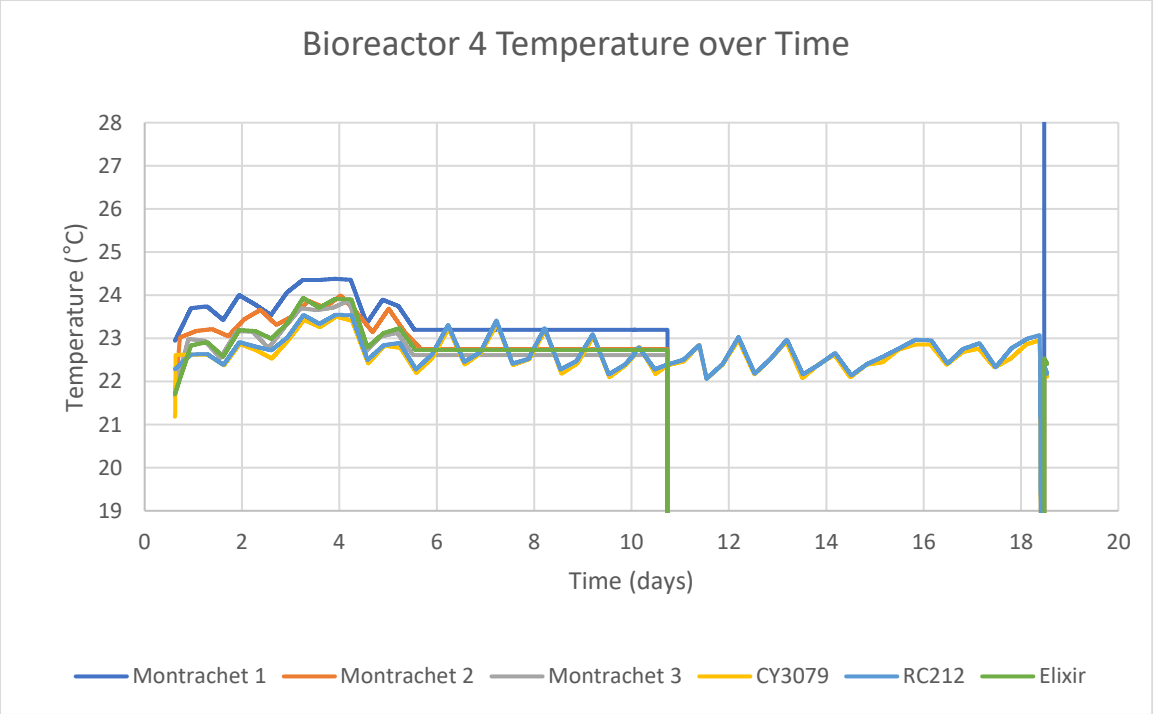

Figure A.4: Temperature (°C) over time for bioreactor experiment 4. See Table A.1 for experiment parameters.

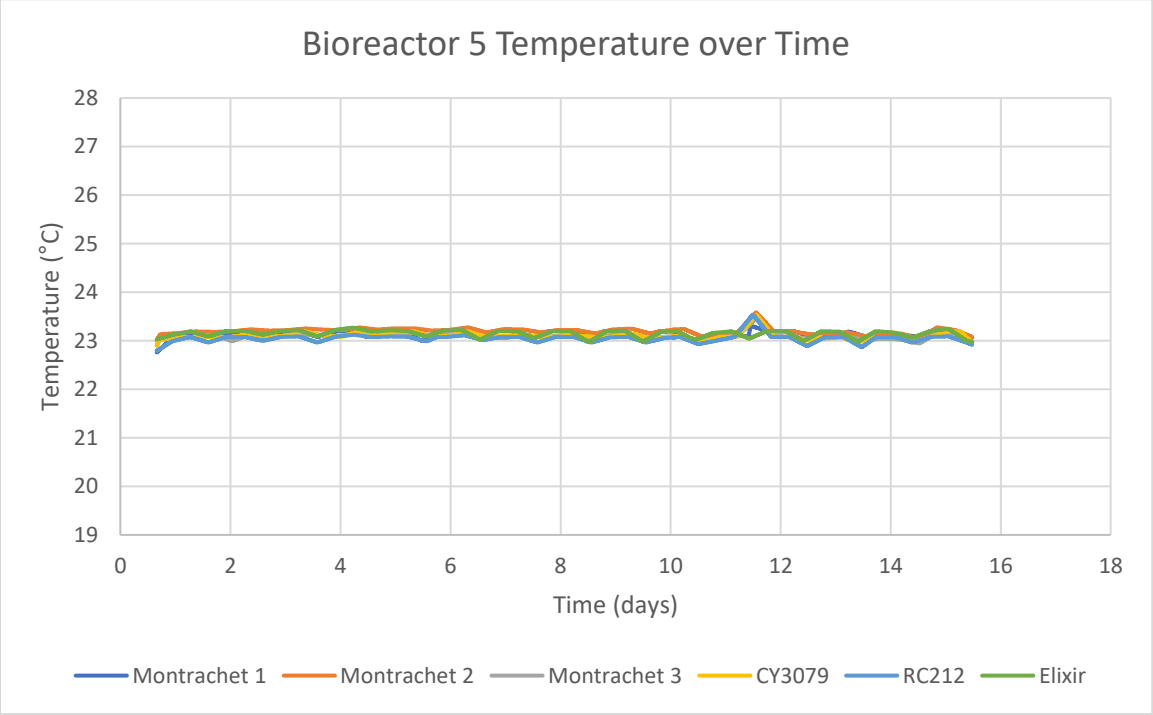

Figure A.5: Temperature (°C) over time for bioreactor experiment 5. See Table A.1 for experiment parameters.

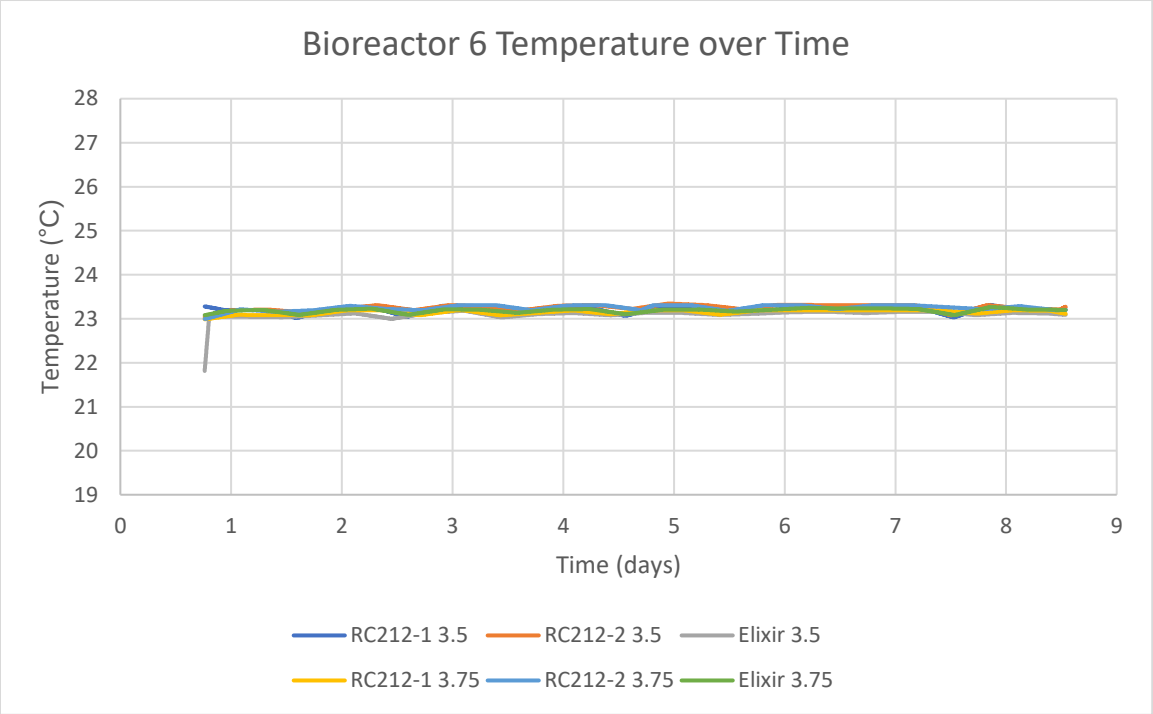

Figure A.6: Temperature (°C) over time for bioreactor experiment 6. See Table A.1 for experiment parameters.

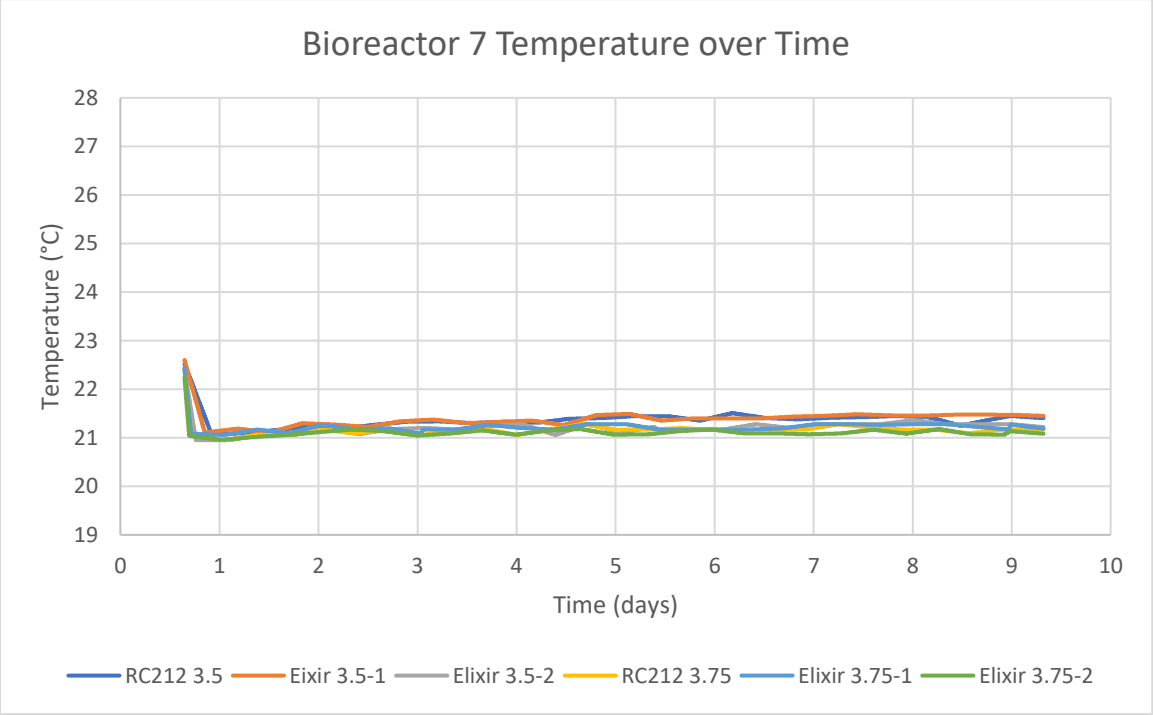

Figure A.7: Temperature (°C) over time for bioreactor experiment 7. See Table A.1 for experiment parameters.

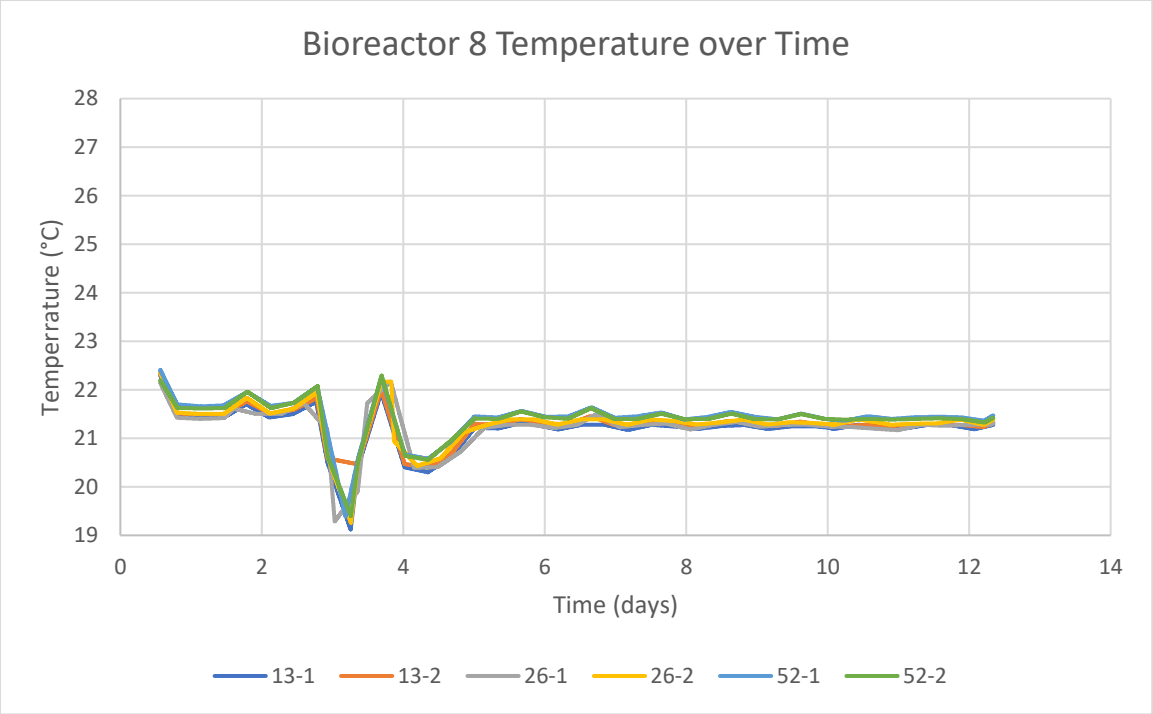

Figure A.8: Temperature (°C) over time for bioreactor experiment 8. See Table A.1 for experiment parameters.

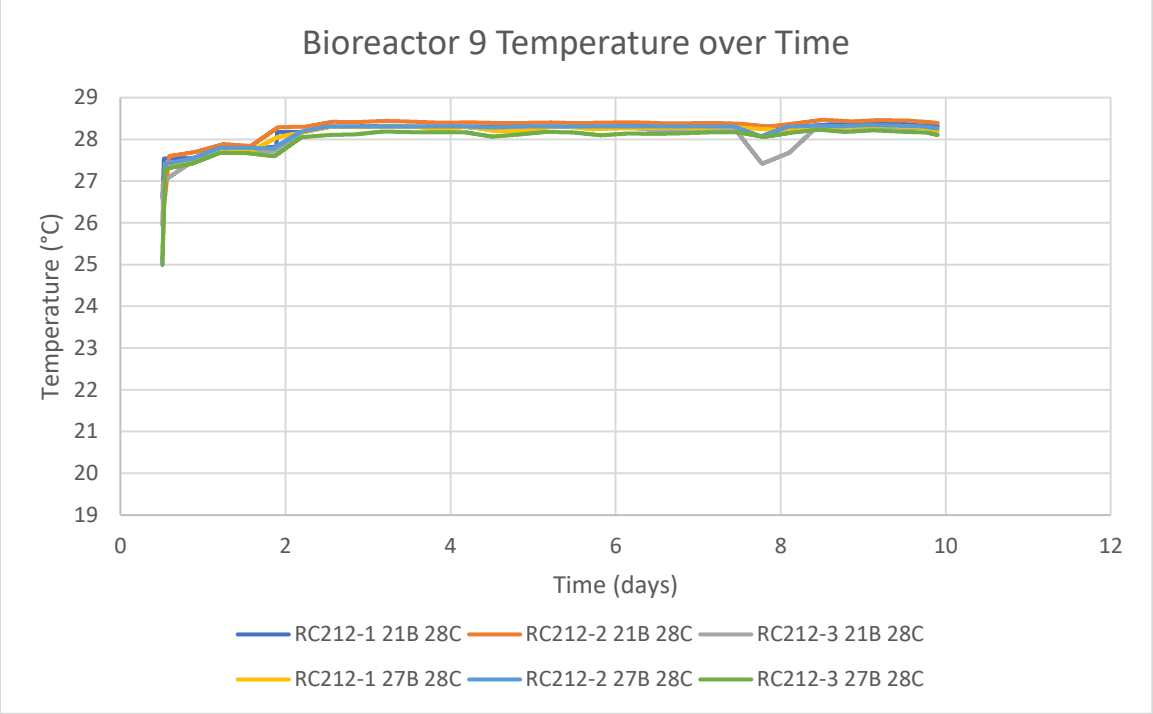

Figure A.9: Temperature (°C) over time for bioreactor experiment 1. See Table A.1 for experiment parameters.

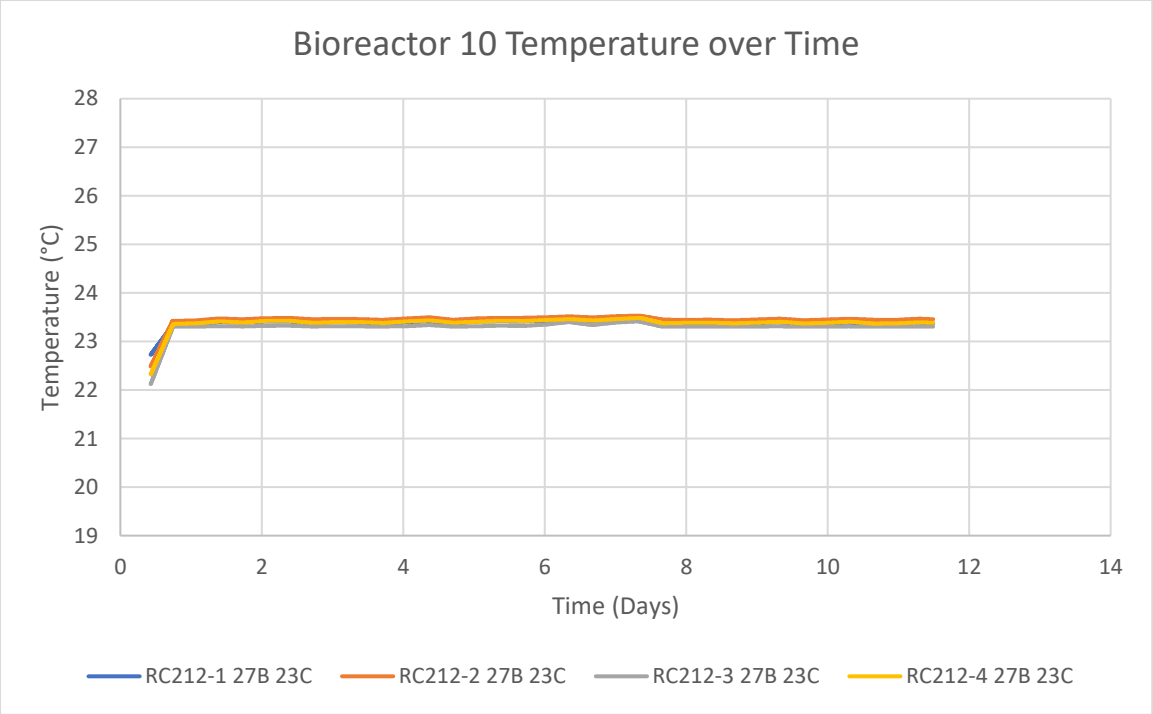

Figure A.10: Temperature (°C) over time for bioreactor experiment 1. See Table A.1 for experiment parameters.