Title
The Effects of Oxygenation on Redox Potential & Fermentation Kinetics

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The Effects of Oxygenation on Redox Potential and Fermentation Kinetics

By

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THESIS

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Committee in Charge

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Abstract

Every year the wine industry faces significant financial losses because of stuck fermentations and the presence of reductive faults in finished wines. Currently, most wineries rely on temperature and °Brix metrics to monitor the progress of a fermentation. However, these parameters do not reflect the chemical and metabolic status of a fermentation in real time. Redox potential, or Oxidation Reduction Potential (ORP), is an emerging process parameter in the wine industry. It offers a valuable real-time indicator of the redox status of a fermentation, which correlates with yeast metabolism and dynamics, fermentation kinetics, and hydrogen sulfide production. Controlling redox via aeration has the potential to facilitate more robust fermentations, help avoid costs associated with problematic fermentations and could be used to make decisions to prevent the formation of unwanted compounds associated with reductive fermentation conditions. The goal of this study was to gain a better understanding of how oxygen introduction, at various times and amounts during fermentation, affects redox status and how this is linked to overall fermentation outcomes. Using juice concentrate as a consistent fermentation medium, the impact of oxygenation on redox potential and fermentation kinetics was investigated at lab scale. This work set out to evaluate when the introduction of air during fermentation is required to see improved fermentation performance. Redox, Brix and cell density data was collected in all trials and compared against un-aerated ferments and across those subject to different oxygenation time and redox set point regiments. Experimental results demonstrated that aerated ferments with higher redox status have faster fermentation kinetics and reach overall greater cell densities than those that were un-aerated. In addition, the timing of aeration was found to be important for this effect.

Acknowledgements

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1. Introduction & Background

Fermentation health is of up most importance for winemakers as problematic ferments can result in stuck fermentations, the development in undesirable sensory attributes, and product loss. Oxidation- reduction potential (ORP) has shown to play an integral role in yeast health and metabolite production, as well as the general chemistry occurring in the fermenting wine matrix. Monitoring ORP in an industry setting offers winemakers a real time indicator of the chemical and metabolic status of their fermentations. Understanding how to employ ORP control is of particular interest as it enables the manipulation of the wine matrix, creating both more favorable environment for fermenting yeast and a less chemically reductive environment overall. This work aims to further investigate and define ORP control to better allow future implementation at the commercial wine scale. Note, all abbreviations used throughout this work are listed in **Appendix Table 6.1** for reference.

1.1 ORP fundamentals

At a chemical level, reduction-oxidation (redox) reactions are a series interconnected reactions involving the oxidation of one compound and the reduction of another [1][2]. Redox potential, also known as oxidation-reduction potential (ORP), is an electrochemical measurement that represents the sum status of the competing transfer reactions in a solution [3]. Many solutions, including wine, possess redox couples. Similar to pH, that indicates the availability of hydrogen ions, the overall redox potential delineates a relative state of gaining or losing electrons [1]. In addition, like pH it affects chemical speciation and therefore chemical, biochemical, and physical rates [4][5]. ORP however is unique in that it is a mixture property and thus a composite measurement that is influenced by pH, dissolved oxygen (DO), temperature and chemical half reactions taking place in a liquid medium [6].

The ORP value is the result of active redox couples moving in a state in which follows the Nernst Equation (Equation 1.1). E_h, a common notation for ORP found in the literature, is dependent on the ratio of activity of oxidized and reduced components of the solution. E^o being the constant of the formal potential of the redox system [1][3][7]. The Nernst equation is as follows where R is the gas constant, T is temperature, n is number of electrons, F is the Faraday constant, ox is the oxidized compound while red is the reduced compound [8].

$$E_h = E^o + \frac{RT}{nF} \ln \frac{a_{\sum_{i=1}^{n} ox_i}}{a_{\sum_{i=1}^{n} red_i}} \quad \text{(Equation 1.1)}$$

In rudimentary terms, a higher ORP value is the result of greater activity of the oxidized forms than the reduced forms, by way favoring oxidation reactions. A lower ORP value is the result of greater activity of the reduced forms than the oxidized forms, thus favoring reduction reactions [2][3][9].

1.2 Measuring ORP

ORP is the measurement of electrochemical potential under zero current and is expressed as voltage in units of millivolts (mV). While ORP measurements can be taken using electrochromic dyes, this method has become obsolete due to the known toxicity of dyes [10]. More modern approaches to measure ORP use electrodes, with the common electrode methods being cyclic voltammetry and the galvanic half-cell method [3].

Cyclic voltammetry (CV) measures the current response of an active redox solution in an electrochemical cell where voltage is in excess to that predicted by the Nernst equation [11][12]. It is performed by cycling the potential of a working electrode and measuring the resulting

current. Using the CV method in wine is challenging as it is a complex solution containing both slow- and fast-moving reactions. This makes CV results difficult to interpret as it is only able to measure the fast-moving species [3][13].

In wine, and other mediums with similar properties, ORP measurement are predominantly taken with ORP probes that employ the galvanic half-cell method. ORP values derived from the galvanic half-cell method are the result of the energy being released during spontaneous redox reactions [3]. The components of the galvanic half-cell include sensing electrodes, a reference electrode, and a salt bridge. The sensing electrodes consist of anodes and cathodes, where the energy of the galvanic cell is the result of difference in sensing electrodes. Platinum is the most used material for sensing electrodes as it is a noble metal with high electroconductivity allowing for sufficient electron exchange [14]. A platinum electrode ORP probe was used in this study.

The reference electrode is considered zero and is used to compare against the ORP of the sensing electrodes. While many reference electrodes exist, the standard hydrogen electrode (SHE) and the silver/ silver chloride (Ag/AgCl) electrode are those most commonly found in modern ORP probes. It should be noted that different reference electrodes result in varying ORP values. The difference in potential between Ag/AgCl and SHE is ~200 mV [1][9][15][16]. When interpreting redox experimental results in the literature understanding difference in reference electrodes is critical in making sound comparisons between works. A salt bridge is essential to mitigate diffusion potentials between half-cells. A potassium chloride (KCl) solution is a typically used as the salt bridge [17]. ORP measurements can be reported via ORP meter or wirelessly through a process control system. The components of the galvanic half-cell method are condensed and embodied within commercial ORP probes **(Figure 1.1)**. A theoretical

visualization of the galvanic half-cell in a wine fermentation is illustrated in **Figure 1.2**, adapted from the master's thesis of David Killeen, 2016 [3].

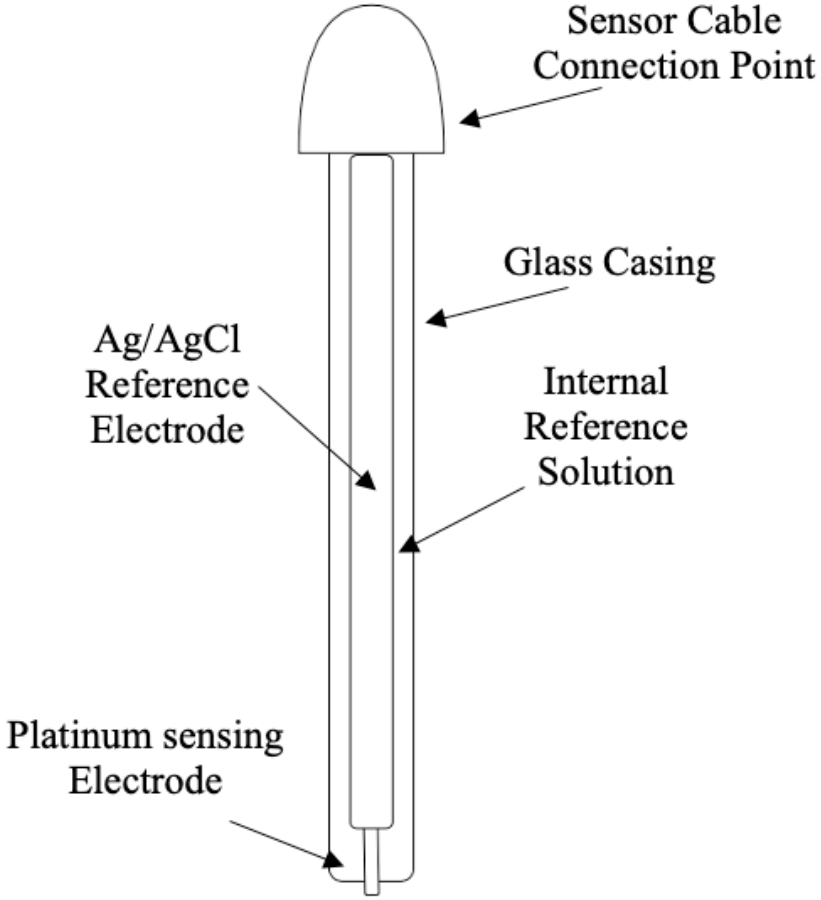


Figure 1.1 Simplified version of the galvanic half-cell components within an Ag/AgCl ORP probe. Diagram based on the ORP probe used in this work.

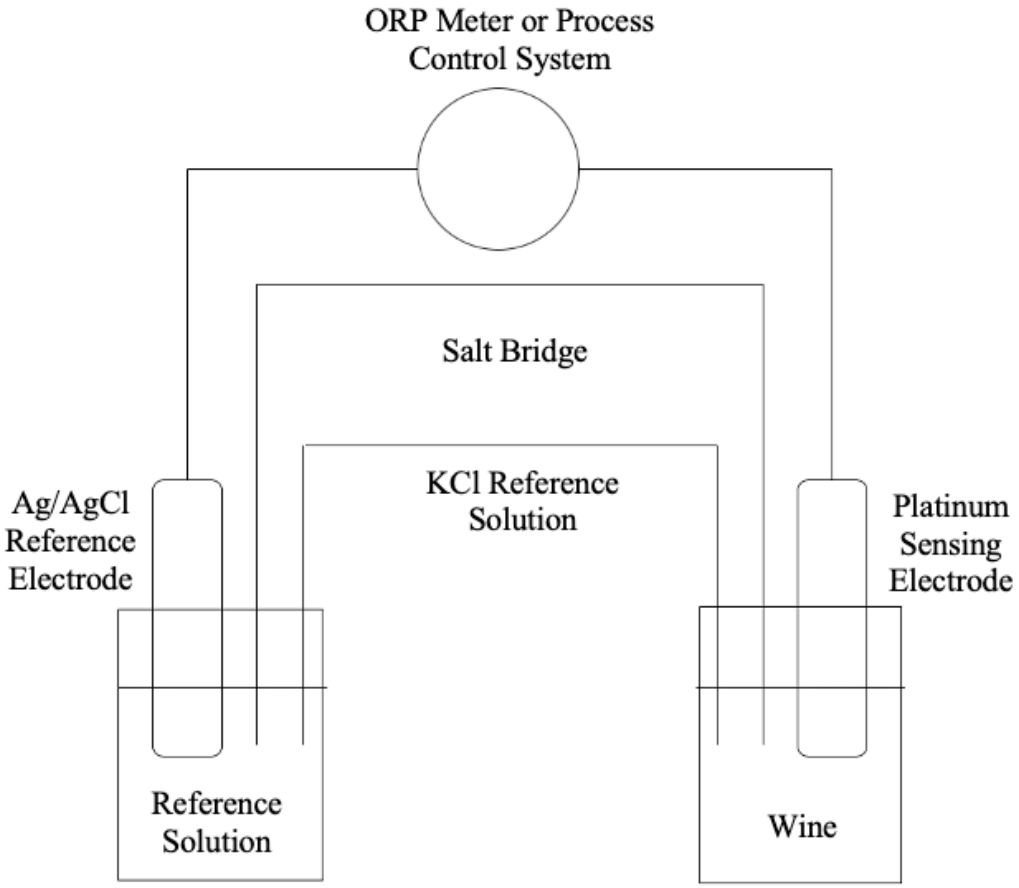


Figure 1.1 Theoretical galvanic half-cell within wine fermentation.

1.3 ORP & wine fermentations

The redox potential of a juice/wine is impacted by many factors, including the metabolic activity of microbes that are present [16]. For example, while ORP is measured in the liquid medium, it is altered by cellular electron transfers and metabolic outputs [1][16][18], as yeast are consistently working to maintain their internal redox (-290 mV) and pH (5-7) [19][20]. This is accomplished via the continuous movement of ions, substrates, and products across their membrane; some of which influence the external redox. Among the most influential reductive species are electrons, glutathione, thiols, and glycerol [1][21][22]. These efforts are directed at

maintaining homeostasis of internal redox pairs, particularly nicotinamide adenine dinucleotide (NAD+/ NADH) as well as its' phosphate derivatives NADP+/NADPH, which are essential for healthy cellular metabolism and stress response. Several biological processes are regulated by intracellular ORP, including subsequent forms of gene expression, enzyme synthesis, protein function and membrane integrity [23]. With prolonged exposure to unfavorable external ORP conditions, internal ORP may not be maintained, causing decreased yeast metabolic function and cell death. In the context of a winemaking, slowed metabolism or premature cell death often result in problematic sluggish or stuck fermentations.

Due to the anaerobic nature of wine fermentations, changes in ORP are predominantly driven by physiological and metabolic activity of the fermenting yeast [6]. As fermentation begins, yeast consume available oxygen in the solution, producing carbon dioxide (CO_2) and other reductive metabolites. This perpetuates a reductive environment, lowering ORP. The ORP profile of a wine fermentation resembles that of a bathtub curve [7]. It is characteristically high at the start of fermentation (~300-400 mV), but when yeast metabolism commences ORP begins to fall [7][16][24]. This slow decline in ORP at fermentation start is correlated with the lag phase of yeast population growth. During the exponential phase of cell growth, ORP falls dramatically and remains low throughout the stationary phase, coinciding with peak cellular metabolism and fermentation. ORP minimums can be as low as -300 to -400 mV [16][24][25]. As glucose is depleted from the system yeast metabolism arrests and a gradual increase in ORP is observed. Adapted from Lin et al. (2010), **Figure 1.3** illustrates typical ORP, °Brix or glucose depletion, and biomass wine fermentation profiles [24].

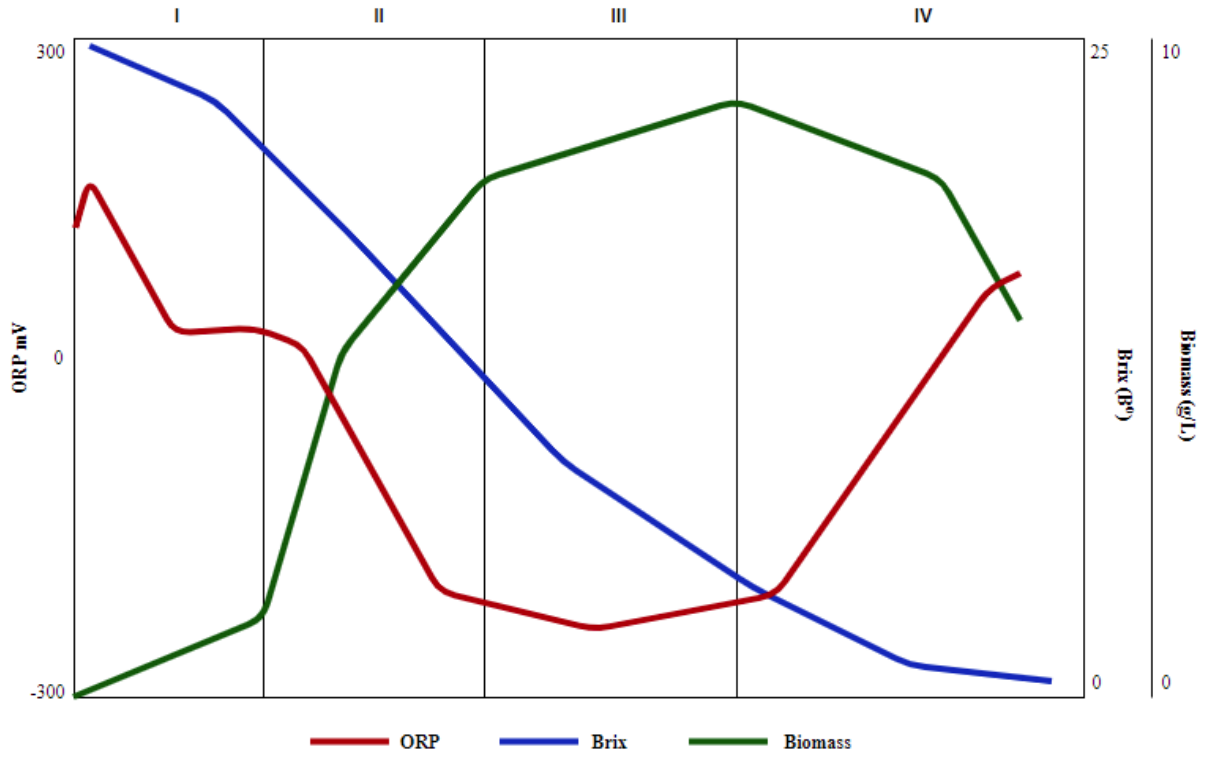


Figure 1.3 Example ORP, brix and biomass curves demonstrating typical fermentation behavior. The four divided regions illustrate the correlation between ORP and yeast growth: lag phase (I), exponential phase (II), stationary phase (III), and death phase (IV).

Wine is a complex matrix that encompasses an array of chemical species and reaction types. Among them are redox reactions, where each redox compound possesses its own unique ORP value [5]. The summation of these redox compounds is indicative of the extracellular ORP, in which determines the oxidative state of the redox compounds in the solution and thus the favorability of redox half reactions occurring [6][25][26]. A half reaction of particular interest to winemakers is the spontaneous formation of hydrogen sulfide (H_2S), a sensorially undesirable compound in finished wines. The formation of H_2S in the presence of elemental sulfur is thermodynamically favored at an ORP of -70 mV [5]. Nearly all active wine fermentations fall below -70 mV, but given the negativity that surrounds H_2S, fermentations below -70 mV are coined to be in the "reductive danger zone" [5][25]. A second half reaction that is of interest is

the reduction of Fe (III) back to Fe (II). Fe (II) is as key catalyst in wine chemistry, playing an essential role in the oxidation of tartaric acid in the initiation phase of Fenton reaction. In regard to ORP, at wine pH Fe (II) acts as an electron carrier perpetuating the formation of hydrogen peroxide (H_2O_2) and its subsequent oxidative products [27]. This reaction raises ORP and serves as a foundation for ORP intervention and control by way of aeration.

1.4 ORP in wine literature

ORP has been a topic of study dating back to the 1920's. Joslyn (1946) was one of the pioneers of ORP research in wine. She assessed 250 lots of wine from 3 viticultural regions in CA [28]. She assessed samples taken from white, red, port and sherry fermentation vats in cellars. Her work yielded some significant results, documented redox behavior in wine where redox starts between ~200-300 mV, falls as fermentation persists (~ -10 mV) and flowing rises again when fermentation arrests (~40 mV). This redox profile, often referred to as a bathtub curve is clearly demonstrated in all unaerated ferments in this work. She observed that white wines tended to have higher initial redox values than red and was one of the first to denote redox maximum (~403 mV) at 15º C. These results were further validated by Bevovic (1988) who conducted a similar experiment at the lab scale observing ORP across temperature in cabernet sauvignon fermentations [10]. Lastly, Joslyn (1946) concluded that aeration of fermentations and oxygen introducing events (i.e., racking, filtration, transfer, etc.) resulted in an increase in ORP [28]. This relationship between aeration and the raise of ORP was further supported by Schanderl (1959), where he showed that the addition of air in wine fermentations resulted in an increase in ORP of ~50 mV [9][29].

Rankine's (1963) work investigated the relationship between ORP and the production of hydrogen sulfide (H_2S), tracking H_2S production throughout red and white wine fermentations at

the lab scale [30]. His work demonstrated that peak H_2S formation coincided with the ORP minimum of a fermentation. These were monumental results in trajectory of ORP research, as it not only highlighted the relationship between low ORP and elemental sulfur but suggested that the formation of H_2S could be mitigated or controlled if a fermentation was held at a higher ORP. Some years later, Schutz and Kunkee (1977), sought to better discern the nature of H_2S, where it be chemical or enzymatic. It was determined that the reduction of elemental sulfur was apparently a non-enzymatic chemical reaction caused by the overall extracellular ORP caused by certain reducing compounds formed by yeast [31].

Kekec et al. (2002) investigated the impact of temperature on ORP in Sauvignon blanc fermentations in the lab at the 10-liter scale [7]. Fermentations were carried out at 15ºC, 18ºC and 24ºC. This work concluded that higher temperature fermentations resulted in a faster fall in ORP and lower ORP minimums. She correlates the effect of temperature on yeast metabolism to ORP, where higher temperature result in an increased fermentation rate, thus accelerated yeast metabolism reflected as lower ORP values. This work identified that ORP measurements could be used to speak to yeast metabolism and in turn fermentation health. Lastly, this work noted the relationship between glycerol production and ORP, where lower ORP results in an increase in the accumulation of glycerol.

There have also been studies that have investigated the relationship between ORP and wine yeast metabolic activity. For example, Farina et al. (2012) sought to investigate the impact of ORP on wine quality [32]. This work was carried out in 125 mL flasks and conducted in a chemically defined “wine like” media. It explored the production of volatile compounds (esters, alcohols, acids and lactones) in both reductive and micro-aerobic conditions. The study showed that redox conditions dramatically affected the accumulation of aroma compounds where less

reductive conditions favored higher alcohol, acids and acetaldehydes while reductive conditions could add a positive reductive component to finished wines but in excess could result in faults. In addition, intracellular ORP has been a topic of interest in both bacteria and yeast. Redox couple ratios of NAD^+/NADH, glutathione (GSH)/glutathione disulfide (GSSG), thioredoxin (TrxSS)/ Trx have been of particular interest, at a membrane protein and genetics level [1][19][20].

Lastly, several recent studies have investigated the relationship between ORP, fermentation kinetics, and cell counts in fermentations under ORP control [3][9][33] [34][35][36]. Previously ORP control experiments at the lab scale had only been conducted using YM and YPD (Yeast Malt & Yeast-Extract Peptone Dextrose) broths as the fermentation mediums [33][34][35][36]. Killen et al. (2018) work demonstrated ORP control at a larger scale, utilizing true red wine fermentation conditions [3][9]. All of which demonstrated a correlation between higher ORP values and accelerated fermentation kinetics as well as increased cell densities.

1.5 ORP as a process parameter

It has long been suggested controlling ORP has the potential to facilitate more robust wine fermentations, help avoid costs associated with problematic fermentations and sensory faults in finished wines. While the use of ORP as a process parameter is still in its emergent stages in the wine industry it has long been utilized in number of other fermentation sectors for a variety of purposes and applications. Indeed, on a day-to-day basis humans depend on aerobic and anaerobic fermentation industries, spanning across the biofuel, wastewater, dairy, and beverage industries, which each use ORP as a process parameter.

ORP is an incredibly useful process parameter as it is a quick measurement to take and provides information in real-time. For example, it allows for the estimation of physiochemical

properties of water, aids in the decision making for the optimal scheme of water treatment and allows for regulation of its antioxidant properties [37][34]. An example of ORP use in the biofuel industry is the utilization of ORP to determine the "antioxidant power" of fatty oils and esters in bio-diesel degradation and refinery [38][35]. In the dairy industry, E_h is a control parameter during yogurt and cheese fermentation aiding in the control of aroma compound formation [39][36]. In all cases ORP is used to more efficiently monitor and/or control the metabolic and chemical behavior of fermentation products.

Every year the wine industry faces significant financial losses as a result of stuck fermentations and the presence of reductive faults in finished wines. Currently, most wineries rely on temperature and °Brix metrics to monitor the progress of a fermentation. However, these parameters do not reflect the chemical and metabolic status of a fermentation in real time, rather they are reflective of fermentation progress up until the point of measurement. The utilization of ORP offers a valuable real-time indicator of the redox status of a fermentation, which correlates with yeast metabolism and dynamics, fermentation kinetics, and hydrogen sulfide (H_2S) production.

1.6 ORP process control

Methods of ORP process control have long been utilized in industries deploying aerobic/ anaerobic bacterial fermentations. One method of ORP control is via bioelectric energy input. Bioelectrical reactors (BERs) and bioelectrical systems have been developed, where the equipped anodes and cathodes act as the electron donors and acceptors in the system ultimately raising the redox [1]. A study conducted by Thomas & Gerson (1985) demonstrated the effectiveness of this method successfully controlling ORP via BERs in aerobic *Escherichia coli*

cultures [8]. This method of ORP manipulation however restricts users to specific and expensive reactors out of the sphere of commercial winery use.

Another method of ORP manipulation is through the direct addition of reductants and oxidants to a redox system. Example additives including iron chloride, hydrogen peroxide, sodium sulfate, etc. [1]. Given the food safety regulations surrounding winemaking, chemical additions of this nature are widely not allowed. In addition, the presence of these control chemicals has the ability to alter the fermentation bioprocess and are often too costly to use at the industrial scale.

A third method of ORP control is via gas sparging. Different gases can be utilized to raise or lower ORP depending on the bioprocess goals (i.e., oxygen raises ORP while the addition of hydrogen or sulfide dioxide (SO_2) lowers ORP) [1][5]. Of the presented methods, gas sparging would be most easily adapted in the wine industry. It is food safe, cost effective, accessible in the winery setting and a method that can easily be adapted in wine fermentation protocols.

Given what is known about relationship between low ORP values and H_2S formation as well as the metabolic stress it imparts on the fermenting yeast, raising ORP via oxygen sparging is of particular interest. It should be noted that the complexity of juice/ wine system presents many challenges. One of which being the varying redox buffering capacity of juices or resistance to change in potential, which ultimately effects the sensitivity of the ORP response to oxygen additions [3][5][9]. In addition, ORP control and the oxygenation response can be limited by the metal and organic acid composition of juice [4][5].

Though the chemistry of ORP control is not the focus of this paper, **Figure 1.4** and **Figure 1.5** present simplified schematics of the chemistry occurring when oxygen is introduced as a means of ORP control. These schematics are adapted from Waterhouse & Laurie (2006)

[40]. In short, the oxidizing potential of molecular oxygen in wine is harnessed by the generation of a reactive oxygen species. The initial electron transfer leads to the formation of a superoxide (O_2•), which at wine pH exists as a hydro-peroxide radical (•OOH) transfer step requires a catalyst, presumably a transition metal (i.e., iron or copper). The transfer of a second electron would then produce a peroxide, H_2O_2 being the specific form generated in wine, raising the ORP [40]. Given the integral role of transition metals in this oxidation process, it becomes clear that wine mediums lacking metals in their composition would elicit a limited ORP response to oxygenation.

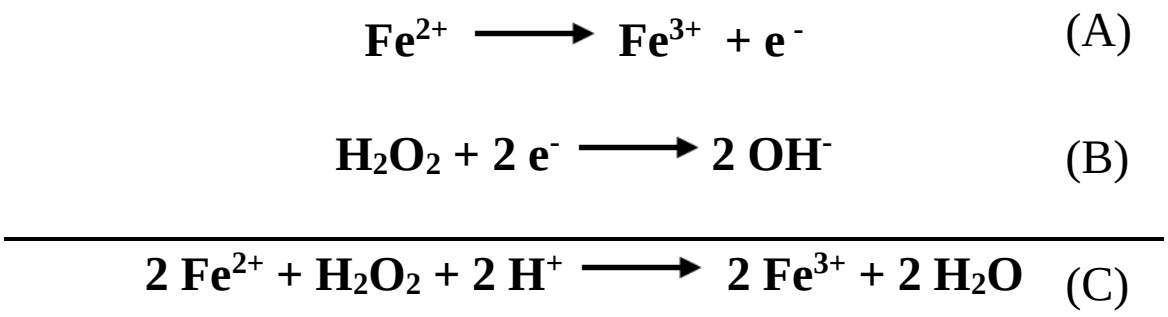


Figure 1.4 Redox system: A) Oxidation of ferrous to ferric ion, B) Reduction of hydrogen peroxide to hydroxide, C) Overall redox reaction. Adapted from [40].

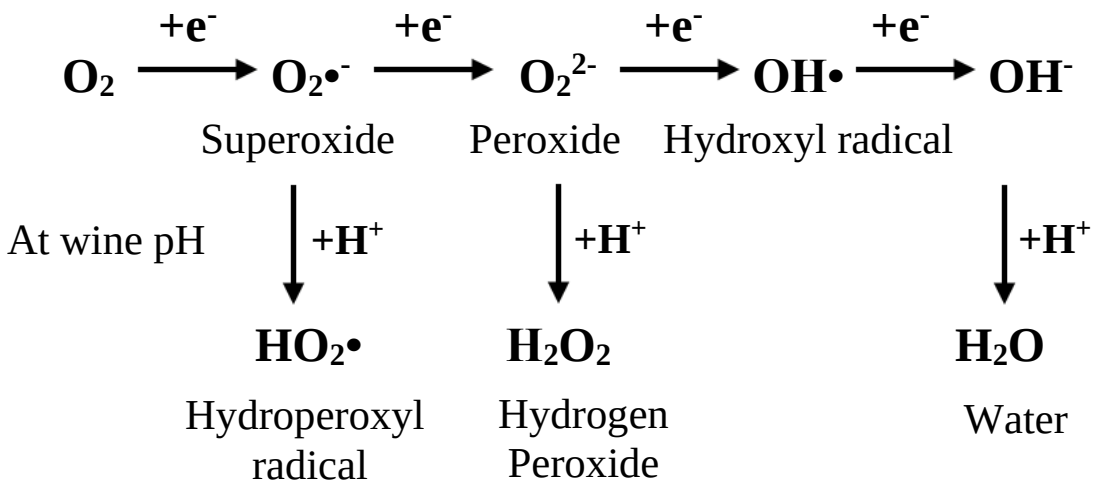


Figure 1.5 Oxygen reduction schematic. Adapted from [40].

ORP control and the effects of oxygen addition on fermentation behavior has been investigated in ethanol fermentation using *Saccharomyces cerevisiae*, the predominant wine yeast. A study conducted by Liu et al. (2015 & 2016) demonstrated the ability to control ORP using YPD media at the lab scale [33][34]. This work validated the ability to control ORP at different levels, (-150 mV, -100 mV, & -50 mV) via oxygenation. It reported the differences in fermentation behavior and outcomes observed between set points. The fermentation controlled at higher ORP levels resulted in accelerated glucose utilization thus increased fermentation kinetics, greater biomass accumulation and enhanced yeast viability. Clear delineation in these outcomes was observed across set points.

Killeen et al. (2016) was the first to employ ORP monitoring and control in a wine fermentation [3][9]. The research was conducted in red wine fermentations at the 30-gallon scale. An ORP pneumatic control system was developed that enabled ORP control at a given set point. In this work ORP was successfully controlled at 215 mV in triplicate and compared against uncontrolled natural ORP curves. Controlled fermentations demonstrated accelerated

fermentation rates, mirroring the work by Lui et al. (2015 & 2016) [33][34]. This increase in fermentation kinetics was attributed to increased yeast activity and metabolism. Direct biomass accumulation and cell viability were not investigated in this study. However, the use of an integrated fermentation model indicated that cell viability and cell maintenance rate were in fact higher in controlled fermentations.

1.7 Project goals

The primary goals of this work were to 1) further define the effects of oxygenation on redox status in wine fermentations and 2) better understand the relationship between redox control via oxygenation and its effects on fermentation outcomes (i.e., fermentation kinetics, cell counts, etc.).

Building on the results of Killeen et al. (2016) research, this work sought to implement and explore pneumatic redox process control at the lab scale [3][9]. White grape juice concentrate was used as the fermentation medium, as it limited variability between experimental runs providing a consistent juice medium. In addition, it allowed this work to continue outside of the bounds of the grape harvest. This work can be broken down into three experimental endeavors, the first being the development and preliminary testing of a new redox control system at the lab scale. The second group of experiments evaluated the effects of different redox control intervals on fermentation outcomes. This set of experiments aimed to better determine when the introduction of air during fermentation is required to see improved fermentation performance. The third group of experiments assessed the effect of redox control across varying set points. The intention here was to better decipher differences or lack thereof in fermentation outcomes across set points or degree of redox control. These results could aid if potentially defining an optimal redox set point in the future.

For the duration of this work redox measurements, temperature, Brix, and cell density data was collected from all experiments. In later, experiments periodic fermentation samples were taken and frozen for future wine chemistry analysis. Data compared against un-aerated ferments and across those subject to different oxygenation time and redox set point regiments.

2. Materials & Methods

2.1 Fermentation medium

For this work, white juice concentrate (Constellation Brands, Inc, Victor, NY) diluted with chlorine free water to ~24°B was used as the fermentation medium. All concentrate used in this work shared the same batch ID but was distributed across two vessels. Dilutions were carried in the UC Davis Teaching & Research Winery (Davis, CA) before being transported to the lab. Juice concentrate was stored at 5°C prior to dilution. The appropriate volume of concentrate for each experiment was diluted the morning of the experimental set up. Room temperature water was used for dilutions and the solution was vigorously whisked until homogenized. If not immediately used, the diluted concentrate was stored in refrigeration and then set out at room temperature to equilibrate prior to use.

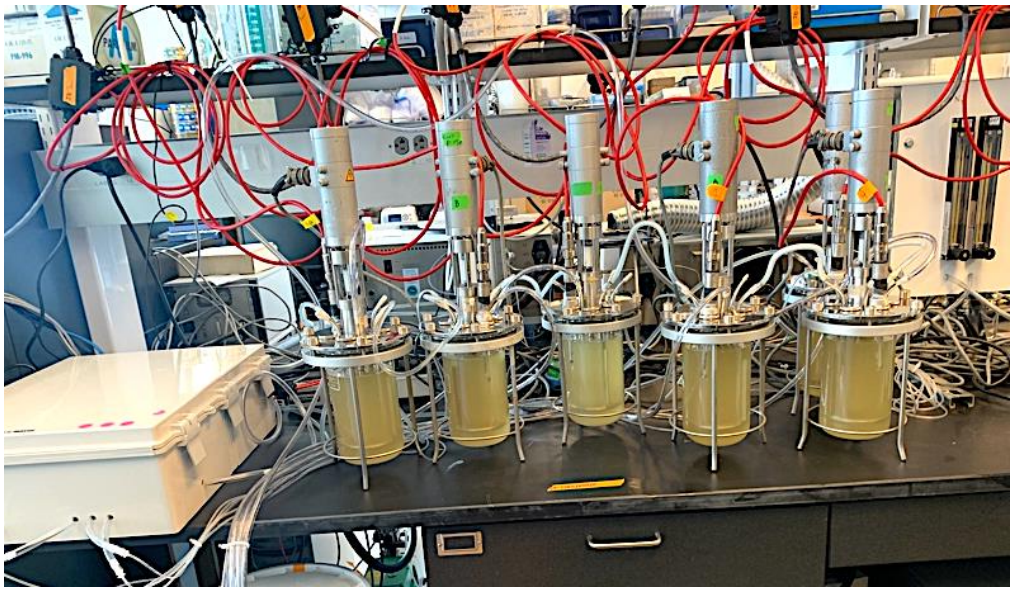
2.2 Experimental set-up & general parameters

Fermentations were carried out in 1 L autoclavable glass bioreactor (BR) vessels (Applikon, Schiedam, Netherlands) (**Figure 2.1**). To ensure homogeneity, bioreactors possessed motors that stirred the mixture at a rate of 100 rpm for the duration of the fermentation (Applikon Motor Controller ADI 1012 & ADI 1032 with P100 Mixer) (**Figure 2.1**). Ferments were inoculated with commercial EC-1118 Lalvin Wine yeast (Lallemand, Montreal, Canada) at an addition rate of 25 g/hL. Go-Ferm (Lallemand, Montreal, Canada) was used as a yeast

rehydration nutrient at an addition rate of 30 g/hL. Go-Ferm was mixed in 10 times its weight in 40°C water. Once homogenized, yeast was added and left to sit for 15 minutes. After this wait time, the hydrated yeast mixture was stirred, and equivalent volumes of diluted concentrate was added every 10 minutes until temperature was within 5°C of the experimental temperature. Additionally, at the start of fermentation SpringFerm (Fermentis, Marcq-en-Baroeul, France) was added to each bioreactor at a rate of 25 g/ hL. In the latter four experiments, once set up was complete, vessels were surrounded with cardboard to prevent any potential light-induced reactions (e.g., redox reactions) from occurring.

For all experiments temperature was controlled at approximately 23°C **(Appendix Figure 6.2)**. This was accomplished by circulating water through the overlay/ addition pipes of the bioreactors from a water bath (NesLab RTE-100 water bath/ circulator). Platinum electrode 120 mm EasyFerm Plus ORP ARC probes (Hamilton Company, Reno, NV) were used to monitor ORP (**Figure 2.2**). ORP probes were placed in the sensor port to ensure stability and minimal movement during experiments. Probes were 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. A time-series database (PI, OSIsoft, San Leandro, CA) was used to read the gateway's internal memory through a Modbus TCP/IP interface. The ORP, probe temperature and probe resistance were recorded as a function of time. The data was processed in MATLAB and retimed to one minute time points.

A)



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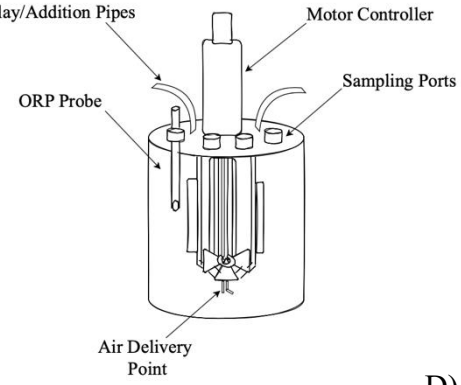


C)

D)

Figure 2.1. A) Comprehensive image of experimental set-up. B) Diagram of bioreactor vessel and notable parts. C) Applikon Motor Controller ADI 1012 & ADI 1032 boxes. D) Applikon P100 mixer motor attachment for bioreactors.

Figure 2.2. Platinum electrode 120 mm EasyFerm Plus ORP ARC probes.

The ORP probes were cleaned before every use. The cleaning regiment consisted of the probes being placed in 1% NaOH for 10 minutes, rinsed with deionized water, placed in 1% citric acid for 10 minutes, and then placed back in the 3 M KCL storage solution for 20 minutes. Immediately before inserting the probe into the bioreactor, each probe was calibrated in an ORP standard solution (+271 mV, Hamilton Redox Buffer).

2.3 ORP control

Redox control was accomplished by pulsing in air as needed to maintain programmed set points. A medical grade compressed air cylinder (UN1002, Airgas, Radnor, PA) was used as the gas supply. The air tank was regulated to 30–40 PSI and connected to a variable area flow meter (Cole Parmer, Vernon Hills, IL). The output of the flow meter was split into two pneumatic lines, with each line connected to a set of three pneumatic solenoids. The output of the solenoids was connected to the bioreactor through the addition/ overlay pipe. The solenoids were controlled by a programmable logic controller (PLC) with integrated relays (C0-11ARE-D, Automation Direct). A schematic of the ORP control and air delivery set up are illustrated in **Figure 2.3**. The ORP set point and duration of air addition was programmed into the PLC. Programming and parameters differed across experiments. Variations in parameters are reported in **Table 2.1**.

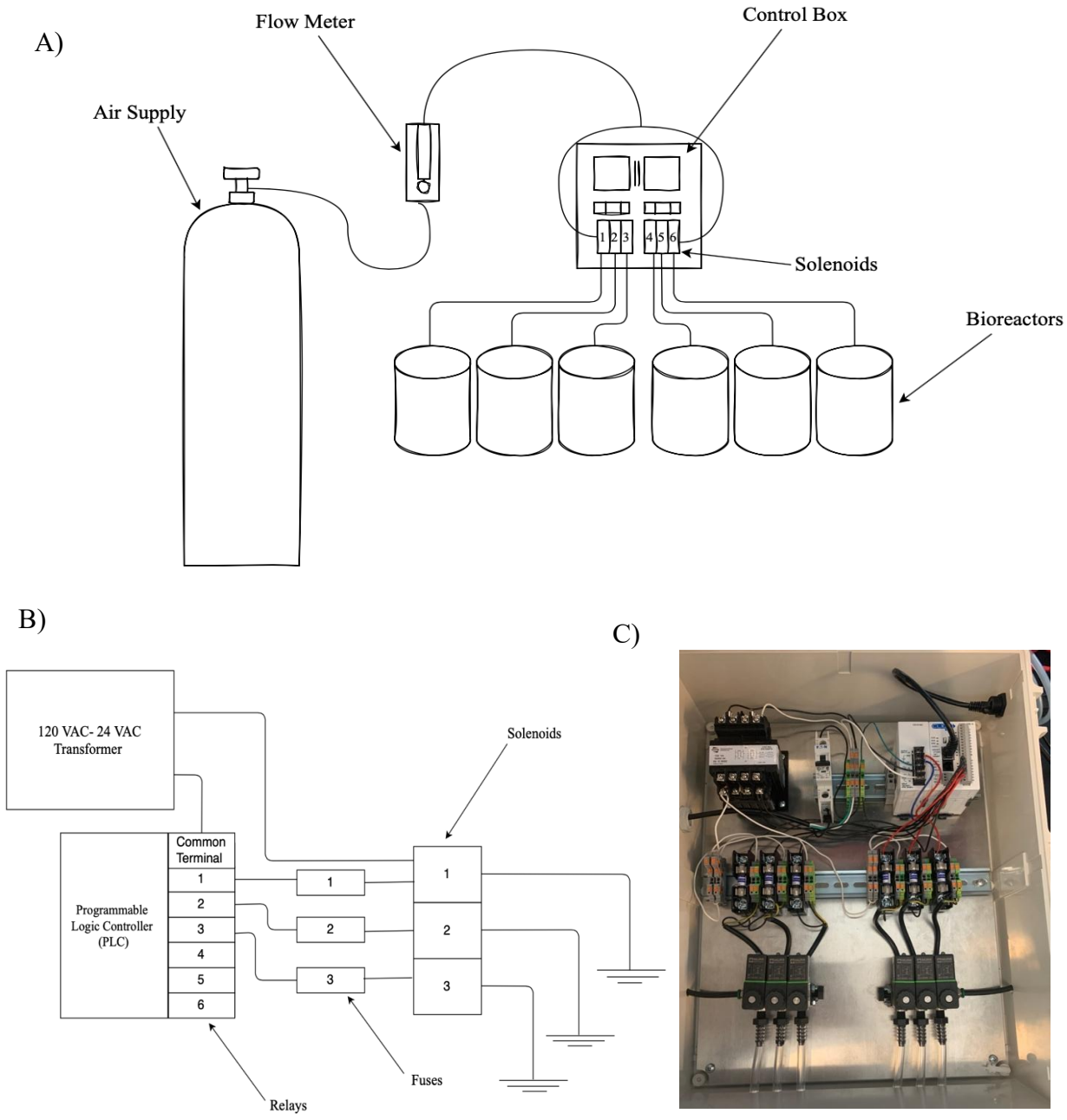
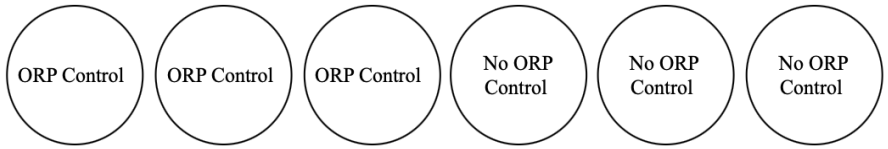
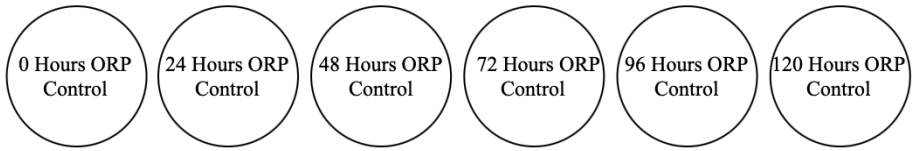
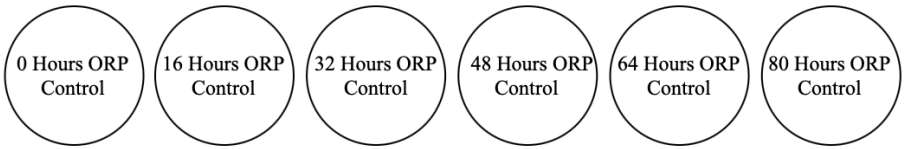
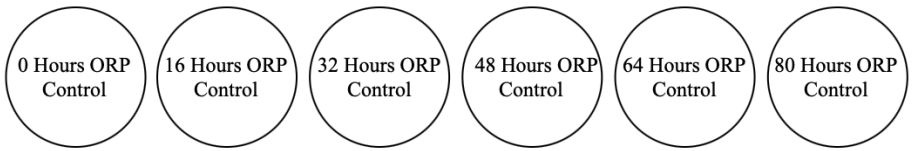
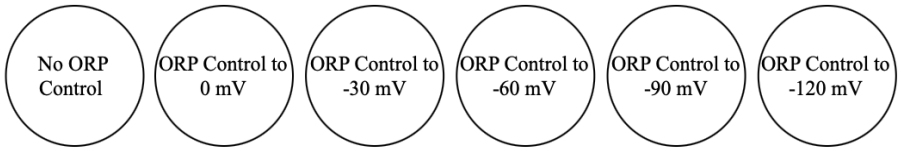
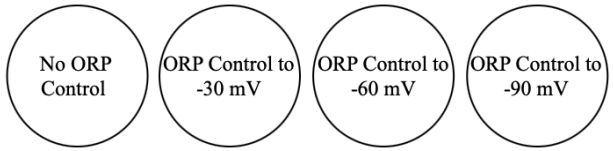
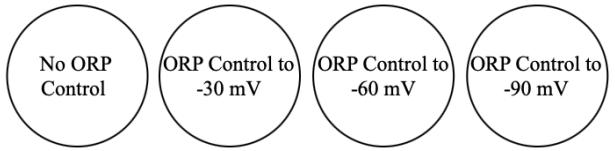


Figure 2.3. A) Schematic of air delivery system for ORP control. B) Schematic of air delivery control box. C) Image of air delivery control box.

Table 2.1. Summary of parameters and design for experiments 1 through 7.

Experiment	Fill Volume (mL)	Air Flow Rate (mL/minute)	ORP Set Point (mV)	Solenoid Opening Time (seconds)	Experimental Design
1	1000	50–200	-20	6 -> 8 as needed*	
2	1000	100–150	-60	8 as needed*	
3	950	75–100	-60	8 staggered*	
4	950	100–150	-60	8 staggered*	

5	950	150	0, -30, -60, -90 & -120	0 mV: -30mV: 12; -60 &-90, -120: 8 staggered*	
6	950	150	-30, -60 & -90	-30mV: 24 -> 16 -60 mV:18 -90: 12 staggered + dummy**	
7	950	150	-30, -60 & -90	-30mV: 24 -> 28, -60 mV:20 -90: 12 staggered + dummy**	
*Solenoid programmed to open on a 60 second loop **Solenoids programmed to open on a 75 second loop					

2.4 Data collection & sampling

A juice panel for each batch of diluted concentrate was conducted by the UC Davis Teaching & Research Winery staff to determine the initial juice conditions of each experiment. The juice panel used assessed juice Brix, pH, titratable acidity, malic acid, NOPA, NH_3 and yeast assimilable nitrogen (YAN). Brix was measured via a refractometer Brix tester (Bellingham Stanley Ltd., RFM 110, Tunbridge Wells, UK). Titratable acidity and pH were determined using an OMNIS auto titrator equipped with OMNIS dosing modules (Metroholm, Herisau, Switzerland). Titratable acidity was measured via titration with 0.1 N sodium hydroxide to pH 8.2. Malic acid, NOPA, NH_3 and YAN were determined enzymatically using a Gallery automated photometric analyzer (Thermo Fisher Scientific, Waltham, MA). Compatible enzymatic kits from Megazyme (Chicago, IL) for malic acid, ammonia, and primary amino nitrogen (PAN and PANOPA) and were used by the Gallery to generate NADH, after which absorbance at 340 nm was measured to indirectly quantify these compounds.

Fermentation progress was tracked using Brix metrics. Brix was taken 2–3 times per day using DMA 35N (Anton Paar, Ashland, VA). Brix sampling protocol varied depending on the experiment between 6 hour or 12-hour time points. Fermentation was considered complete when 0 °Brix was reached. A Petroff-Hausser Counting Chamber (Hausser Scientific, Horsham, PA) was used to collect yeast cell count numbers under a microscope. Cell counts were taken at three main time points during experiments: day 1 to determine an initial cell count, day 3 when the ORP minimum was most often reached and at the time of fermentation completion which is referred to as “final” in this work. In early experiments, cell viability was also evaluated. This was done via methylene blue staining on day 1, day 3 and final cell count time points.

Optical density (OD) measurements were taken at the morning and evening sampling time points using UV-VIS Spectrophotometry (Shimadzu UV 1280, Kyoto, Japan). A 1/20 dilution with deionized water was used when taking OD measurements. Collected samples were centrifuged in a Spectrafuge 24D (Labnet, Edison, NJ) for 5 minutes at 5,000 RPMs to remove yeast and other debris. The supernatant was then decanted from the pellet and stored at 20°C.

When fermentation reached 0 °Brix, samples were taken for a final wine panel. Again, wine analysis was conducted by the UC Davis Teaching & Research Winery staff. The final wine panel assessed ethanol, pH, titratable acidity, residual sugar, malic acid and acetic acid. Ethanol was measured via near-infrared spectroscopy using an Alcolyzer (Anton Paar, Ashland, VA). Residual sugar and acetic acid were determined enzymatically, again using the Gallery automated photometric analyzer (Thermo Fisher Scientific, Waltham, MA). Compatible enzymatic kits from Megazyme (Chicago, IL) for *D*-glucose, *D*-fructose, and from Thermo Fisher (Waltham, MA) for acetic acid.

3. Results

To better understand the use of ORP as a winemaking process parameter, this work explored the relationship between ORP and oxygenation. Specifically, the use of aeration as a means of ORP control and its subsequent effect on fermentation outcomes. To reiterate, aeration is a practice commonly used to raise the redox state and is a method of control that is both compatible with the winemaking process and widely available in the industry [35]. Controlling ORP is a useful tool as it has the potential to both mitigate the formation of reductive compounds and avoid costs associated with problematic or stuck fermentation [9]. This work looked to

establish ORP control at the lab scale and examine the effects of oxygenation at various times and amounts during fermentation, as well as across ORP control setpoints.

3.1 Summary of experimental results

For all experiments, an initial juice panel was conducted on each batch of diluted concentrate to determine the initial juice conditions. A summary of juice panel results across experiments is reported in **Table 3.1**. It should be noted that, experiment 1 is the only batch of diluted concentrate that was taken from the first vessel, the remainder of the experiments were taken from the second vessel. Concentrate used in each experiment was within +/- 0.5 of the target 24 °B. Results show that the fermentation medium used was a low pH environment ranging between 3.13 and 3.29. In all events, apart from experiment 3 and 5, YAN was reported to be between 250–260 mg/L. Experiment 3's YAN was reported to be uncharacteristically high at 314 mg/L, while experiment 5's YAN was lower than the other runs at 226 mg/L. Experiment 3 also demonstrated an uncharacteristically high NH_3 value.

Table 3.1. Summary of initial juice analysis results for experiments 1 through 7.

Experiment	Brix (°B)	pH	TA (g/L)	Malic Acid (mg/L)	NOPA	NH_3	YAN (mg/L)
1	23.5	3.14	6.70	3318	196	75	258
2	24.1	3.16	6.37	3486	184	85	255
3	23.2	3.13	6.38	3532	155	192	314
4	24.1	3.16	6.37	3486	184	85	255
5	23.9	3.19	6.13	4430	175	85	226
6	23.7	3.25	6.05	2325	102	85	257
7	23.2	3.29	6.41	3513	176	76	256

When fermentations completed, a wine panel was conducted for experiments 4 through 7 to evaluate final wine conditions. Results of all wine panels are reported in **Table 3.2**. Little variation in final alcohol, pH and TA were found across experiments. At the time of fermentation completion, a majority of vessels that received aeration were reported to be dry (<0.5 g/L) across experiments, with the exception of experiment 5. In all experiments BR1, those that did not receive ORP control, had greater final residual sugar values than vessels that were subject to aeration. In fact, in experiments 5 through 7, uncontrolled vessels did not go dry.

Table 3.2. Summary table of final wine analysis results from experiments 4 through 7. Wine analysis was not conducted on experiments 1, 2 or 3.

Experiment	Vessel	Alcohol (%)	pH	TA (g/L)	Residual Sugar (g/L)	Malic Acid (mg/ L)	Acetic Acid (g/L)
4	1	14.25	3.14	8.42	0.35	3017	0.84
	2	14.29	3.13	8.27	0.12	2930	0.59
	3	14.14	3.11	8.28	0.10	2956	0.72
	4	14.08	3.12	8.47	0.11	2848	0.58
	5	13.92	3.24	8.35	0.12	2814	0.59
	6	13.94	3.21	8.04	0.12	3276	0.55
5	1	13.35	3.11	8.36	1.95	3858	0.80
	2	12.95	3.17	8.33	0.12	3596	0.51
	3	13.28	3.16	8.23	1.24	3820	0.66
	4	13.37	3.11	8.27	1.08	3706	0.50
	5	13.68	3.17	8.32	0.59	3932	0.68
	6	13.54	3.14	8.23	1.92	3943	0.82
6	1	13.21	3.08	8.79	0.93	3421	0.77
	2	13.65	3.09	8.48	0.17	3419	0.73
	3	13.39	3.05	8.44	0.14	3364	0.60
	4	13.65	3.01	8.36	0.14	3391	0.73
7	1	13.76	3.14	8.55	1.01	3086	0.80
	2	13.11	3.11	7.85	0.18	2861	0.44
	3	13.60	3.05	8.05	0.19	2964	0.52
	4	13.82	2.99	8.21	0.22	3078	0.68

3.2 ORP control at the lab scale

Given the ORP control infrastructure was new to the lab and a new fermentation medium was being used, the first step of this work was to ensure that ORP could be controlled via aeration. In experiment 1, ORP was controlled at -20mV or left uncontrolled in triplicate fermentations. For this run the flow rate of air ranged between 50–100 mL/min. Solenoids were initially opened for 4 seconds as needed to maintain the desired -20 mV set point. When this control was not achieved, the solenoid opening time was increased to 8 seconds at 2.5 days (**Table 2.1)**.

Figure 3.1 demonstrates both the ORP and Brix curves over the course of the 7-day fermentation. Observing the uncontrolled vessels, this figure shows a typical redox curve where redox begins high (between ~350–400 mV) prior to the start of fermentation and abruptly decreases as yeast metabolism increases and fermentation gets under way. ORP eventually reaches an ORP minimum between -100 and -250 mV where it tends to stay throughout the remainder of the fermentation. ORP values are expected to increase once fermentation completes and yeast metabolism arrests. The typicity of this ORP trajectory can be observed in Appendix **Figure 6.**3, an ORP composite figure of all vessels without redox control across experiments.

While redox control was not achieved at -20 mV with the air introduction regime used, ORP controlled vessels did hold at approximately -80 mV. This was a significantly higher ORP than that of the vessels left uncontrolled by aeration, which reached an ORP minimum of -198 mV. On day 2, an overflow event occurred resulting in volume loss from aerated vessels, which likely contributed to changes in ORP observed during and following the incident. Though the target set point was not achieved using these experimental parameters, it was concluded that there was some ability to control ORP minima via aeration. Importantly, the same ORP trends

were demonstrated within the controlled and uncontrolled triplicates, which demonstrates that this fermentation setup provides for high reproducibility.

In reference to the Brix curves (**Figure 3.1 and Table 3.3)**, fermentations that were under ORP control demonstrated faster fermentation kinetics with the uncontrolled vessels finishing 12 hours later. While all controlled vessels were considered to have completed fermentation at the same time point, variation across Brix curves was observed during the course of the fermentation. For example, on day 3 BR3 was approximately 3°B ahead of BR1 and BR2 (**Table 3.3)**. This difference was not observed in the uncontrolled vessels as they demonstrated similar Brix curve trajectories.

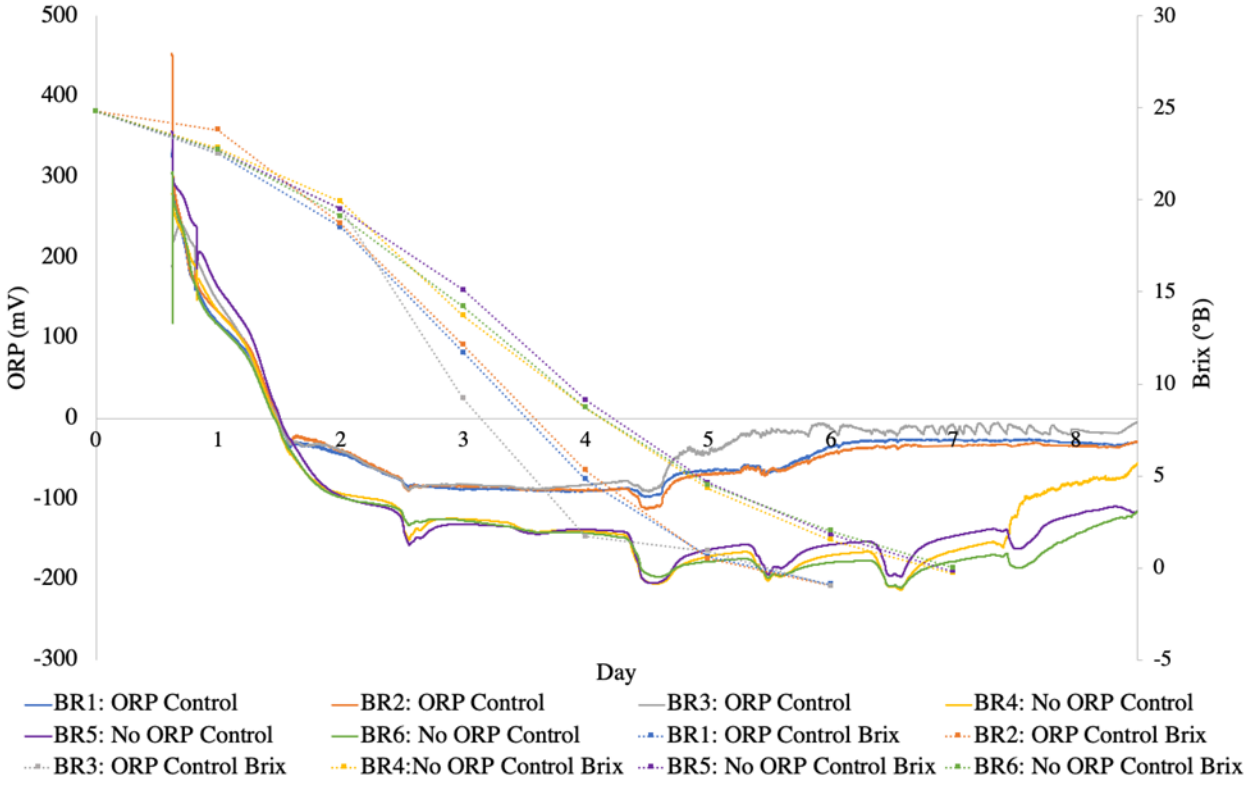


Figure 3.1. A composite figure of ORP curves (solid line) and brix curves (dashed line) from experiment 1, where ORP controlled and ORP set point for this experiment was -20 mV.

Table 3.3 A summary of notable experimental results from experiment 1. Brix, OD, cell counts and percent viability on day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count vs. the experiment's uncontrolled vessel's final cell count is shown.

Experiment	Vessel	Results														
		Brix (°B)			OD			Cell Count (cells/ mL)								
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 1 Cell Viability (%)	Day 2	Day 2 Cell Viability (%)	Day 3	Day 3 Cell Viability (%)	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Un-controlled Count
1	BR1	22.5	11.7	-0.9	4.7	9.8	17.6	3.85E+07	93%	1.83E+08	94%	1.54E+08	89%	300%	1.48E+08	160%
	BR2	23.8	12.1	-1	6.6	8.1	18.1	4.30E+07	97%	1.59E+08	96%	1.55E+08	94%	260%		
	BR3	22.5	9.2	-1	5.1	10.4	16.3	3.88E+07	95%	1.33E+08	93%	1.35E+08	92%	248%		
	BR4	22.8	13.7	-0.3	3.8	4.0	11.5	2.95E+07	96%	7.70E+07	95%	7.85E+07	91%	166%	9.25E+07	
	BR5	22.7	15.1	-0.2	2.6	4.1	12.2	2.90E+07	90%	8.80E+07	93%	9.40E+07	90%	224%		
	BR6	22.7	14.2	0	4.4	6.0	12.0	2.98E+07	94%	9.10E+07	92%	9.45E+07	95%	296%		

OD measurements and cell counts were taken to discern changes in cell density over the course of fermentation and between ORP treatments **(Table 3.3)**. While all vessels possessed similar cell densities on day 1 of the fermentation, the vessels that were under ORP control possessed greater OD and cell density values by fermentation completion. In controlled vessels changes in cell counts from day 1 to dryness ranged from 260–300%, while changes in uncontrolled vessels were between 166–224%. These changes are illustrated in **Figure 3.2**. Overall, the final cell count average of redox controlled vessels resulted in values an order of magnitude greater than those left uncontrolled, with a final cell count percent difference of 160% greater than the average of the uncontrolled vessels **(Table 2.3)**. All vessels demonstrated high cell viability throughout the fermentation, > 85% **(Table 3.3)**. No differences in viability were observed across ORP conditions.

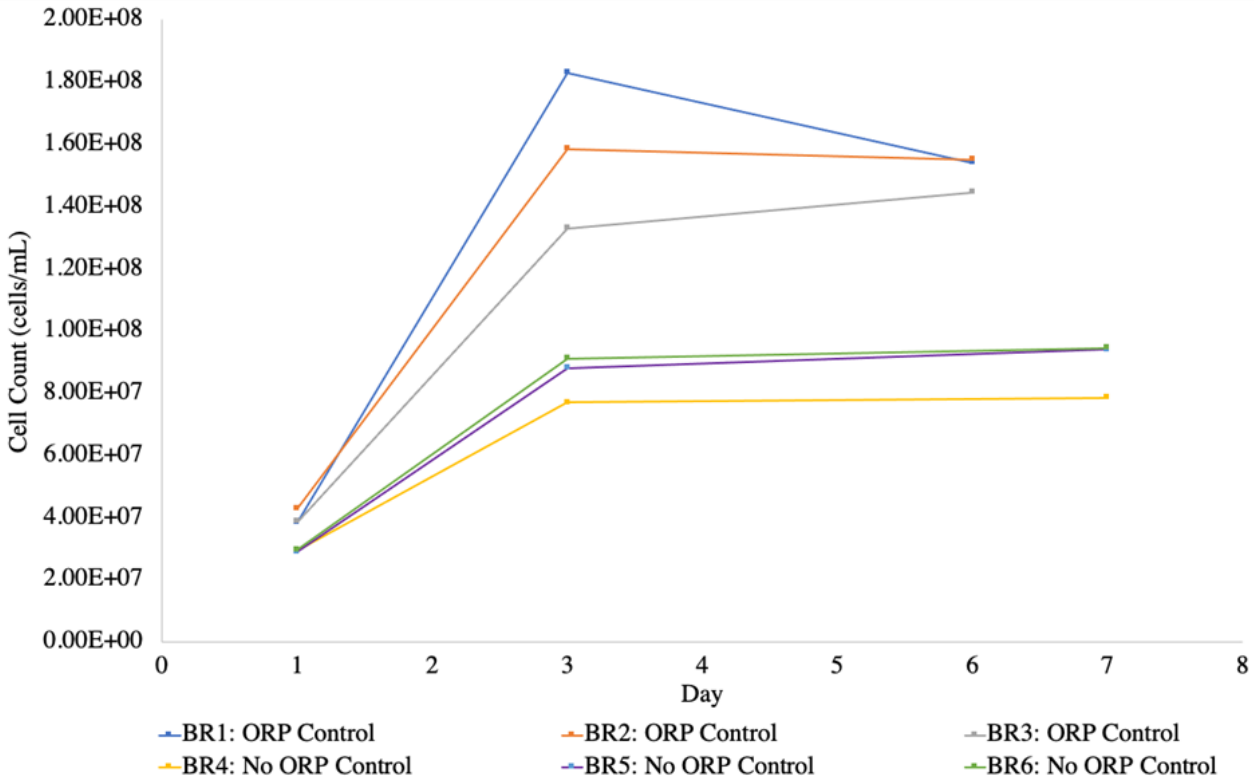


Figure 3.2. A composite figure of vessel cell densities from experiment 1, where ORP controlled and uncontrolled fermentations were performed in triplicate. Cell counts were taken on day 1, day 3 and the day of fermentation completion.

3. 3 ORP control across varying aeration intervals

3.3.1 Experiment 2 - ORP control in 24 hour intervals

After confirming that ORP control could be accomplished using this system, the next part of this work addressed the time period during fermentation ORP control was needed to achieve observed fermentation outcomes. This set of experiments (experimental runs 2–4) encompassed six vessels that were exposed to ORP control aeration for different amounts of time over the course of the fermentation.

In the second experiment this was accomplished by shutting off ORP control across vessels in 24-hour intervals. Vessels where controlled to maintain a minimum ORP set point for 0, 24, 48, 72, 96 and 120 hours after the inoculation time point by introducing air as needed.

Based on the outcomes of experiment 1, the control set point was changed to -60 mV, the air flow rate increased to 100–150 mL/min and the solenoids opened for 8 seconds all in an attempt to achieve a controllable ORP set point (**Table 2.1)**.

As seen in **Figure 3.3** desired ORP control was accomplished in BR3, where the -60-mV set point was held until control was terminated at 48 h post-inoculation. This result was not observed in BR4, BR5 and BR6 where ORP control to the set point was lost prior to aeration shut off time points despite air delivery. Again, this point of control loss coincides with overflow of media from the vessels. BR1 and BR2 displayed similar ORP curves. This is because as of 24-hour shut off point for BR2, this bioreactor had not yet dropped below the ORP set point. These data again highlight the reproducibility of the system setup because these were essentially duplicates from an aeration standpoint. Though BR4 fell below -60 mV prior to control shut-off, the ORP did remain above that of the unaerated vessels. In both BR3 and BR4, once the control was terminated, the ORP decreased with a similar trajectory of the vessels under no ORP control. BR5 and BR6's ORP remained above the other vessels during the majority of the fermentation and eventually returned to the control set point. Fermentation completion at 96 hours in both BR5 and BR6 coincided with the 96 hours shut off point of BR5, which negated the need for control through 120 hours. These vessels are also essentially duplicate runs, as both received ORP control for the entirety of their fermentation.

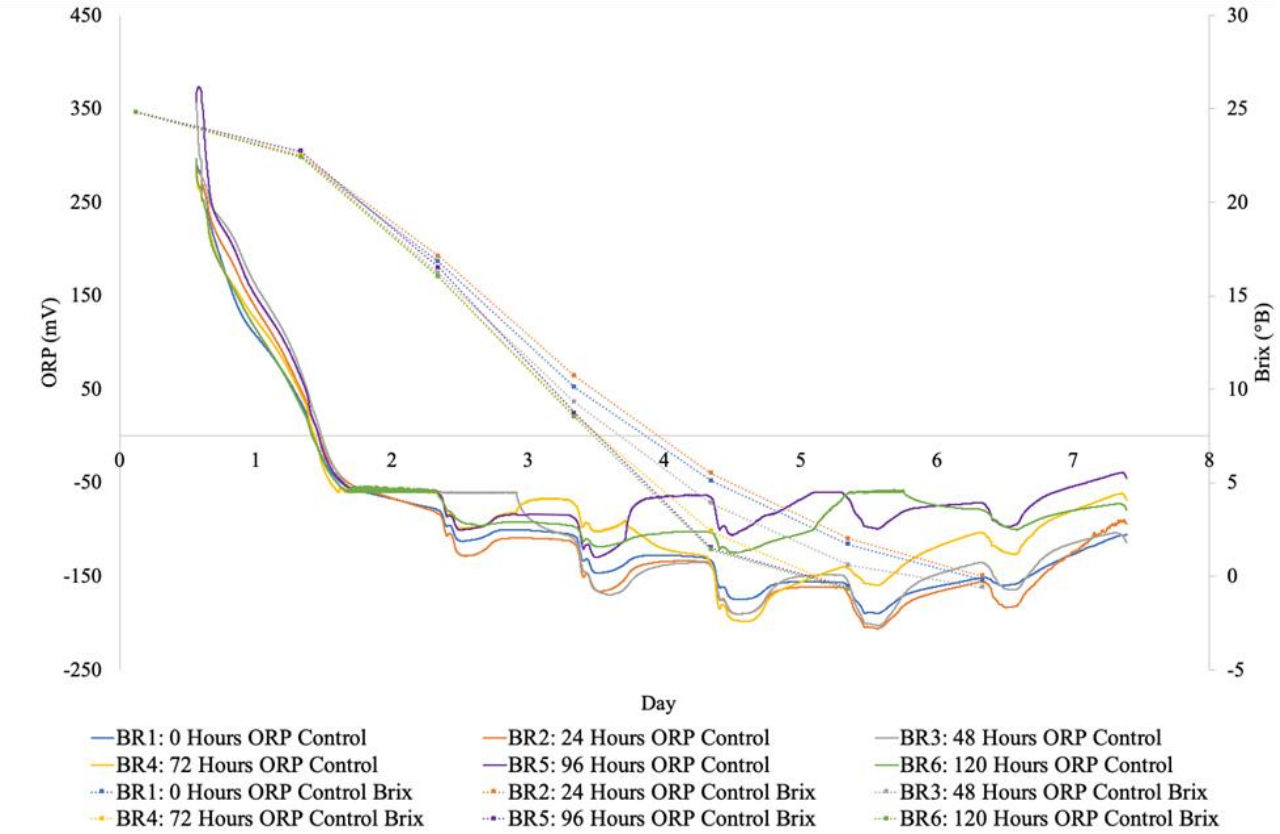


Figure 3.3. Impact of ORP control period by aeration on measured ORP (solid line) and brix (dashed line) from experiment 2, where vessels received 0, 24, 48, 72, 96 and 120 hours of ORP control. The programmed ORP set point for this experiment was -60 mV.

Compared to the vessels that did not receive air, BR1 and BR2, the aerated vessels again displayed increased fermentation kinetics. In addition, the length of ORP control correlated with faster fermentation kinetics and the timing of fermentation completion (**Figure 3.3)**. BR5 and BR6 demonstrated the same brix trajectory and the fastest fermentation kinetics of all vessels. BR4 demonstrated slower kinetics towards the end of the fermentation but shared the same fermentation completion time point as BR5 and BR6. BR3 showed an intermediate fermentation rate compared to BR5/6 and BR1/2, while BR1 and BR2 were the slowest fermenters. Note that for this experiment Brix sampling was only conducted once a day, so it is possible that with a denser sampling protocol further differences in fermentation rates and completion times would

have been observed. To further support this hypothesis, the final sampling time point for both BR5 and BR6 were reported complete at -0.8°B, while BR4 was at -0.3 °B **(Table 3.4)**. Another possibility could be that there is no difference in fermentation kinetics after 72 hours of ORP control, as BR4, BR5 and BR6 fell below 4.8-5.1°B **(Table 3.4).**

In agreement with fermentation changes, there was an upward trend observed in both ODs and cell counts, where vessels that were under ORP control for longer periods of time reached greater cell densities faster and demonstrated higher cell densities over all **(Figure 3.4)**. This trend was further supported by final cell count percent differences of bioreactors BR3-BR6 as compared to the vessel without ORP control (BR1) **(Table 3.4).** Despite differences in ORP conditions, no discernable differences in cell viability were observed. Again, all vessels demonstrated high cell viability, > 85%, for the entirety of the fermentation **(Table 3.4)**.

Table 3.4 A summary of notable experimental results from experiment 2. In addition, brix, OD, cell counts and percent viability from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results														
		Brix (°B)			OD			Cell Count (cells/ mL)								
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 1 Cell Viability (%)	Day 2	Day 2 Cell Viability (%)	Day 3	Day 3 Cell Viability (%)	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Un-controlled Count
2	BR1	22.2	8.6	-0.1	0.237	0.54	0.725	3.80E+07	89%	1.08E+08	91%	1.65E+08	87%	334%	N/A	-
	BR2	21.3	6.4	-0.5	0.153	0.591	0.665	4.53E+07	93%	1.74E+08	94%	1.95E+08	92%	330%		118%
	BR3	22.5	6.0	-0.1	0.169	0.714	0.791	4.83E+07	96%	1.65E+08	98%	2.03E+08	93%	321%		123%
	BR4	21.1	5.1	-0.3	0.185	0.788	0.908	4.85E+07	90%	1.95E+08	95%	2.18E+08	91%	348%		132%
	BR5	21.7	4.9	-0.8	0.182	0.728	0.892	4.73E+07	88%	1.87E+08	91%	2.47E+08	87%	422%		149%
	BR6	22.6	4.8	-0.8	0.25	0.822	0.956	4.73E+07	95%	1.84E+08	96%	2.50E+08	95%	429%		152%

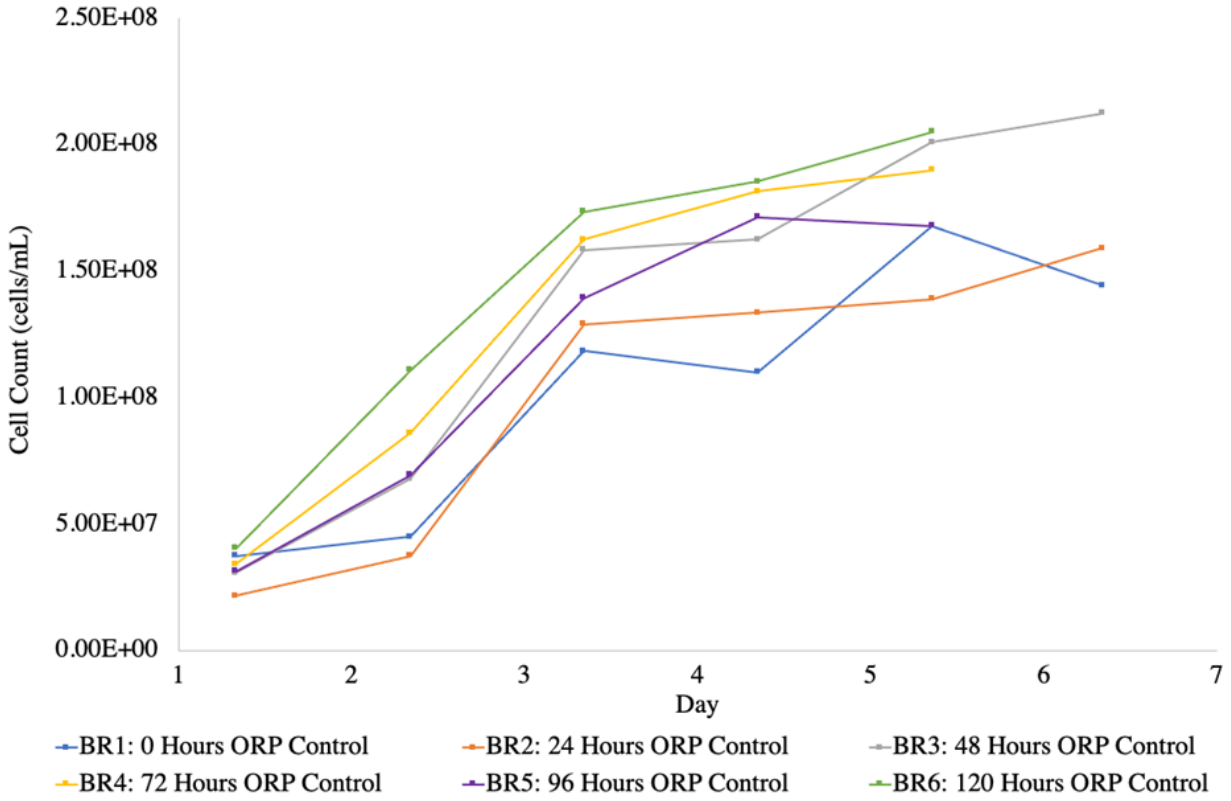


Figure 3.4. **C**ell densities from experiment 2, where vessels received 0, 24, 48, 72, 96 and 120 hours of ORP control. Cell counts were taken at every sampling time point until fermentation completed.

3.3.2 Experiment 3 - ORP control in 16 hour intervals

The results of experiment 2 aided in narrowing the windows of time to apply ORP control. In experiment 3, ORP control intervals were shortened to 16-hours to better match the timing of fermentation completion. Vessels received 0, 16, 32, 48, 64 and 80 hours of ORP control by aeration as needed to maintain the -60 mV set point. To ensure BR2 would be subject to aeration, the timing interval was counted from the point where aeration was needed to regulate ORP (i.e., BR2's first solenoid opening), which occurred at 16 hours after inoculation. The flow rate ranged from 75–100 mL/min and solenoids again opened for 8 seconds (**Table 2.1)**. Solenoid programming was adjusted for this experiment by actuating in a staggered manner. This strategy resulted in opening solenoids 1 through 6 consecutively on the 60 second loop.

Solenoids opened one at a time for 8 seconds as needed with a 2 second close time between each opening event. This programming aimed to avoid the opening of multiple solenoids at the same time and to ensure air was delivered to each vessel at the same flow rate. The fill volume of the vessels were also adjusted from 1000 mL to 950 mL to eliminate overflow events.

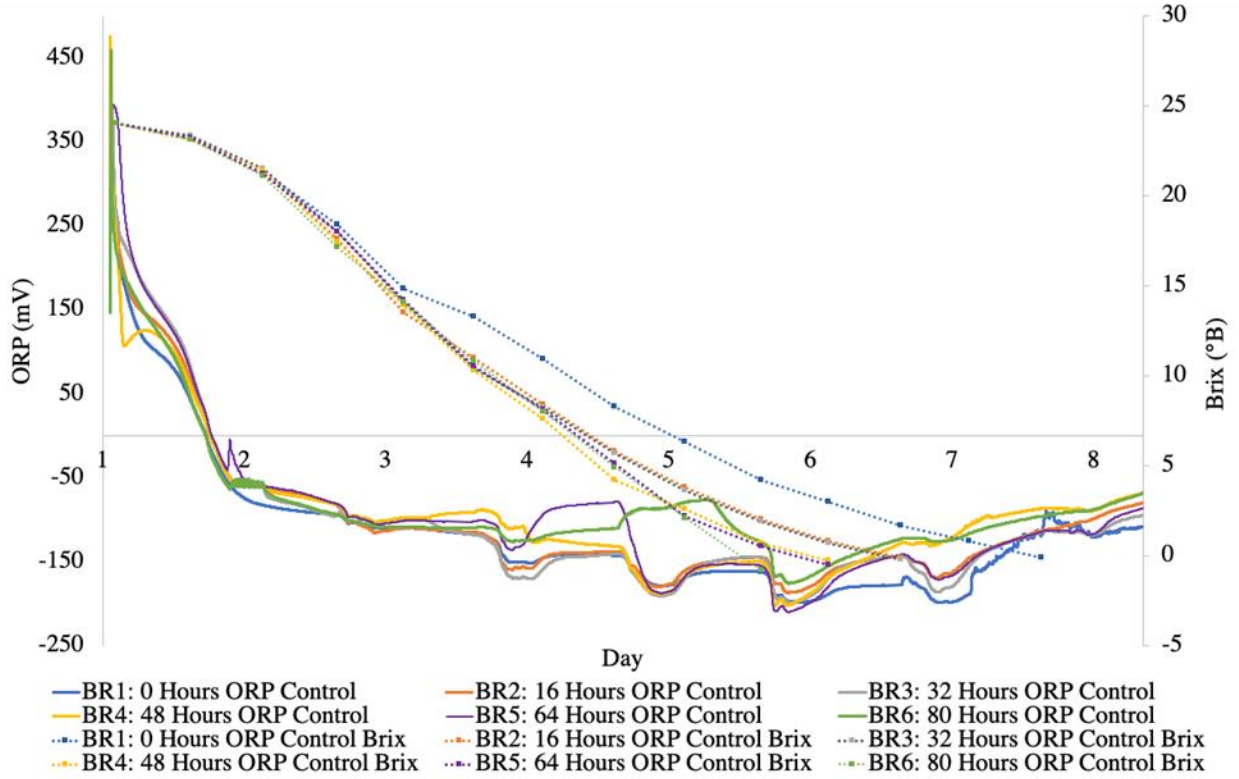


Figure 3.5. Impact of ORP control period by aeration on measured ORP (solid line) and brix (dashed line) from experiment 3, where vessels received 0, 16, 32, 48, 64 and 80 hours of ORP control. The programmed ORP set point for this experiment was -60 mV.

ORP control at -60 mV was only accomplished for a short period of time in all aerated vessels **(Figure 3.5)**. Once control was not achieved by aeration, ORP values in BR2 and BR3 continued to decrease until they were similar to that of the uncontrolled vessel, BR1. BR5 and BR6 oscillated around slightly higher ORP values (~ -100 mV) but, once control was shut off ORP values fell to those of the uncontrolled vessels. Yeast metabolism was anticipated to be

most active by day 3 of the fermentation, thus acting as the driving force bringing ORP down. It is likely that higher ORP values were achieved in BR5 and BR6 because aeration continued past the time of peak fermentation rate and closer to the point of metabolic arrest. Knowing that the -60 mV set point was achievable, but not accomplished, suggests that there was not enough air being delivered to the system. The combination of the flow rate adjustment to 75–100 mL/ minute and the change to a staggered solenoid regiment was likely too great of a reduction in air flow to achieve the desired control. Repeated oscillations patterns in the ORP curves of this experiment as well as previous experiments were recognized and were hypothesized to be correlated with diurnal weather events (e.g., sunlight shining on equipment in the laboratory).

Again, vessels subject to longer periods of ORP control demonstrated faster fermentation kinetics, with BR6 completing 48 hours sooner than BR1 **(Figure 3.5).** Brix curves showed that BR2 and BR3 demonstrated similar fermentation kinetics throughout the fermentation. This trend was also observed in BR4 and BR5 **(Table 3.5)**. These results suggest that there is little difference in fermentation completion times whether vessels are under control for 16 or 32 hours, as well as 48 or 64 hours. A12-hour difference in completion times was observed between groupings.

Table 3.5 A summary of notable experimental results from experiment 3. In addition, brix, OD and cell counts from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results											
		Brix (°B)			OD			Cell Count (cells/ mL)					
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Uncontrolled Count
3	BR1	23.2	13.3	-0.1	0.159	0.555	0.511	3.25E+07	7.35E+07	1.14E+08	249%	N/A	-
	BR2	23.2	11.0	-0.1	0.155	0.553	0.669	2.73E+07	9.70E+07	1.13E+08	313%		99%
	BR3	23.3	10.4	-0.2	0.166	0.495	0.773	3.50E+07	1.14E+08	1.14E+08	226%		100%
	BR4	23.1	10.3	-0.3	0.176	0.572	0.889	4.05E+07	1.22E+08	1.35E+08	233%		119%
	BR5	23.2	10.5	-0.5	0.151	0.476	0.808	3.73E+07	9.70E+07	1.63E+08	338%		144%
	BR6	23.1	10.8	-0.8	0.189	0.571	0.817	4.63E+07	1.26E+08	1.70E+08	268%		150%

Cell count data also trended with ORP control, the general pattern being that cell numbers increased with increasing times of aeration **(Figure 3.6)**. It is likely that this trend exists due to the impact aeration is having on cell growth and cell maintenance. Notably, BR6 and BR5 demonstrated similar cell growth rates for the entirety of the fermentation, yet BR6 completed fermentation faster, suggesting it may not be total cell number that is the only cause of increased fermentation rates **(Figure 3.6)**. This was also reflected in final cell count percent differences when compared to the uncontrolled vessel, with 144% and 150% respectively **(Table 3.5)**. Due to the lack of variability in previous experiments, cell viability was not collected.

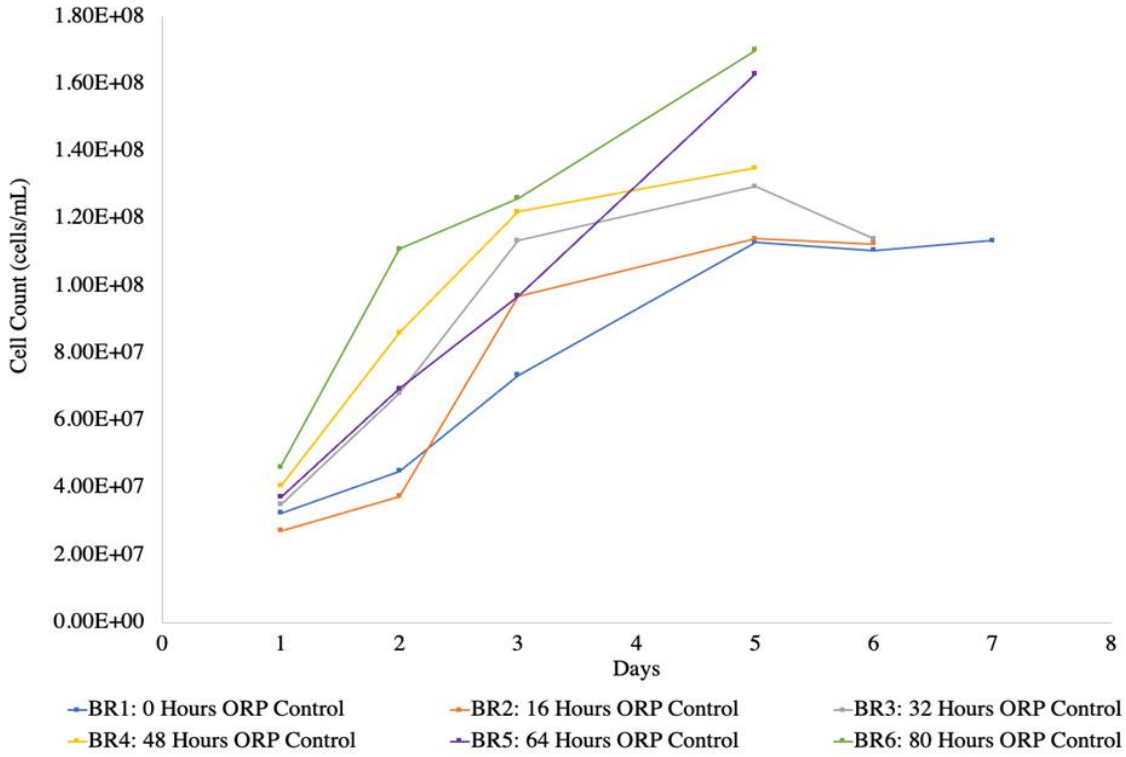


Figure 3.6. Cell densities from experiment 3, where vessels received 0, 16, 32, 48, 64 and 80 hours of ORP control by aeration. Cell counts were taken at every sampling time point until fermentation completed.

3.3.3 Experiment 4 - ORP control in 16 hour intervals with increased aeration

Experiment 4 replicated the experimental design of the previous experiment, using 16-hour time intervals between shut off points. Again, the vessels received 0, 16, 32, 48, 64 and 80 hours of aeration as needed. Attempting to accomplish ORP control at -60 mV once again, the flow rate was increased to 100–150 mL/minute. Solenoids were staggered, opening for 8 seconds. Having no overflow events occur in experiment 3, a 950 mL fill volume was used for the remainder of this research (**Table 2.1)**. To shade from potential sunlight effects, especially on ORP, the vessels were surrounded with cardboard.

Experiment 4 was the most successful of the set point experiments. Though some vessels fell slightly below the -60 mV set point, ORP control was accomplished in nearly all vessels for the entirety of their control intervals. BR2 and BR3 were able to maintain the desired set point until their 16 and 32 hour shut off points. At the 16-hour time point, the ORP of BR4, BR5 and BR6 fell to -85 mV **(Figure 3.7)**. At the 48-hour shut-off time (BR4), the ORP of BR5 rose back to the desired set point. This jump in ORP is also observed in BR6 at the 64-hour shut-off time. These rises in ORP are observed immediately after aeration was shut off in the previous vessel. This pattern of rising ORP values post aeration shut off was also observed in the results of experiment 2 **(Figure 3.3)** and experiment 3 **(Figure 3.5)**. A possible explanation for this pattern could be the accumulation of back pressure prior to the opening of the proceeding solenoid. If this were the case, the vessel following the control shut off would receive a greater burst of air which would ultimately raise its ORP. Lastly, it should be noted that experiment 4 data did not have the oscillations that were observed in previous experiment's curves (**Figure 3.1, Figure 3.3 & Figure 3.5).** This result suggests there is an undefined light reaction occurring that was affecting the ORP and causing these oscillation patterns. Because shading the experiment

reduced noise in the ORP curves, cardboard was used to shade the vessels from diurnal effects for the remainder of this research.

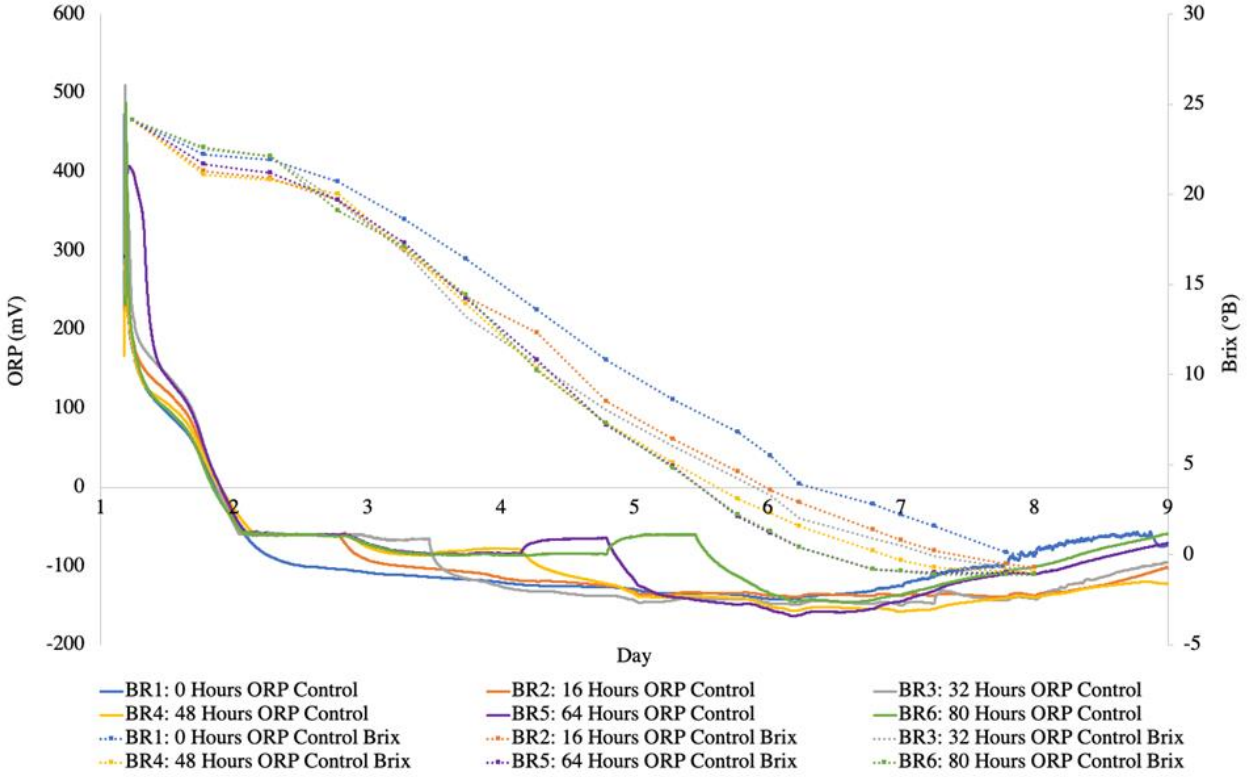


Figure 3.7 A composite figure of ORP curves (solid line) and brix curves (dashed line) from experiment 4, where vessels received 0, 16, 32, 48, 64 and 80 hours of ORP control. The programmed ORP set point for this experiment was -60 mV

As with previous experiments, increased fermentation kinetics were observed in vessels that received ORP control, with all ORP-controlled vessels completing at least 6 hours earlier than uncontrolled BR1. Again, longer ORP control correlated with faster finishing times. BR5 and BR6, the vessels under ORP the longest, completed 30 hours earlier than BR1, demonstrating a 20% acceleration in fermentation completion times. BR5 and BR6 demonstrated very similar Brix curves and completing fermentation prior to the 80 hours sampling point, which suggests that ORP control after 64 hours yields no difference in fermentation kinetics of

these experiments. BR2, BR3, BR4 and BR 5 all demonstrated a 6-hour difference in completion times.

Accelerated fermentation kinetics appear to be correlated with cell count. This trend is clearly depicted in **Figure 3.8.** BR5 and BR6 possessed very similar cell densities throughout the fermentation and the overall highest cell count values of the experiment, 2.75E+08 cells/ mL and 2.78E+08 cells/mL respectively and showed the greatest percent difference from the uncontrolled vessel, BR1 **(Table 3.6)**. These results further support the hypothesis that there is no difference or added benefit when these ferments were controlled at 64 versus 80 hours. BR1, which received no aeration, again demonstrated the lowest cell density of the vessels throughout the course of the fermentation.

Table 3.6 A summary of notable experimental results from experiment 4. In addition, brix, OD and cell counts from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results											
		Brix (°B)			OD			Cell Count (cells/ mL)					
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Uncontrolled Count
4	BR1	22.2	16.4	-0.1	0.191	0.413	0.711	3.80E+07	1.08E+08	1.65E+08	334%	N/A	-
	BR2	21.3	14.3	-0.5	0.197	0.514	0.809	4.53E+07	1.74E+08	1.95E+08	330%		118%
	BR3	22.5	13.2	-0.1	0.187	0.578	0.794	4.83E+07	1.65E+08	2.28E+08	372%		138%
	BR4	21.1	13.9	-0.3	0.127	0.599	0.933	4.85E+07	1.95E+08	2.58E+08	431%		156%
	BR5	21.7	14.2	-0.8	0.171	0.620	0.919	4.73E+07	1.87E+08	2.75E+08	482%		167%
	BR6	22.6	14.4	-0.8	0.132	0.618	0.921	4.73E+07	1.84E+08	2.78E+08	487%		168%

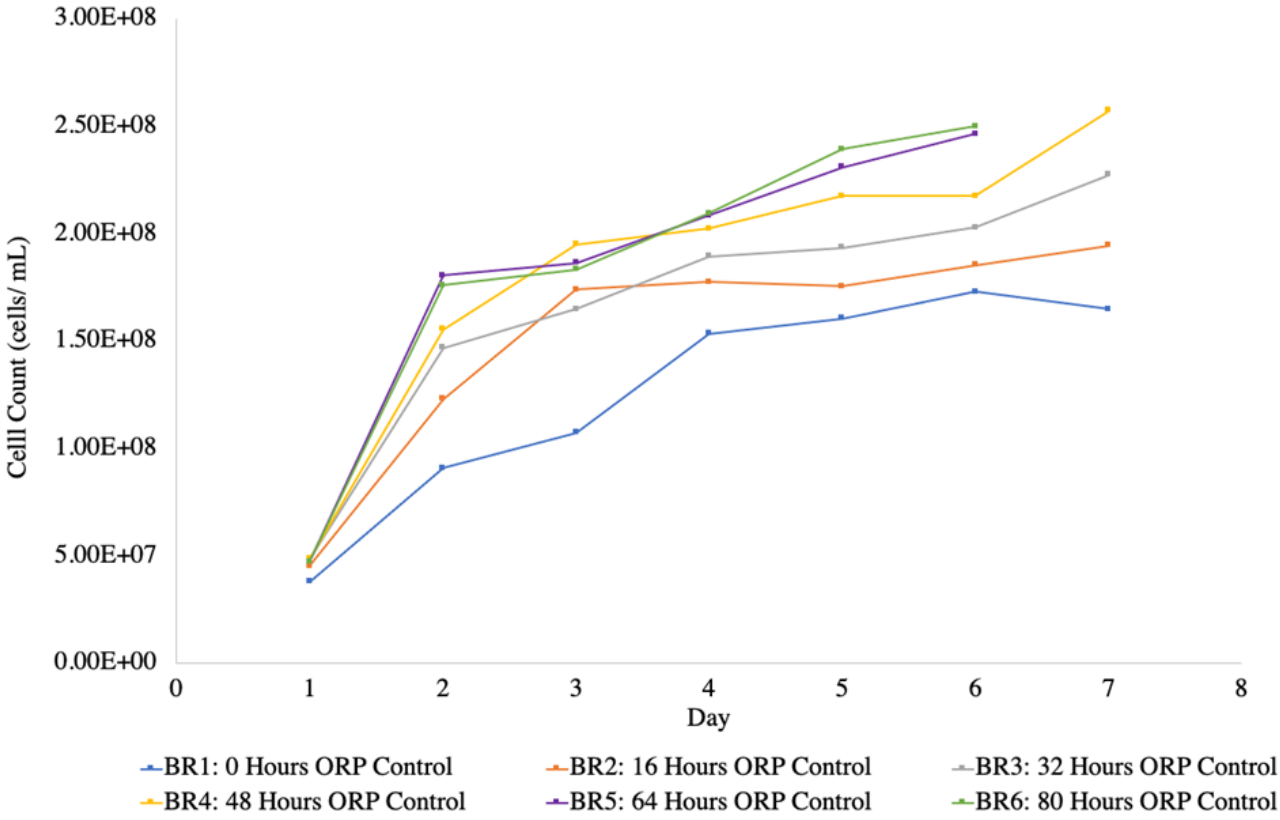


Figure 3.8. A composite figure of vessel cell densities from experiment 4, where vessels received 0, 16, 32, 48, 64 and 80 hours of ORP control. Cell counts were taken during the morning sampling time point until fermentation completed.

3.4 ORP Control across various set points

3.4.1 Experiment 5 - impact of 0 to -120 mV ORP set points on fermentation

Experiment 5 was the first of the three experiments (experiments 5–7) that investigated the ability to obtain ORP control across various set points. This set of experiments sought to further define the relationship between ORP control and fermentation outcomes, specifically the ORP control threshold required to achieve previously observed outcomes. Six vessels were controlled at various set points, 0, -30, -60, -90 and -120 mV. The air flow rate was set to 150 mL/minute and solenoids opened for different amounts of time across vessels depending on the set point value. The strategy is that the higher ORP set point would require more air for control. Solenoids were opened for 12 seconds in vessels controlled at 0 and -30 mV, while open 8

seconds in vessels controlled at -60 mV, -90 and -120 mV. Solenoids again opened on a 60 second loop **(Table 2.1)**.

Desired ORP control was achieved in BR5 at the -90 mV set point (**Figure 3.9)**. In BR2, 0 mV control was not able to be achieved after 6 hours of the solenoids opening for control. This immediate loss of ORP control likely meant that opening solenoids for 12 seconds was not long enough to deliver the amount of air needed to control at 0 mV. Though the set point value 0 mV was not achieved BR2 maintained the highest ORP throughout the ferment, reaching an ORP minimum of -55 mV. Interestingly, regardless of receiving aeration the entirety of the fermentation BR3 did not respond to the ORP control, reaching the lowest ORP minimum of all the vessels, -130 mV. BR3's ORP, despite aeration, fell lower than BR1 the vessel left uncontrolled. The reason for this observation is currently unknown. For BR4, control to -60 mV was not achieved, but held at -77 mV nearly the entire fermentation. ORP control at -120 mV was not needed in BR6, as the ORP never hit this set point value, reaching an ORP minimum of only -117 mV. This was an unanticipated outcome, as ORP minimums observed in all previous experiments were between -170 and -200 mV. Because ORP control was never turned on in BR6 and BR1 were replicate runs and thus shared similar ORP curves. Drastic spikes in the ORP curves at the end of fermentation correspond with volume loss due to final sampling at each vessel's completion time point.

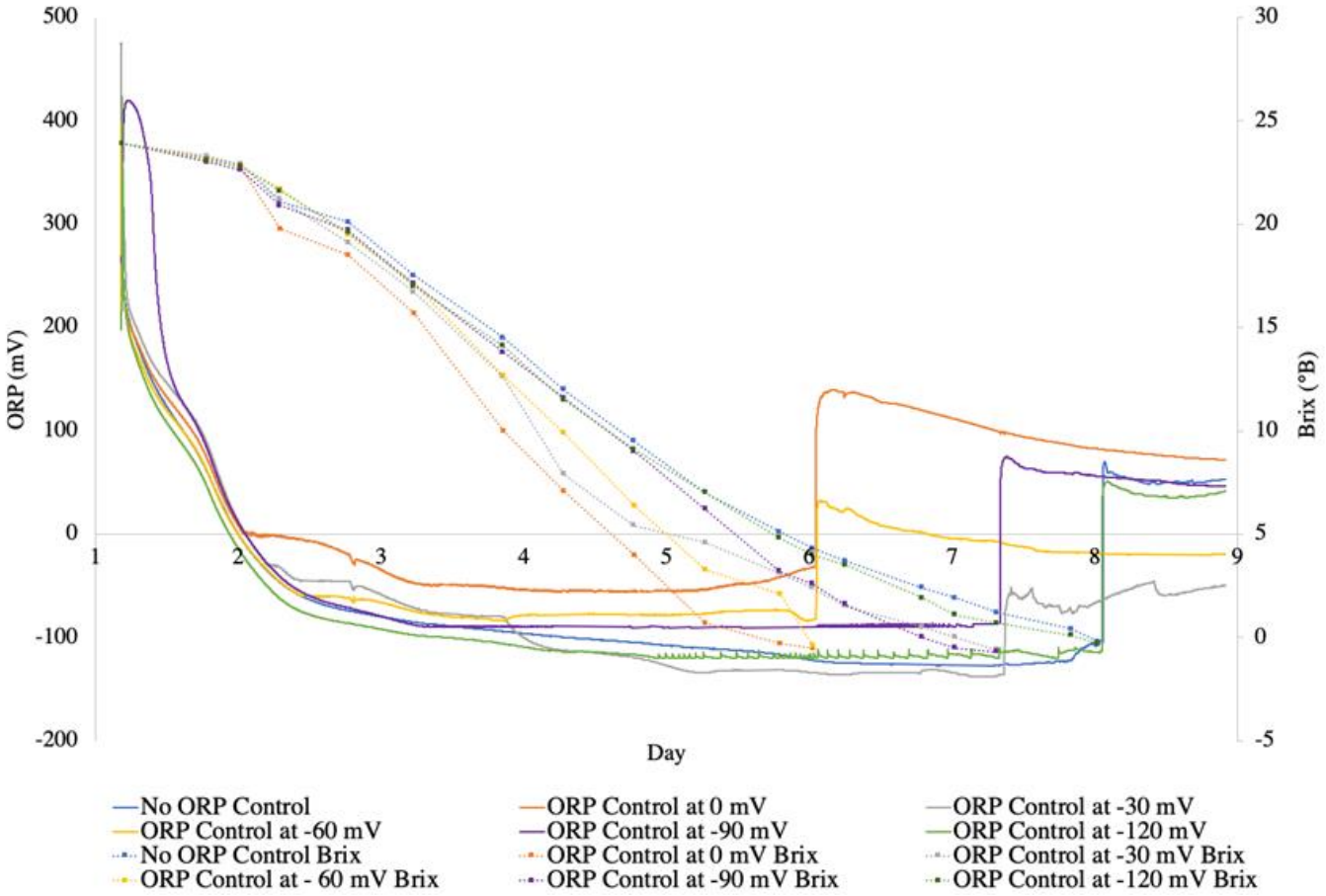


Figure 3.9 Impact of ORP control set-point by aeration on measured ORP (solid line) and brix (dashed line) from experiment 5, where ORP was controlled at various set points, 0, -30, -60, -90 and -120 mV across vessels. The large increases in ORP starting on day 6 was due to probe removal from the vessel.

Table 3.7 A summary of notable experimental results from experiment 5. In addition, brix, OD and cell counts from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results											
		Brix (°B)			OD			Cell Count (cells/ mL)					
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Uncontrolled Count
5	BR1	23.2	14.5	-0.2	0.142	0.399	0.627	3.08E+07	1.08E+08	1.28E+08	316%	N/A	-
	BR2	23.2	10	-0.3	0.132	0.569	0.924	3.50E+07	2.05E+08	2.47E+08	604%		193%
	BR3	23.3	12.6	-0.6	0.12	0.462	0.823	3.35E+07	1.65E+08	2.02E+08	501%		157%
	BR4	23	12.7	-0.4	0.119	0.349	0.802	3.60E+07	1.86E+08	2.26E+08	528%		177%
	BR5	23	13.8	0	0.148	0.399	0.648	3.38E+07	1.79E+08	2.12E+08	528%		166%
	BR6	23.1	14.1	-0.3	0.132	0.399	0.679	3.00E+07	1.33E+08	1.42E+08	372%		111%

BR2 demonstrated the fastest fermentation kinetics and earliest finishing time, finishing 30 hours earlier than uncontrolled vessels. As replicates, both receiving no ORP control, BR1 and BR6 demonstrated analogous fermentation kinetics and possessed similar Brix curve profiles, including a shared completion time point. Vessel finishing times correlated with ORP, where vessels that maintained greater ORP minimums throughout the fermentation demonstrated faster fermentation kinetics. The only vessel this was not observed in was BR3, who finished prior to the vessels left uncontrolled despite ultimately reaching a lower ORP minimum. This difference in finishing times could be attributed to the overall greater cell density in BR3 in comparison to that of BR1 and BR6.

As anticipated, BR1 and BR6 demonstrated the lowest cell counts throughout fermentation, reaching 1.28E+08 and 1.42E+08 respectively (**Table 3.7)**. BR2 reached the highest cell population overall and possessed the greatest cell densities throughout fermentation of all vessels (**Figure 3.10)**. These cell numbers correlate with the greatest ORP minimum values and thus greatest control intervention via aeration. Though BR3 and BR4 had similar cell counts on day 2 of fermentation, it is clear that once ORP control was lost in BR3 a decline in cell population was observed. BR2, BR4 and BR5 demonstrated the same pattern as observed in previous experiments, where greater ORP control correlates with greater cell densities and earlier finishing times.

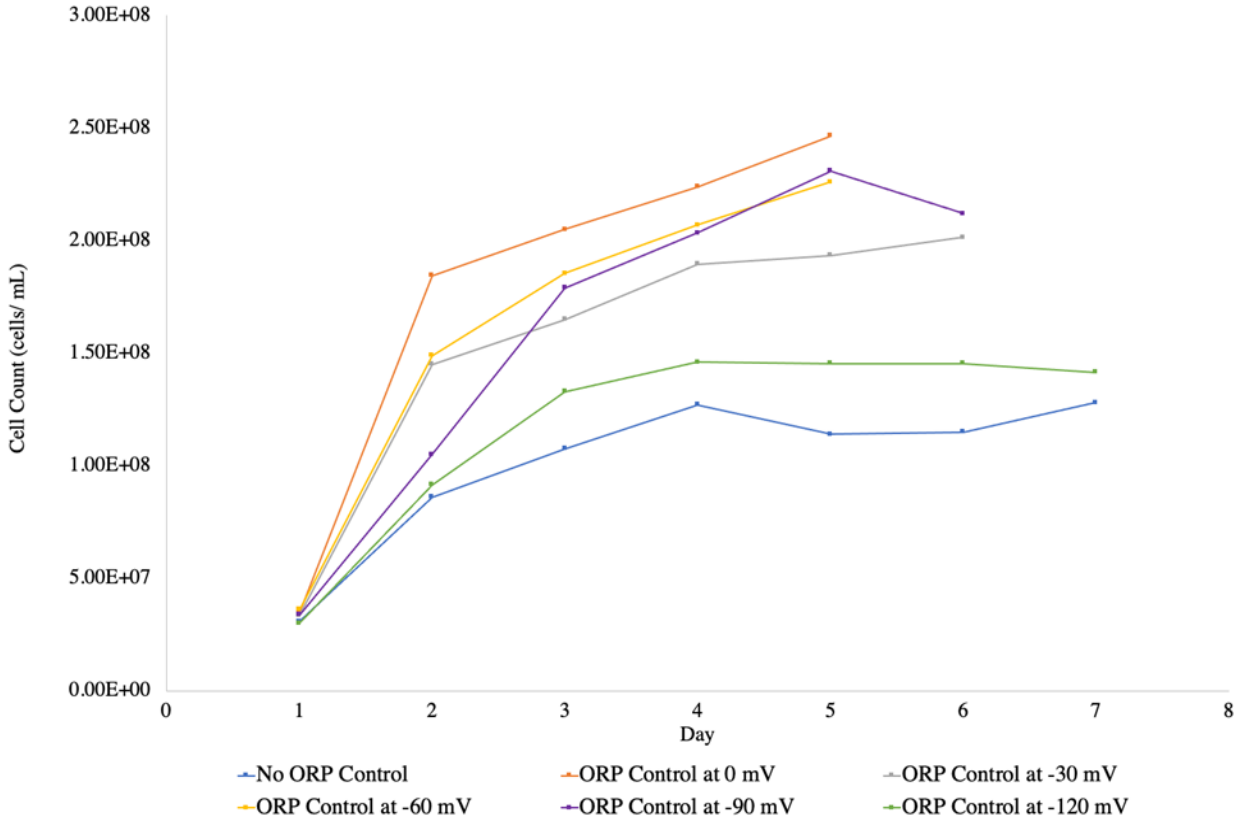


Figure 3.10 Cell densities from experiment 5, where ORP was controlled in vessels at various set points: 0, -30, -60, -90 and -120 mV. Cell counts were taken at each morning sampling time point until fermentation completed.

3.4.2 Experiment 6 - impact of -30 to -90 mV ORP set points on fermentation #1

Experiment 6 consisted of 4 vessels, one with no ORP control and the latter three controlled at -30mV, -60 mV and -90 mV (**Table 2.1**). With minimal control achieved under experiment 5's parameters, the solenoid opening regiments were adjusted. Solenoids now opened for varying times depending on the desired control set point, with higher set points having longer opening periods. At the start of this experiment, the solenoid for the -30 mV set point was originally opened for 24 seconds but was adjusted to 16 seconds later in the fermentation. For control at -60 mV and -90mV, solenoids opened for 18 and 12 seconds, respectively. In this experiment, a "dummy" solenoid was introduced. This "dummy" solenoid would open for 2

seconds prior to each vessel's pneumatic solenoid in order to release any back pressure that might have accumulated in the air lines. Dummy solenoid 1 was paired with solenoid 4 in BR2, solenoid 2 with 5 in BR3 and solenoid 3 with 6 in BR4. With the availability of the extra ORP probes, two probes were put in both BR1 and B3. This allowed for variability between probes to be observed **(Figure 6.4.2)**.

As pictured in **Figure 3.11**, ORP control was achieved at the approximate set points in BR2, BR3 and BR4. Again, BR3 held at the -90 mV set point steadily throughout the course of the fermentation. These replicate results confirm that solenoid opinion regiment and flow rate do deliver adequate air to maintain this set point. BR3 held at -60 mV for the first 2 days before falling to -75 mV for the remainder of the fermentation. Though control at exactly -60 mV was not achieved, the results yielded useful insight on how to adjust air delivery in future runs. Control in BR2 at the -30 mV set point was achieved throughout the course of the fermentation. The saw tooth pattern observed in the ORP curve is indicative of excess air delivery, causing the ORP to spike and fall after each delivery event. This unexpected response can be attributed to an overflow event that occurred shortly after BR2's first solenoid opening. This event was significant, resulting in one third of the vessel's volume lost. With a considerably less volume in the system, the programmed air delivery resulted in a dramatic ORP response. In an attempt to account for this loss, the solenoid opening time was reduced from 24 to 16 seconds. This overflow was the result of a flowmeter malfunction delivering air at a rate of 450 mL/ min for the first 6 hours of control. After this initial period, the flowrate was reset to the desired 150 mL/ min flow for the duration of the ferment. Though less dramatic, this increased flow can be observed in BR3 and BR4 ORP curves as a shorter and less notable saw tooth pattern. BR1, under no ORP control, reached the lowest ORP values, reaching a minimum of -155 mV.

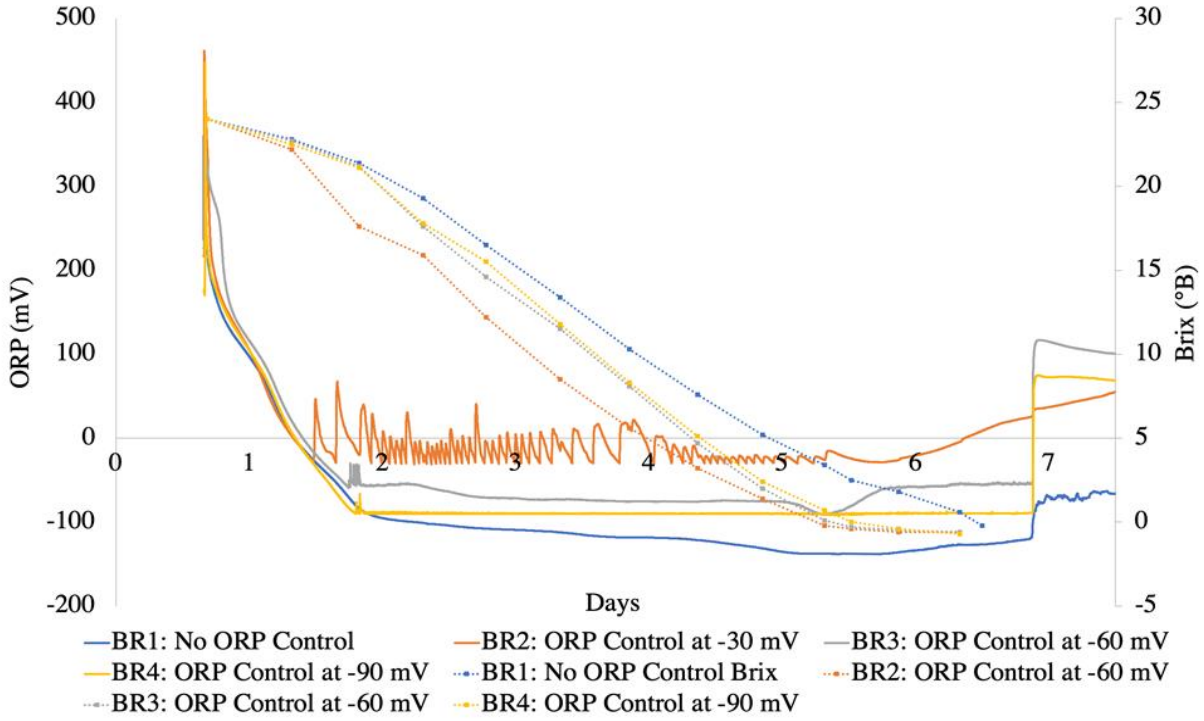


Figure 3. of ORP control set-point by aeration on measured ORP (solid line) and brix (dashed line) from experiment 6, where ORP was controlled at various set points-30mV, -60 mV and -90 mV across vessels.

This pattern of increased fermentation kinetics and greater ORP control persisted in Experiment 6 (**Figure 3.11**). A 6-hour difference in fermentation completion times occurred consecutively across the -30, -60 and -90 set points. BR2 demonstrated a 20% acceleration in fermentation finishing time in comparison to BR1, completing 30 hours earlier. BR2's accelerated fermentation kinetics could be observed as early as day 3 of the fermentation, reaching 8.5°B, while others ranged between 11.5–13.4°B (**Table 3.8**). BR3 and BR4 demonstrated comparable Brix measurements on day 3, 11.5°B versus 11.8°B, suggesting they were showing similar fermentation kinetics up to that point before diverging.

Difference in finishing times can be interrelated with difference in ORP values and cell densities. Cell count data from experiment 6 demonstrated obvious differentiations in cell densities across treatments (Figure 3.12). Higher ORP values and greater magnitudes of ORP

control correlated with higher cell densities. When comparing BR2 and BR3, the vessels controlled at -30 mV and -60 mV, respectively, a notable final cell count percent difference from BR1 was observed for each of, 429% and 331% (Table 3.8). Final cell count numbers of these two vessels demonstrated a magnitude of ten-fold difference in cell numbers in comparison to that left uncontrolled. This marginal difference in cell numbers is a large contributor to the accelerated fermentation kinetics, resulting in the 30 hour difference in finishing time between vessels.

Table 3.8 A summary of notable experimental results from experiment 6. In addition, brix, OD and cell counts from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results											
		Brix (°B)			OD			Cell Count (cells/ mL)					
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Uncontrolled Count
6	BR1	22.8	13.4	-0.2	0.18	0.493	0.615	2.93E+07	7.10E+07	7.00E+07	139%	N/A	-
	BR2	22.2	8.5	-0.2	0.22	0.733	0.768	3.10E+07	1.23E+08	1.64E+08	429%		234%
	BR3	22.7	11.5	-0.3	0.202	0.682	0.787	3.43E+07	1.09E+08	1.48E+08	331%		211%
	BR4	22.5	11.8	-0.4	0.188	0.654	0.716	3.23E+07	7.55E+07	9.05E+07	181%		129%
	BR5	-	-	-	-	-	-	-	-	-	-		-
	BR6	-	-	-	-	-	-	-	-	-	-		-

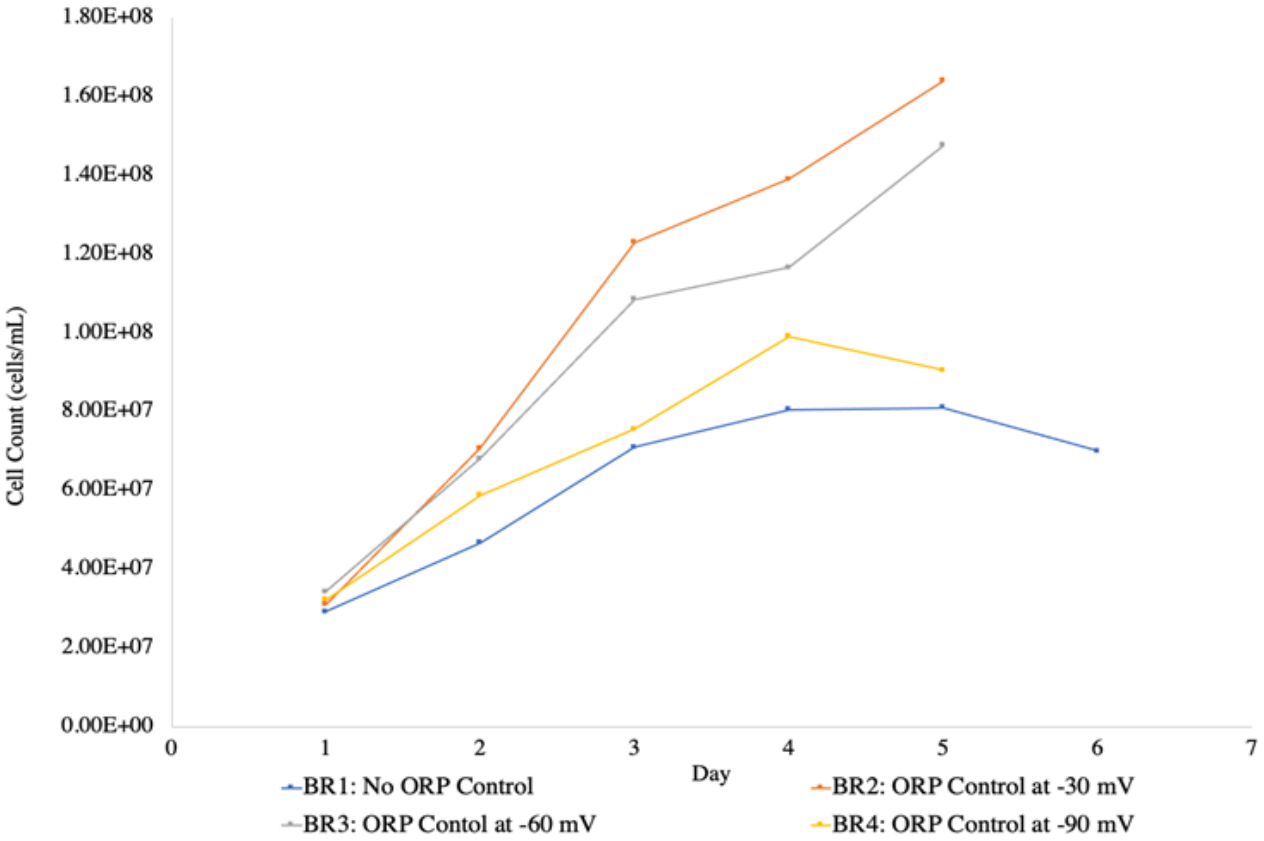


Figure 3.12 Cell densities from experiment 6, where ORP was controlled in vessels at various set points, -30, -60 and -90 mV. Cell counts were taken at morning sampling time points until fermentation completed.

3.4.3 Experiment 7 - impact of -30 to -90 mV ORP set points on fermentation #2

Experiment 7 served as a duplicate of experiment 6, where ORP was controlled at -30, -60 and -90 mV across vessels on a 75 second loop (**Table 2.1**). Adjustments to the solenoid regiments were made in attempts to gain better control at the programmed set points. At the start of the experiment, BR2 had an opening time of 24 seconds which was adjusted to 28 seconds midday on day 1 as its ORP began to fall below the -30 mV set point. BR3's opening time was increased from that used in experiment 6 to 20 seconds. Due to the success in experiment 6, the solenoid opening time for BR4 was kept at 12 seconds. Solenoid opening was staggered on a 75

second loop. The same “dummy” solenoid pairing was used in this regiment, where solenoid 1 was paired with solenoid 4 in BR2, solenoid 2 with 5 in BR3 and solenoid 3 with 6 in BR4. Again, with the availability of ORP probes, two probes were put in both BR2 and BR4 to observe any variability between them (**Figure 6.4.2)**

Desired ORP control was only accomplished in BR4 at -90 mV (**Figure 3.13**). Despite increasing the solenoid opening time in BR2 to 28 seconds ORP control at -30 mV was never accomplished. The ORP minimum in BR2 reached as low as -75 mV. While control at this set point was accomplished in experiment 6, it is likely the differences in the ratio of vessel volume to air proved inadequate to achieve control. Though air delivery was increased in BR3 for this experiment, ORP control at -60 mV was not accomplished. BR3 ORP minimum fell to -85 mV, 10 mV lower than observed in experiment 6. This result was unexpected as BR3’s air delivery time was increased by 2 seconds in response to the results of experiment 6. When under no ORP control, BR1’s ORP fell to an ORP minimum of -165 mV. ORP curves yielded from this experiment demonstrate a wavy pattern. This result was anticipated as surrounding the vessels with cardboard during setup was neglected. These results further support the hypothesis that light reactions are occurring during diurnal shifts that do affect ORP values.

Vessels controlled at higher set points again demonstrated overall faster fermentation kinetics and earlier finishing times **(Figure 3.13)**. When comparing the vessel controlled at the highest set point, BR2, and that left uncontrolled, BR1, BR2 demonstrated a 38% acceleration in fermentation finishing times. The acceleration observed in this experiment was prominent as it was nearly double that observed in other experiments (i.e., ~20%). BR2, BR3 and BR4 finished consecutively in 12-hour intervals. Comparatively to experiment 6, this run demonstrated longer differences in finishing times between -30 mV, -60 mV and -90 mV set points.

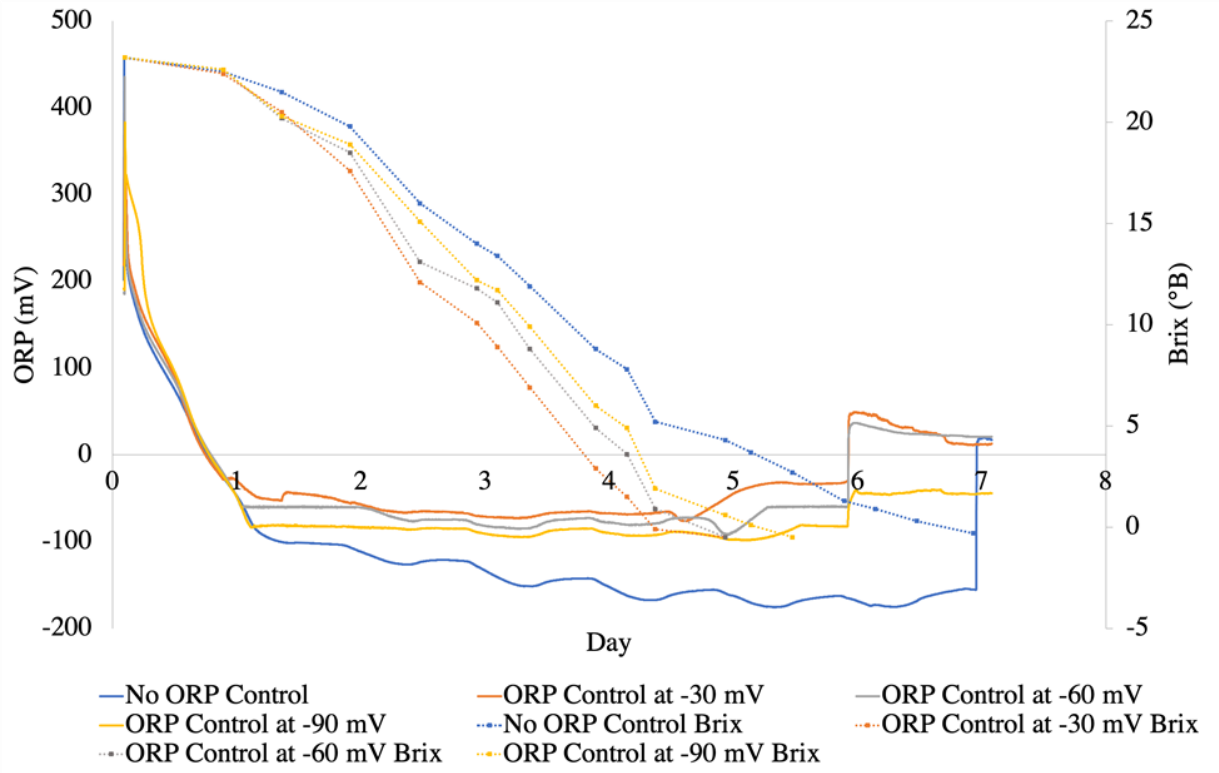


Figure 3.13 Impact of ORP control set-point by aeration on measured ORP (solid line) and brix (dashed line) from experiment 7, where ORP was controlled at various set points-30mV, -60 mV and -90 mV across vessels. Large increases in ORP starting on day 6 was due to probe removal from the vessel.

Table 3.9 A summary of notable experimental results from experiment 7. In addition, brix, OD and cell counts from day 1, day 3 and day of fermentation completion, "final", are reported. Percent differences in final cell count versus vessel's initial cell count, as well as percent difference of vessel's final cell count versus the experiment's uncontrolled vessel's final cell count is also shown.

Experiment	Vessel	Results											
		Brix (°B)			OD			Cell Count (cells/ mL)					
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Final Cell Count % Difference from Initial Count	Triplicate Average Final Cell Count	Final Cell Count % Difference from Uncontrolled Count
7	BR1	22.5	14.0	-0.3	0.208	0.511	0.588	3.78E+07	8.05E+07	1.40E+08	271%	N/A	-
	BR2	22.4	10.1	-0.7	0.491	0.822	0.847	4.13E+07	2.24E+08	2.59E+08	527%		185%
	BR3	22.6	11.8	-0.5	0.329	0.701	0.761	4.23E+07	1.31E+08	2.40E+08	467%		171%
	BR4	22.6	12.2	-0.5	0.339	0.599	0.699	3.48E+07	8.20E+07	1.71E+08	392%		122%
	BR5	-	-	-	-	-	-	-	-	-	-		-
	BR6	-	-	-	-	-	-	-	-	-	-		-

As observed in experiment 6, there is clear differentiation in cell count values across the ORP set points. This similarity is clearly illustrated when comparing the trends observed in both Figure 3.12 and **Figure 3.14.** One difference between the experiments is that cell count numbers in experiment 7 were comparatively higher overall than that in experiment 6 (**Table 3.9**). Regardless, cell densities proved to be greater in vessels controlled at higher ORP values. BR2 showed a rapid increase in cell population over the course of its 4-day fermentation, demonstrating a 527% difference from its initial cell population on day 1 (**Table 3.9**). This jump in cell numbers surely contributed to rapid fermentation kinetics that BR2 displayed. It is interesting to note that large differences in cell growth were exhibited between BR2 and BR3 versus BR4. Referencing the percent difference in cell counts from the uncontrolled vessel, BR2 and BR3 demonstrated 185% and 171% difference, while BR4 demonstrated only a 122% difference (**Table 3.9**). Referring back to the previous runs (experiment 5 and 6), this trend in cell numbers and percent differences was also observed. While the intended difference between BR3 and BR4 was 30 mV, a difference of only 5 mV was accomplished. These dramatic differences in achieved cell density may be due to the differences in solenoid opening times, as BR3 received 8 seconds more aeration each solenoid opening.

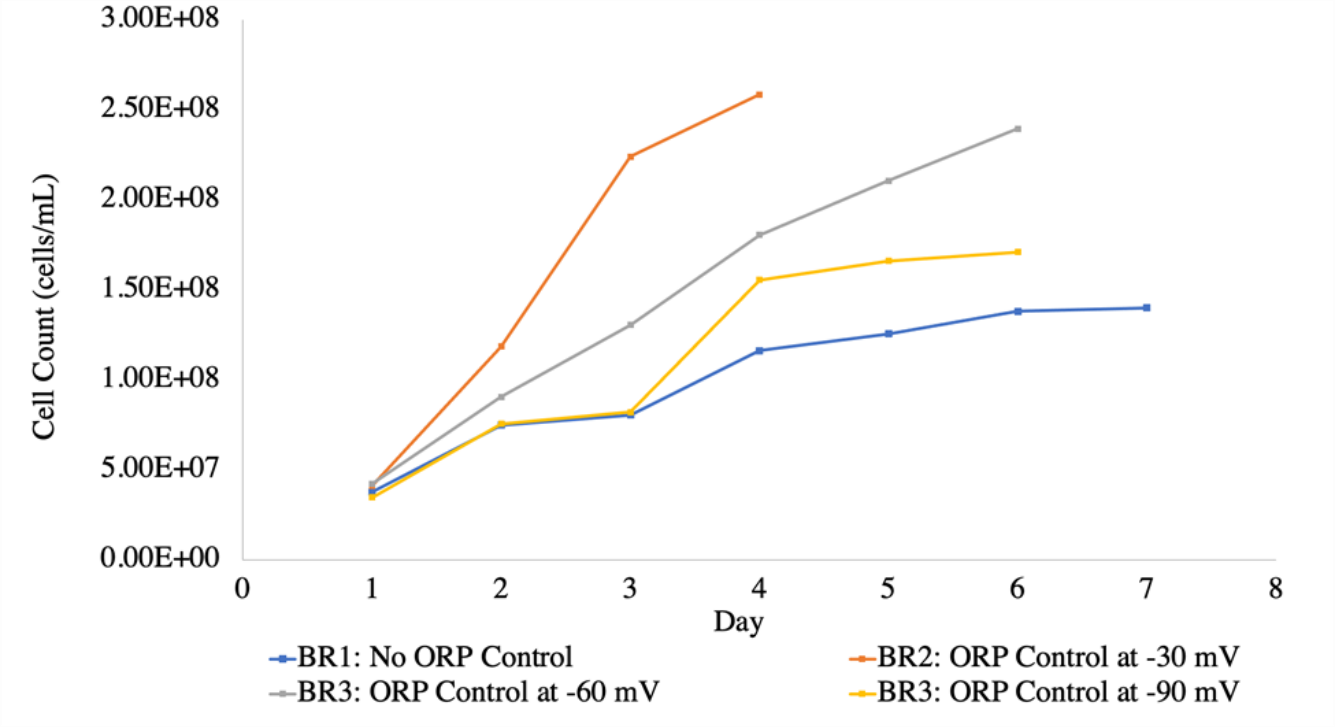


Figure 3.14 Cell densities from experiment 7, where ORP was controlled in vessels at various set points, -30, -60 and -90 mV. Cell counts were taken at morning sampling time point until fermentation completed.

4. General Discussion

ORP, redox status, is a real time metric that reflects both the chemical and metabolic state of a fermentation. Understanding redox status and its impacts on fermentation health and wine quality will better aid winemakers in making more informed and timely fermentation decisions. It is long been established that aeration is a viable method to raise ORP. Controlling ORP is a useful tool as it has the potential to facilitate more robust fermentations which in turn may avoid costs associated with sluggish or stuck fermentations as well as undesirable reductive wine character. Despite the wine industry's increasing adaptation of this emerging parameter, ORP is still relatively undefined and not well understood. This work

investigates the effects of oxygenation on ORP at the lab scale across varying aeration control intervals and setpoints.

4.1 The fermentation medium

While previous studies have explored ORP and its control mechanisms, those works were conducted across varying mediums (e.g., wine, nutrient medias, etc.) and have thus elicited varying ORP responses and results. Given that ORP is a matrix dependent variable that is affected by factors such as nutrient status and chemical composition, it becomes challenging to make true comparisons across studies. In response to these discrepancies, this work was conducted using a white juice concentrate as the fermentation medium. The goal in using concentrate was to mitigate variation in juice conditions between experiments. Using concentrate at the 1 L lab scale also allowed for multiple experiments and replicate runs to be conducted in a controlled setting which enabled continued research outside the bounds of the short harvest season.

Overall, nitrogen levels in the concentrate used here were relatively high in comparison to the initial juice conditions of concentrates used in other work [41][42][43]. Independent of the nitrogen status of the juice panel, SpringFerm was added to each ferment to account for any sterols, minerals and vitamins that may have been altered in the concentrate production process [44].

One of the biggest challenges working with the concentrate was homogenizing it before taking aliquots for each experiment. Given the shape of these particular concentrate vessels, mixing prior to sampling was not an option. Differences in appearance, color and viscosity of the concentrate were observed, indicating separation had occurred. This likely contributed to slight differences observed in initial juice panels **(Table 3.1)**. To combat the temperature control issues

in the winery's cold storage, concentrate was diluted the morning of the experiments. Keeping the concentrate in its high Brix form: 1) provided protection of the concentrate lot in storage, as most fermenting microbes cannot prevail in an environment with such a high osmotic pressure and 2) limited the window in which spontaneous fermentation could occur in the diluted form prior to being inoculated [41][43][45].In future work, to ensure best practice the entirety of the concentrate vessel should be diluted to a desired Brix, stored in cold room (<25°F) and remixed before use.

Though previous work has successfully demonstrated the ability to control ORP in both synthetic media and wine fermentation mediums, the concentrate medium is unique from the two and dramatically differs from a red wine ferment [3][9][33][34][35][36]. Wine represents a complex redox system due to its chemical composition. From a redox control perspective, some components of interest include Cu, Fe, and Mn as the oxidation state of these metal cations determines the redox response to oxygenation [6][11]. These metals are significant in that they act as catalysts in oxidation reactions. Electron carriers, metal ions complex with oxygen to produce hydrogen peroxide and subsequently a hydroxyl radical, raising redox state [2][6][11][27]. Wine oxidation is therefore mediated by the redox cycling of the Cu/ Cu (II) Fe (III)/Fe (II) couples between oxidized and reduced states [11][27]. To date, no ORP control research has been conducted using grape juice concentrate. The initial experiment sought to confirm that the concentrate medium possessed the chemical complexity necessary to elicit redox response to oxygenation. Though the -20-mV control set point was not achieved in experiment 1 (**Figure 3.1)**, the ORP of controlled vessels demonstrated continuous response to aeration. These findings confirmed that the concentrate possessed chemical components necessary to support

redox cycling. It was concluded that this concentrate was a suitable fermentation medium for this work.

Lastly, it is recognized that the concentrate fermentation media does not encapsulate the complexity of a true wine matrix that may impact ORP and its' response to oxygenation, especially in red ferments. The use of concentrate does, however, offer a sound environment for debunking ORP fermentation behavior in "wine like" conditions. In addition, it enabled repeated troubleshooting of a newly developed redox control system, an ongoing challenge throughout this work.

4.2 Troubleshooting a new ORP control system

The first part of this work aimed to put in place and develop an ORP control system at the lab scale. The first step of this pursuit included putting an air delivery line in place and building a pneumatic control box, like that which is used at the UC Davis Research Winery for the lab **(Figure 2.3).** This control system was built around a bench top where the bioreactor vessels, a water bath and functioning ORP probes were already in place. Experiment 1 served as an initial assessment of the functionality of the new control system. It sought to confirm that all electronics were working properly, that no issues persisted in terms of remote control and communication, and lastly that air was successfully delivered from the cylinder to each of the bioreactors. The results of experiment confirmed a working system. It also highlighted the sensitivities associated with air delivery and affirmed that the optimal flow rate needed to be further explored.

A significant challenge faced throughout this work was the series of overflow events associated with excessive air flow or insufficient gaseous pressure release. These events occurred in experiments 1, 2, and 6. On day 1 of experiment 1, at the time of the overflow event an increase in air flow from 50 mL/ min to 200 mL/min was noted. The cause of this fluctuation is

unknown. It was initially assumed that this increased flow rate was the cause of the overflow. At this time the flow rate was readjusted to 50 mL/min and solenoid opening times were increased **(Table 2.1)**. This resolved the overflow issue but was not sufficient to achieve ORP control at the desired set point, -20mV.

In experiment 2, flowrate was initially set to 100 mL/min and was further adjusted to 150 mL/min when ORP fell below the -60 set point **(Figure 3.3)**. ORP control was initially achieved in BR3-6 for nearly 2.5 days before another overflow event occurred. This outcome led to the re-evaluation of the carbon dioxide (CO_2) output hose. In order to avoid gaseous pressure, build up in the initial setup, each vessel had a polyethylene tube attached to an overlay pipet that fed into a bucket of water **(Figure 2.1)**. In experiment 1 and 2, ¼" ID tubing was used. The outcomes of these experiments resulted in adjustments to the experimental setup. To avoid pressurization and overflow events in future work the following changes were made the ¼" ID tubing was replaced with 1" ID tubing, the fill height of the vessels was decreased to 950 mL, solenoid opening times were staggered and the flow rate was adjusted. It should be noted that loosening of the sampling ports was a sign of pressurization. For this reason, double checking that all sampling ports were tightly closed at each sampling point became habit.

Experiment 3 was the first run with no overflow event. This experiment confirmed that 950 mL was a sufficient fill volume to measure ORP in a 1L BR. Previously, it was unknown how much the vessel volume could be decreased before interfering with the ORP measurements. It is worth noting that the flow rate range documented for experiment 3 in **Table 2.1** was not the result of an adjustment, but of drift. This repetitive drift from the set value resulted in the addition of a daily flow meter check when sampling. Given the lack of control at -60mV at 75-100 mL/min, the flowrate was increased back to 150 mL/min for experiment 4. This experiment

demonstrated the greatest control ability to date. The success of experiment 4 demonstrated that 150 mL/min was an adequate flow rate for control at -60 mV. It also confirmed hosing changes allowed for sufficient pressure release. The output house with the 1” ID was also permanently adapted in this setup.

When the overflow event in experiment 6 was discovered there had been a jump in flow rate from 150 mL/min to 400 mL/min in the first 12 hours between sampling times. This event resulted in the greatest volume loss of all the experiments. The cause of this momentous jump in flow is unknown. Reoccurring drift incidents called the integrity of the flowmeter into question. Given that overflow events seem to perpetuate loss of ORP control, it is imperative that a reliable flowmeter is used. From this work it was concluded that a flowrate of 150 mL/min was a sound flowrate for this lab setup. It is interesting to note that flowrate used in Killeen et al. (2018) work was reported as ~40 L/min [3][9]. This work differed in that aeration occurred via a nominal 20 µm sparging stone. The benefit of using a sparging stone is that it creates smaller bubbles which increases mixing and enlarges bubble surface area thus increasing the rate of oxygen dissolution in juice [3][9]. It should be noted this work was conducted using 100L volumes, significantly larger than the volume at hand. Other works have demonstrated ORP control in 4.75 L working volumes at a flow rate of 2.5 L/min [36]. At the 100 mL working volume scale an additional study demonstrated ORP control at a flow rate of 0.5L/min [33][34]. Discrepancies between research suggest further work needs to be conducted to determine optimal flowrate as well as the incorporation of sparging stones at the lab scale.

4.3 Interpreting the relationship between ORP control, fermentation kinetics, cell counts and viability

4.3.1 ORP control

Experiment 1 differed in design from those to follow in that it explored just two experimental conditions, ORP control at -20 mV and no ORP control, in triplicate **(Table 2.1)**. This work sought to develop a new pneumatic ORP control system at lab scale. While other pneumatic ORP control at lab scale has been reported in the literature, this works system design was adapted from Killeen et al. (2015 & 2018) work done at the UC Davis research winery. Using the knowledge gained from each experiment, the system and protocol evolved throughout this work. Killeen et al.'s previous work has demonstrated the ability to control ORP at a -20 mV set point (200 mV SHE) [3][9]. For this reason, -20 mV set point was chosen to test the new system design and parameters. Though clear differences in triplicates were observed, ORP control at -20 mV was not achieved **(Figure 3.1)**. This failure to obtain control served as a starting point for equipment and parameter adjustment. The differentiation observed in aerated and unaerated triplicates confirmed two things, 1) oxygen, even if an incorrect dosing, was being introduced to the system and 2) that the concentration was a compositionally sound medium for this work as it demonstrated a clear response to oxygenation.

Once the functionality of the pneumatic ORP control system was assessed, the next set of experiments (experiments 2-4) aimed to investigate the effects of ORP control over various time intervals throughout the fermentation **(Table 2.1)**. To do so, the optimal length of the ORP control interval as well as control set point required further exploration. Using the fermentation completion times observed in experiment 1 (i.e., ~7 days) and the number of vessels available, 24-hour ORP control intervals were examined in experiment 2. This set of experiments aimed to

explore 6-time interval conditions per run. However, given experiment 2 was the initial attempt employing the new experimental design, both the fermentation completion time and time intervals were overestimated. This resulted in duplicate 0 hours control BRs, a 48 hour control BR, 72 hour controlled BR, and duplicate 96 hour control BRs. The interval time was then shortened to 16 hours for experiments 3 and 4 to better encompass shutoff intervals within the time constraints of a fermentation (<96 hours).

Given the -20 mV set point used in experiment 1 was unsuccessful, the ORP set point for experiment 2 was decreased to -60 mV in hopes of more easily obtaining ORP control. This ORP set point value was chosen keeping the spontaneous formation of hydrogen sulfide or ORP "danger zone" in mind [4][5][25]. To reiterate, the reduction potential for the sulfur-hydrogen couples at wine pH is -70 mV [2][5]. Meaning that when ORP falls below -70 mV the spontaneous chemical reduction of elemental sulfur into H_2S occurs [2][5][6]. In choosing a target set point above -70 mV, this work aims to remain in the scope of practical use of for ORP control in the wine industry. The -60 mV set point was used in experiments 2-4. While only experiment 4 demonstrated clear and continuous control at -60 mV across shut off points **(Figure 3.7)**, experiment 2 and 3 aided in establishing best flow rate, solenoid opening time, fill height and gaseous evacuation set up **(Table 2.1)**.

Once control at -60 mV was accomplished, experiments 5-7 sought to examine ORP control across various set points. The goal of these experiments was to 1) define the parameters in which to achieve desired set point control and 2) investigate the impact across set points on fermentation outcomes. Experiment 5 controlled across 0, -30, -60, -90, and -120 mV. This range of set points was chosen with goals of reaching higher ORP control ability as seen in the literature while remaining above the ORP minimums observed in previous experiments

[1][3][9][33][34]. With only 4 vessels being available, experiments 6 and 7 controlled across -30, -60 and -90 mV set points. To accomplish control across desired set points solenoid opening times were adjusted, where longer opening times coincided with greater ORP set points.

With the adjusted parameters, experiment 5 proved to be overall unsuccessful as desired control was only achieved at the -90-mV set point. The results of this experiment were perplexing in that BR6 ORP never fell to -120 mV set point. This was far above the ORP minimum observed in the previous runs. The initial inclination is to look for any difference is initial juice conditions that may have persisted **(Table 3.1).** Differences in conditions that could have affected yeast metabolism thus ORP profile (i.e., nitrogen sources, pH, etc.), but no outstanding differences were observed in experiment 5 versus previous juice panels.

In addition, this result was peculiar in that BR3, set to -30 mV, fell below the ORP minimum of BR6 to -130 mV despite oxygenation (**Figure 3.7)**. These results, called into question the air delivery system for BR3 and resulted in a more vigorous cleaning protocol of air lines to the protocol. In the case that cell debris and tartrates were clogging the line, the lines would now be soak in alcohol the morning before use. In addition, despite no change in parameters or protocol from experiment 4, controlling ORP at -60 mV was not achieved (**Table 2.1)**. The results of experiment 5, resulted in the reassessment and fine tuning of solenoid opening time regiment.

Experiment 6 appeared to be the most successful run of the series (experiments 5-7), maintaining ORP control at approximately -30, -60 and -90 mV set points. However, after observing the results of experiment 7, its ‘success’ of controlling ORP at -30 mV can likely be attributed to the overflow event that resulted in 1/3 volume loss. The change in volume to aeration ratio resulted in a saw tooth pattern (**Figure 3.11),** a dramatic ORP response not seen in

previous experiments [46]. Despite further adjustments in solenoid opening times, ORP control was only successful at the -90 mV set point.

It is clear that controlling across ORP set points requires further exploration that was outside the scope of a master's thesis. Further work investigating media oxygenation capacity (i.e., transition metal availability) at higher control set points and further lab scale pneumatic ORP control system development is required moving forward.

4.3.2 Fermentation kinetics

While substantial time was devoted to system development and protocol evolution, an additional goal was to investigate the effects of these different ORP control efforts on fermentation outcome, fermentation kinetics and cell density. Throughout the entirety of this work a few common themes were observed. Fermentations under ORP control 1) demonstrated faster kinetics and thus earlier finishing times, 2) had accelerated biomass accumulation and reached overall greater cell densities, and 3) the extent at which fermentation kinetics and cell density accelerated was dependent on the timing and amount of oxygenation fermentations were subjected to.

In experiment 1, the Brix curves depict a clear separation in fermentation behavior between treatments, with the vessels under ORP control demonstrating faster fermentation kinetics and earlier finishing times **(Figure 3.1)**. Vessels with no ORP control completed fermentation 12 hours later than those that received ORP control via aeration. The observed differences in Brix curves between treatments is similar to what was reported in Killeen et al. (2018) [9].

A clear differentiation in Brix curve trajectories between treatments was observed in experiments 2-4. Vessels subject to ORP control for longer time intervals demonstrated

consecutively faster fermentation kinetics **(Figure 3.3, Figure 3.5 & Figure 3.7)**. Given the sampling protocol used in experiment 2 it was difficult to decipher differences in fermentation finishing times between BR1, BR2 and BR3 and BR4, BR5 and BR6 Brix samples were only taken once a day.

The general relationship between longer control intervals and accelerated fermentation kinetics persisted in both experiment 3 and 4. In particular, BR5 and BR6 completed fermentation 24 and 30 hours earlier than the uncontrolled vessel (**Figure 3.5 & Figure 3.7)**. Despite experiment 3's successful execution of 6 control conditions. Differentiations in fermentation kinetics and finishing times between conditions was not overtly clear. Overlap in fermentation behavior and finishing was observed in BR2 and BR3 as well as BR4 and BR5. To further extrapolate differences in finishing times sampling was adjusted from 2/ day in experiment 3 to 3/day in experiment 4. Experiment 4 not only had a more robust sampling protocol but had also demonstrated the greatest success at controlling ORP at the -60 set point to date **(Figure 3.7)**. This combination resulted in clear 6 hour differences in finishing times between BR1 though BR4. BR5 and BR6 again shared the same fermentation completion time point suggesting there is no significant difference in fermentation kinetics between 64- and 80-hour control periods.

The final series of experiments (experiments 5-7), examining differences in fermentation outcomes across control set points demonstrated similar trends in finishing times and fermentation kinetics. While the most accelerated fermentations were those under ORP control for the longest period in experiments 2-4, in experiments 5-7 it was the fermentations that possessed the higher control set point.

When reflecting on this work as a whole, the common thread between all experiments was that the quantity of oxygenation a fermentation was subject to seemed to correlate to not only greater ORP control, but faster fermentation kinetics. A clear delineation in kinetics was exemplified between treatments in experiment 1, 2-4 and 5-7. In experiments 2-4 fermentations subject to ORP control for longer amounts of time were subject to greater oxygenation. In experiments 5-7, fermentations were subject to ORP control the entirety of the experiment, however the degree of oxygenation was dependent on the ORP set point value, where higher set points required more oxygenation which thus determined the degree of acceleration for fermentation kinetics.

While ORP control has been the focus of past studies, many do not speak to the accelerated fermentation kinetics observed throughout this work. Killeen et al.'s (2018) work did highlight differences in fermentation kinetics and finishing between ferments under and not under ORP control. However, the scope of these differences remained between just the two conditions. These results are both directly comparable and in agreement with experiment 1, despite differences in achieved set points [3][9]. Other studies that demonstrate ORP control, focus other components of interest such as dissolved oxygen, ethanol, and biomass accumulation, hinting minutely at fermentation kinetics by way accelerated glucose consumption [33][34][36]. No work to date has spoken to the differences in fermentation kinetics across varying degrees ORP control or the effects of differing magnitudes of oxygenation. The increasingly robust fermentation outcomes observed in this work is likely a cumulative result of greater ORP status and higher biomass accumulation. Both of which can be attributed to the degree of oxygenation.

4.3.3 Cell counts

Vessels under ORP control reached overall greater cell densities. The degree of biomass accumulation was again dependent on the magnitude of ORP control and extent of oxygenation. In experiment 1, final cell densities of controlled vessels yielded cell values a magnitude greater than that of the uncontrolled **(Table 3.3)**. This was observed across triplicates.

In experiment 2, when controlling ORP across time intervals clear difference in biomass accumulation and growth trajectory was observed between vessels under no ORP control (BR1 & BR2) and those under ORP control. While BR6, the vessel under ORP the longest demonstrated the greatest cell density and growth kinetics, differentiations between other vessels were not outwardly clear **(Figure 3.3)**. In the experiments to follow clearer separation in cell densities was more readily observed. In experiment 4, BR5 and BR6 demonstrated near identical growth trajectories **(Figure 3.7)**, correlating to the similarities observed in both fermentation kinetics and finishing times. These results support the hypothesis that there is little difference in fermentation outcome when ORP control persists past 64 hours.

When controlling ORP across set points, differences in final cell counts were a magnitude of ten greater when comparing control at the highest redox set point and no ORP control. In experiments 6 and 7, a clear differentiation in biomass accumulation between vessels with set points above -60 mV and vessels set below -90 mV was discerned **(Figure 3.11 & Figure 3.13)**. This trend was not observed in experiment 6, where differentiation between groupings fell between vessels set to -120 mV and below.

To reiterate, the ORP values of a fermentation do correspond with population growth dynamics. Yeast populations demonstrate four stages of growth during a wine fermentation: lag phase, exponential growth phase, stationary phase and decline stage [24][25]. Oxygen is most

critical during the lag and exponential phases of growth as yeast utilize it as an essential nutrient for membrane synthesis of phospholipids and fatty acids [6][33][45]. This action drives redox status down, which is coupled with increasing metabolic activity, to reach an ORP minimum that is maintained during stationary phase by ongoing metabolism [10][24][25].

In this work, it can be inferred that the oxygenation through the lag and exponential growth stages favored healthier membranes better equip to handle environmental stress and thus resulted in rapid cell regeneration, higher biomass, and faster rate of fermentation. These results support that of previous work where greater biomass accumulation correlates with higher ORP values [33][34][36]. When comparing the results of Lui et al. (2016 & 2015) to the current study, less differentiation cell densities between set points was observed [33][34]. Throughout a majority of the experiments at hand clear delineations between ORP control intervals and set points could be observed. It should be noted this study utilized higher ORP set points and was thus likely subjected to greater quantities of oxygenation.

It is also important to recognize the effect of ORP on cellular stress. To cope with stressful conditions, yeast alter their metabolism maintain their internal redox state (~ -290 mV) and the use of this alterative metabolic pathway often results in the production of reductive metabolites [19][20]. This ultimately creates a more reductive fermentation environment, perpetuating environmental stress. Fermenting yeast are only equipped to function within the limits of -200 to 350 mV, with values encroaching upper and lower ORP limits inhibiting yeast function [10][25]. By controlling ORP via oxygenation, the load imposed on the yeast population is lessened.

4.3.4 Cell viability

Lastly, cell viability was also assessed in experiment 1 and experiment 2. In experiment 1, no notable differences between triplicate controlled and uncontrolled vessels were observed

(**Table 3.3)**. At the fermentation completion time point, when viability would have been expected to be the lowest, both treatment groups yielded high viability values between 89-95%. Experiment 2 differed from experiment 1 in that each vessel was intended to be under ORP control for differing lengths of time in 16-hour intervals, rather than the entirety of the fermentation. To reiterate, this was not accomplished as BR1 and BR2 ended up being uncontrolled replicates. BR5 and BR6 were 96-hour replicates as fermentations completed prior to the 120-hour shut off point. Given the experimental design of experiment 2, clear differences between the different lengths of control were anticipated. However, viability results remained relatively constant across treatments, throughout the fermentation **(Table 3.4)**. Like experiment 1, all vessel's demonstrated viability was between 87-95% at the fermentation completion time point. Overall, high cell viabilities, >85%, were observed through all phases of fermentation across treatments and experiments.

Though these results demonstrate that ORP control via oxygenation did not affect cell viability, previous studies have reported conflicting data in the literature. One study, which controlled ORP at-150 & -100 mV reported no relationship between viability and aeration [35]. However, these works differ from study at hand in that 1) ORP was controlled at much lower set points than used here and 2) a pH neutral medium was used making data comparison between studies challenging due to the pH effect. In addition, a second study controlled ORP at -150 mV and reported a viability of 85% throughout the course of the fermentation in YPD media [36]. Conversely, an early study reported both increased cell density and viability with a one-time aeration in a wine-like media [47]. More recent work conducted by Lui et al. (2015 & 2016), reported increased viability in YPD media ferments controlled at -150 mV in comparison to its uncontrolled counterparts [33][34]. It was reported that with higher ORP control set points high

viability and increased biomasses persisted, while uncontrolled reached substantial biomasses, but demonstrated lower viability. Viability was determined via methylene blue staining, the same method as used in the current study. Killeen et al. 2018, while not measuring viability directly integrated fermentation modeling to investigate it [9]. The fermentation curves indicated both the rate of cell maintenance and cell viability to be increasingly higher in ORP controlled ferments. These fermentations were conducted using a grape juice medium and were controlled at 215 mV (HSE), thus more comparable to the current studies conditions. Given the discrepancies between the results at hand and the literature, the relationship between cell viability and ORP control via aeration requires further exploration.

5. Future Perspectives

5.1 Conclusions

Throughout this work it was demonstrated that fermentations under ORP control possessed faster fermentation kinetics, earlier finishing time and greater biomass accumulation. The magnitude of these outcomes appeared to be directly proportional to the degree of ORP control or oxygenation the fermentation endured. This phenomenon was demonstrated in both the second set of experiments (experiments 2-4) where ORP was controlled across varying time intervals as well as the third set of experiments (experiments 5-7) where ORP was controlled across varying set points. In both sets more oxygenation (i.e., longer intervals or higher set points) demonstrated faster fermentation kinetics and greater cell densities. Given these results it is challenging tease apart if these fermentation outcomes were due strictly to controlling ORP, to the increased oxygenation, or the combination of both. In addition, cell viability was examined in the first two experiments. Again, differences in cell viability between treatments was not

observed, resulting in no further exploration in this work. These finding further contribute to the conflicting results surrounding the relationship between ORP control and viability found in the literature.

5.2 New research directions

While this work offered valuable insight into the benefits and outcomes of controlling ORP by way of oxygenation, there is still a significant amount of research needed to understand ORP as fermentation metric, better define the necessary methods and parameter needed to control ORP as well as the long-term impact of these fermentation outcomes. There are many directions future research could take, such as expanding on this work to better define ORP control set points as well as further testing the relationship between ORP and viability.

Other avenues of research could include further investigating the effects of ORP control on hydrogen sulfide production as well as other chemical components such as organic acids and amino acids. Considering wine is a consumer product no research is complete without the incorporation of sensory analysis. Additionally, further work is necessary to better establish the relationship between ORP and yeast strain as well as ORP and fermentation medium. The effect of vineyard site and ORP is largely undefined as well. There is room for exploration of alternative ORP control methods, such as the use of hydrogen peroxide in place of oxygenation, which offers a potential means regulate ORP and to separate the impact of oxygen on yeast metabolism from ORP control. Lastly, it is absolutely crucial that research surrounding ORP and ORP control be examined under true production conditions (i.e., grape juice medium and production scale) to make these research outcomes both relevant and accessible for industry use.

5.3 Industry impacts

While there is still much to be investigated around the causal effect behind the fermentation outcomes reported in this work; ORP control via oxygenation pose valuable real-time and potential benefits for the wine industry. For example, the observed acceleration in fermentation kinetics could directly translate to an increased rate of tank turnover time during harvest. This could relieve winemakers of stresses associated with tank space limitations and fermentation planning.

In addition, the observed increase in biomass accumulation in combination with the accelerated kinetics suggests that fermentations under ORP control are more robust. This benefit could ultimately help winemakers avoid issues and costs associated with sluggish and stuck fermentations. ORP has been proposed as a real time indicator of fermentation health. Looking forward and with a better understanding of ORP, these results pose the question if ORP could be used as real time predictive parameter for stuck fermentations?

Lastly, millions of dollars are lost every year to the presence of undesirable or faulty sensory characters in finished wines. Among the most common is the presence of hydrogen sulfide. By controlling ORP above the “danger zone” of -70 mV, winemakers can avoid conditions associated with spontaneous formation of hydrogen sulfide in the presence of elemental sulfur. Hydrogen sulfide has long been known to produce the undesirable reduced or rotten egg organoleptic character in finished wines. Controlling ORP would save wineries from having to perform the taboo like remediation practice of copper fining. This poses a financial incentive, as it would not only reduce the need for added winemaking intervention but would also reduce cost associated with remediation products as well mitigate the financial burden associated with product loss.

6. Appendix

6.1 Abbreviations & acronyms

Table 6.1 A reference list of abbreviations found throughout this work.

Abbreviation	Explanation	Page
Ag/AgCl	Silver/Silver Chloride	3
BERs	Bioelectrical Reactors	11
BR	Bioreactor	16
CO_2	Carbon Dioxide	66
CV	Cyclic Voltammetry	2
DO	Dissolved Oxygen	1
GSH/ GSSG	Glutathione/ Glutathione Disulfide	10
H_2S	Hydrogen Sulfide	7
KCl	Potassium Chloride	3
mV	Millivolts	2
NAD^+/NADH	Nicotinamide Adenine Dinucleotide	6
NADP/NADPH	Nicotinamide Adenine Dinucleotide Phosphate	6
NOPA	Nitrogen by O-Phthaladhyde	23
OD	Optical Density	24
ORP	Oxidation Reduction Potential	1
PAN	Primary Assimilable Nitrogen	23
PANOPA	Primary Assimilable Nitrogen by O-Phthaladhyde	23

PLC	Programmable Logic Controller Primary Assimilable Nitrogen	19
Redox	Reduction-Oxidation	1
SHE	Standard Hydrogen Electrode	3
SO_2	Sulfur Dioxide	12
TA	Titratable Acidity, g/L as tartaric acid	23
TrxSS	Thiredoxin	10
YAN	Yeast Assimilable Nitrogen	23
YM	Yeast Malt	10
YPD	Yeast-Extract Peptone Dextrose	10

6.2 Experimental temperature graphs

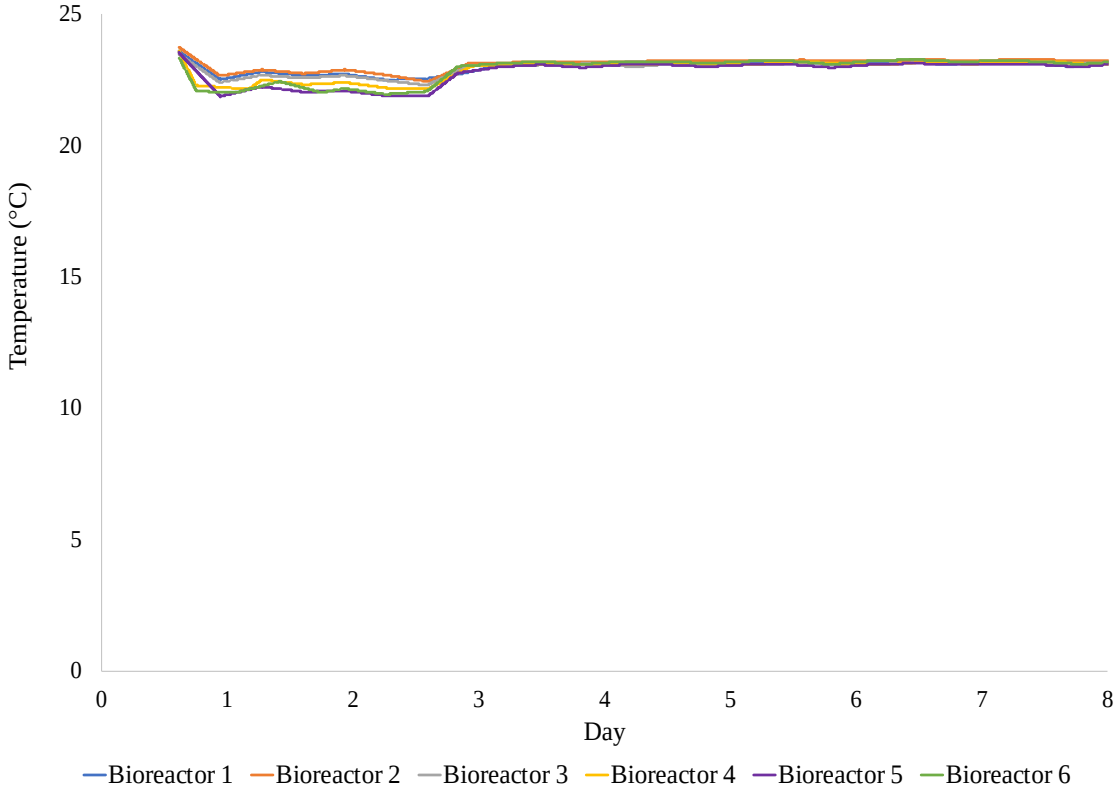


Figure 6.1 A composite figure of temperature graphs of all bioreactors from experiments 1 through 7. Graph numbers match the corresponding experiment number.

6.3 Composite figure of uncontrolled vessel's ORP curves across experiments

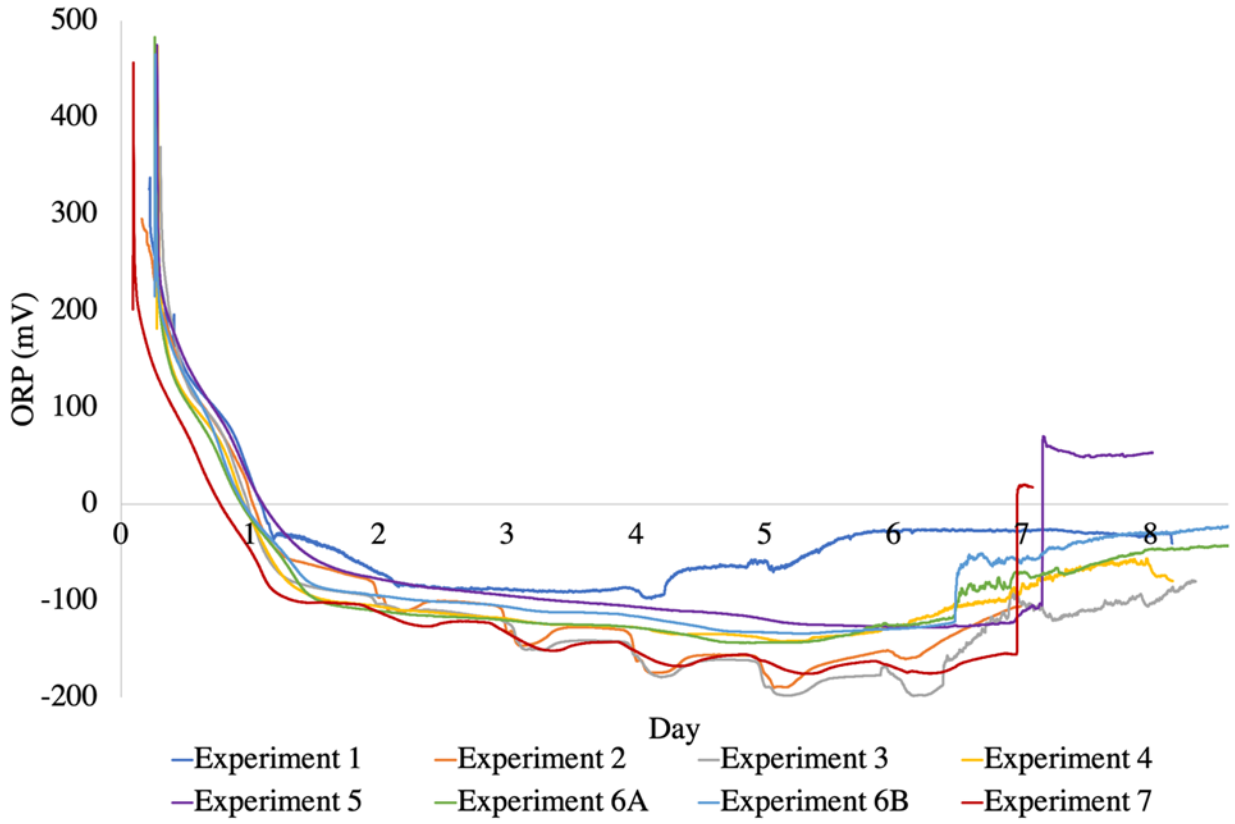


Figure 6.2 A composite figure of all uncontrolled vessel's ORP curves from experiment 1 through 7.

6.4 Assessing ORP probe variability within vessels

6.4.1 Experiment 6

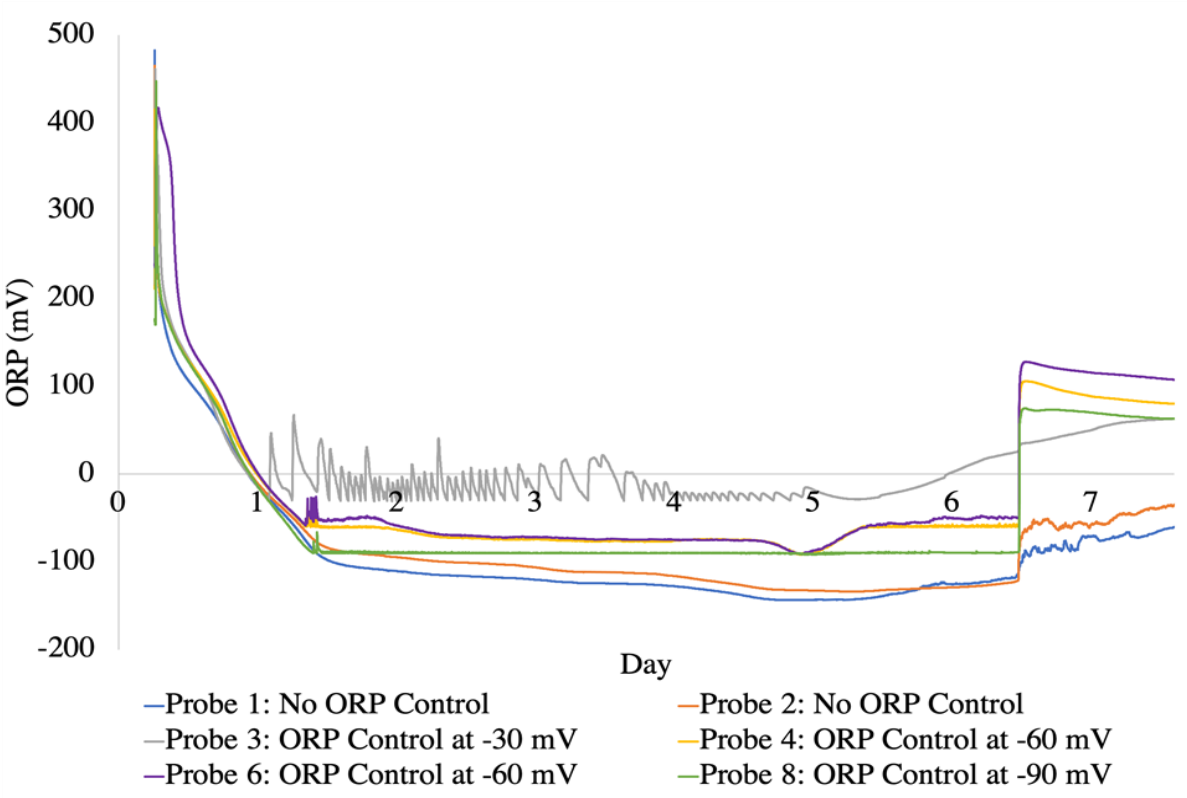


Figure 6.3 A composite figure that demonstrates variability between probes in experiment 6. For this experiment 2 probes were placed in the BR1, the vessel with no ORP control as well as BR3, the vessel controlled at -60mV.

6.4.2 Experiment 7

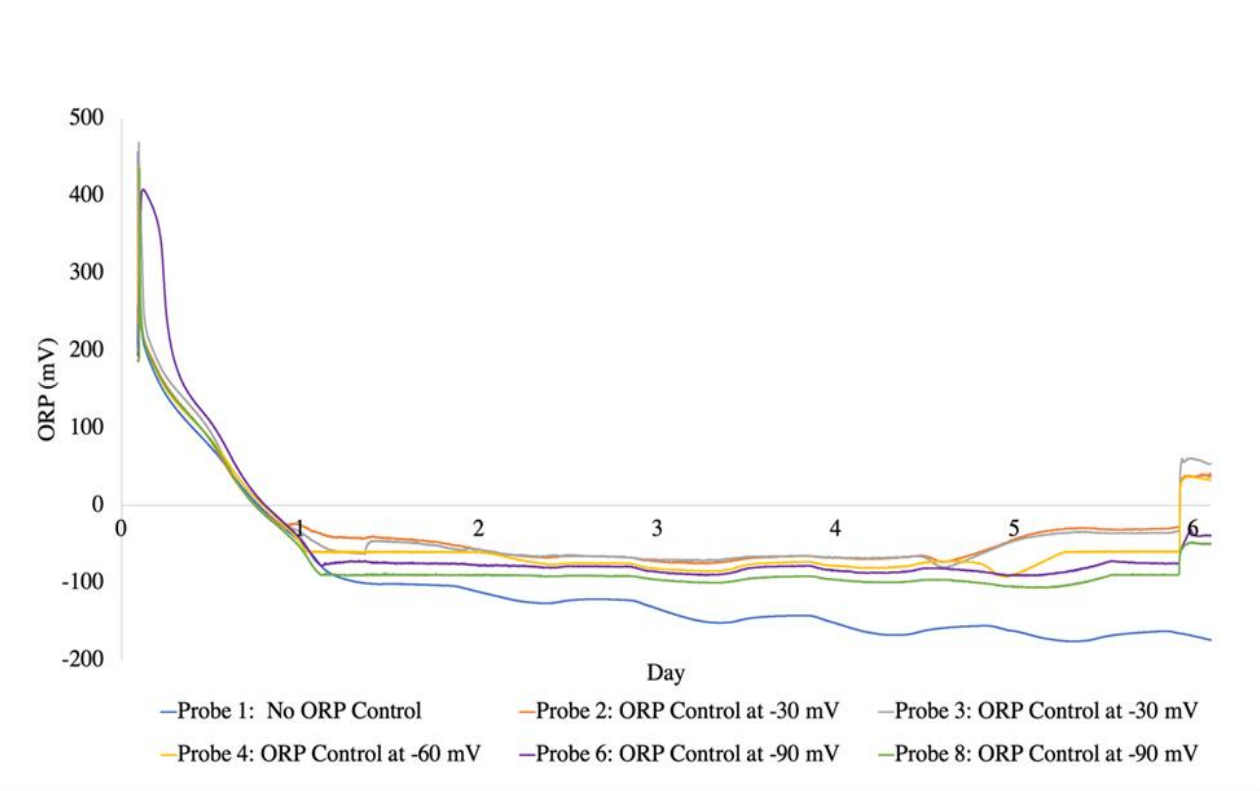


Figure 6.4 A composite figure that demonstrates variability between probes in experiment 7. For this experiment 2 probes were placed in the BR2 the vessel controlled at -30mV as well as BR4, the vessel controlled at -90mV.

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