CARBON DIOXIDE GENERATION, TRANSPORT AND RELEASE DURING THE FERMENTATION OF BARLEY MALT

by

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

at

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DEDICATION

In the vastness of space and immensity of time, it is my joy to share a planet and an age with all of the characters in my life. I dedicate this work to my family who have all helped to support and influence this work (whether you realized it or not). Specifically, my brother Ian MacIntosh (who is rather adept at editing), my mother and father, Lise and James MacIntosh (without their foresight, guidance and support I would not be walking this path), and my partner Mengyu Li, who has been my emotional anchor and companion (even when the going was rough). Mengyu, I am so fortunate to have had you by my side, we have shared in so many adventures and I am looking forward to many, many more.

Thank-you all.
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ABSTRACT

Carbon Dioxide (CO₂) is a major fermentation product generated during the production of beer, the subsequent release of this gas within the fermentor results in agitation that is necessary for sustained industrial fermentation. CO₂ is sometimes monitored allowing brewers to stoichiometrically relate CO₂ released to other products. In this manner the rate of gas release from the fermentor may be used to assess, control and predict other aspects of fermentation. The dynamics of CO₂ generation, transport and release are explored throughout this thesis over several studies. The tools used to examine CO₂ production were scrutinized including a miniature assay using various modeling techniques.

A miniature scale fermentation assay included in the methods of the American Society of Brewing Chemists was compared to industrial scale fermentations. It was found that discrepancies were possibly due (at least in part) to fermentor geometry. Following this study, a literature review of CO₂ solubility in aqueous sugar, and ethanol solutions was conducted. This study exposed previously undescribed inaccuracies in literature, i.e., it was found that several gas solubility tables were empirical derived and are therefore unlikely to accurately reflect all styles of beer. The next study scrutinized the consumption of sugars during barley fermentation and found that these fermentations often exhibit asymmetric sigmoidal attenuation. A five parameter logistic model was introduced to model this sugar consumption more accurately than previously described techniques. Using methods refined during the aforementioned studies, a fermentation was conducted where a mass balance was used to track all major fermentation parameters (the consumption of individual sugars, and the production of ethanol, carbon dioxide, yeast biomass and glycerol). This allowed an assessment of Balling’s theorem as compared to modern theory. It was shown that while accurate in predicting original extract, Balling’s theorem incorrectly quantified other fermentation parameters. This has large ramifications for both industry and research as the estimation of fermentation parameters (such as ethanol and fermentation time) is now better understood.

From these studies, the production of beer becomes less of a “black box” operation, and CO₂ saturation, transport and release can be better explained. Of the many fermentation aspects monitored during these studies, most were predicted by theory, however, there were notable exceptions. For instance, it was found that both the inhibition of maltose consumption and yeast sugar consumption dynamics (which remained relatively constant throughout the fermentation at ~ 50 pg·h⁻¹ for cells with an average mass of ~ 40 pg) were found to deviate from previously described reports. These, and other findings improve our understanding of brewing fermentations allowing for additional applications of theory and recommendations in industrial operations.
# List of Abbreviations Used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
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<tbody>
<tr>
<td>Adenosine Triphosphate</td>
<td>ATP</td>
</tr>
<tr>
<td>Akaike’s corrected Information Criterion</td>
<td>AICc</td>
</tr>
<tr>
<td>Alcohol Percentage (by weight compared to volume of water)</td>
<td>$A_{w/v}$</td>
</tr>
<tr>
<td>Alcohol Percentage (by volume compared to volume of water)</td>
<td>$A_{v/v}$</td>
</tr>
<tr>
<td>American Society of Brewing Chemists</td>
<td>ASBC</td>
</tr>
<tr>
<td>Apparent Degree of Fermentation</td>
<td>ADF</td>
</tr>
<tr>
<td>Apparent Extract</td>
<td>AE</td>
</tr>
<tr>
<td>Asymptotic Standard Error</td>
<td>ASE</td>
</tr>
<tr>
<td>Brewers Association of Canada</td>
<td>BAC</td>
</tr>
<tr>
<td>Brewing and Malting Barley Research Institute</td>
<td>BMBRI</td>
</tr>
<tr>
<td>Canadian Grain Commission</td>
<td>CGC</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>$CO_2$</td>
</tr>
<tr>
<td>Degrees Celsius</td>
<td>°C</td>
</tr>
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<td>Degrees Fahrenheit</td>
<td>°F</td>
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<td>4P</td>
</tr>
<tr>
<td>Four Parameter</td>
<td>5P</td>
</tr>
<tr>
<td>Free Amino Nitrogen</td>
<td>FAN</td>
</tr>
<tr>
<td>High-Pressure Liquid Chromatography</td>
<td>HPLC</td>
</tr>
<tr>
<td>Incomplete Beta-Function</td>
<td>IBF</td>
</tr>
<tr>
<td>Institut Français de Brasserie et Malterie</td>
<td>IFBM</td>
</tr>
<tr>
<td>Term</td>
<td>Abbreviation</td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
</tr>
<tr>
<td>Journal of the American Society of Brewing Chemists</td>
<td>JASBC</td>
</tr>
<tr>
<td>Master Brewers Association of the Americas</td>
<td>MBAA</td>
</tr>
<tr>
<td>Nicotinamide Adenine Dinucleotide</td>
<td>NAD+</td>
</tr>
<tr>
<td>Nicotinamide Adenine Dinucleotide + hydrogen</td>
<td>NADH</td>
</tr>
<tr>
<td>Original Extract</td>
<td>OE</td>
</tr>
<tr>
<td>Premature Yeast Flocculation</td>
<td>PYF</td>
</tr>
<tr>
<td>Real Extract</td>
<td>RE</td>
</tr>
<tr>
<td>Residual Sum of Squares</td>
<td>RSS</td>
</tr>
<tr>
<td>Root Mean Squared</td>
<td>RMS</td>
</tr>
<tr>
<td>Standard Pressure and Temperature (1 atm, 0 °C,)</td>
<td>STP</td>
</tr>
<tr>
<td>Total Yeast</td>
<td>TY</td>
</tr>
<tr>
<td>Yeast Extract Peptone Dextrose</td>
<td>YEPD</td>
</tr>
<tr>
<td>Yeast In Suspension</td>
<td>YIS</td>
</tr>
</tbody>
</table>
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First and foremost I would like to acknowledge my supervisor, Dr. Alex Speers, who has been my supervisor, mentor and friend over the last several years. We both have undergone major life changes over the last few years, taking on new opportunities and challenges. Dr. Speers gave shape to my research and provided the character to make it interesting, such as the time spent pouring over old manuscripts, or contemplating the physical chemistry of fermentation over coffee (scrutinizing the inconsistencies and the unexplained). I have enjoyed my time under your guidance, and sincerely hope that one way or another, we will have the opportunity collaborate once again.

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Throughout this thesis, the support of co-workers has been essential. From assistance with laboratory measurements to cross-field expertise, our brewing lab has been a hub for highly qualified personnel from a variety of backgrounds. I would especially like to thank Maria Josey, Josh Adler, Greg Potter, Jessica Forbes, Alex McKinnon, Chris Bourque, Sarah Singer, Emily Eck and Ankita Mishra for their assistance and support.

Finally, I would like to acknowledge the participation of Rogues Roost brewpub, and the Propeller Brewing Company who graciously allowed us to conduct trials using their equipment and made available their expertise.

Thank-you all.
CHAPTER 1  INTRODUCTION

Carbon dioxide (CO$_2$) is a major fermentation product generated during the production of beer, the subsequent release of this gas within the fermentor results in agitation that is necessary for sustained industrial fermentation. As the rate of CO$_2$ generation is stoichiometrically related to other products, the rate of gas release from the fermentor may be used to assess, control and predict other fermentation parameters. Ultimately the CO$_2$ released from the fermentor is either vented or captured and utilized within the finished product. The aim of this thesis was to investigate CO$_2$ in the brewing process; from generation and relationship with other fermentation products, to use in finished beer. Various relationships between reactants and products were also explored while modern modeling techniques were applied to experimental and historical data. Several procedures used by industry and within this thesis were scrutinized, for example, the use of a miniature fermentation assay outlined in the American Society of Brewing Chemists (ASBC) standard methods (ASBC Yeast-14) and related mathematical models that were used to fit equations to fermentation data. Novel experiments were completed to examine the consumption of sugars and generation of fermentation products such as CO$_2$. Research was conducted at Dalhousie University in conjunction with two local brewing establishments who graciously allowed samples to be drawn from active fermentations.

1.1  RESEARCH OBJECTIVES

The primary goal of this thesis was to closely monitor and model the generation and transport of CO$_2$ during fermentation. This was completed with the aim of refining our understanding of alcoholic fermentation with potential application for improving
control over industrial fermentations. This study was unique in that the high sampling frequency, number of parameters tracked, and the degree of measurement precision which allowed for greater insight into the role of CO₂ within the fermentation process.

Specific objectives are summarized by chapter in Table 1.1 and were to:

I. Assess the miniature fermentation method (Detailed in Chapter 2.4) and its use in characterizing industrial fermentations,
II. Estimate CO₂ generation during fermentation using density attenuation,
III. Estimate the shear within fermentations using a model of CO₂ generation,
IV. Investigate the origin and utility of CO₂ solubility charts,
V. Scrutinize techniques, and model sugar consumption,
VI. Model the production of ethanol, glycerol, and CO₂ during fermentation,
VII. Measure and model the release of CO₂ from the fermentor.

Table 1.1 Table linking each objectives to corresponding Chapters.

<table>
<thead>
<tr>
<th>Chapter/Objective</th>
<th>CH3</th>
<th>CH4</th>
<th>CH5</th>
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<tr>
<td>VII</td>
<td>X</td>
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</table>

Chapter 2 of this thesis provides a brief introduction to the economical, historical and practical role of CO₂ in beer, including background theory concerning CO₂ generation and several modeling techniques. The purpose of this chapter is to provide additional background information to topics discussed later within the thesis. Detailed methods of a miniature fermentation assay (including barley, barley malt and yeast
analysis) have been included in this section as this assay (ASBC Yeast-14) was commonly utilized throughout the thesis.

Subsequent chapters detail the results of three years of research and are presented in manuscript format. In Chapter 3, the use of the miniature fermentation assay for direct comparison to industrial brewing operations was assessed experimentally. The amount of shear generated through the release of CO₂ gas was measured and modeled (using non-linear regression techniques) in order to examine the effect upon miniature and industrial fermentations. In Chapter 4, the use of CO₂ in the packaging of beer is discussed through a review of saturation dynamics published in literature. Non-linear modeling techniques were used to determine the likely origin of several historical equations and their industrial uses. In Chapter 5, modeling techniques used in the miniature fermentation assay and throughout this thesis are explained and compared to other commonly used predictive models. The results of this analysis are applied in Chapter 6; this chapter details experiments designed to closely monitor the generation and release of CO₂ and other fermentation products. This was accomplished through empirical assessment of CO₂ saturation, release, sugar consumption and product generation. The implications and potential applications of these results are discussed.

Finally, in Chapter 7 the results from each chapter are examined and discussed with respect to potential impact upon industry and research. This portion of the thesis forms the basis for recommendations and suggestions for future research.
CHAPTER 2 LITERATURE REVIEW

Some materials in this chapter are drawn from a manuscript that has been accepted for publication in the Journal of the American Society of Brewing Chemists.


2.1 INTRODUCTION

The tradition of brewing is thousands of years old and has played an important social and economic role in many cultures. With this historical significance, it is unsurprising that brewing has been highly scrutinized and that many of the process and mechanisms that take place during fermentation are well understood and documented. However, as scientific methods and tools evolve, there are opportunities to reevaluate and improve our understanding, even in topics that are well understood. Often, apparent discrepancies observed between theoretical and observed results can be explained with a greater understanding of the process.

2.1.1 Impact of Brewing on the Canadian Economy

Brewing and related industries are an important sector of the Canadian economy such that the production and export of Canadian beer is an economic success story (BAC, 2013). According to the Brewers Association of Canada (BAC), brewing contributes over $14 billion annually to the Canadian economy (BAC, 2013). While the consumption of beer in Canada has remained steady during the last decade at approximately $2.3 \cdot 10^7$ hL, the global total beer consumption has steadily been increasing (BAC, 2007).
In 2013, Canadian brewers had product export sales to the United States of America of approximately $2.5 \times 10^6$ hL, representing 11.34% of total sales (BAC, 2012). While Canadian beer export sales are strong, they face intense competition. With the expanding size and complexity of industrial fermentations, even small changes in efficiency can have huge effects upon the profitability and viability of large scale brewing operations. It is vital that Canadian brewers have access to the latest scientific research, as improving our understanding of the brewing process is essential to remaining competitive within the world market.

2.2 **The Fermentation Process**

The vast majority of industrial brewing operations utilize batch fermentations, where yeast is added (pitched) at concentrations of approximately $12-15 \times 10^6$ cells·mL$^{-1}$ (Briggs et al., 2004). While continuous industrial fermentations do exist, i.e., Morton Coutts’ method used in New Zealand (Virkajärvi and Kronlöf, 1998), these were not the focus of this thesis and are likely to have different characteristics than those described herein. Brewing fermentations are either completed using *Saccharomyces cerevisiae* (ale yeast) or *Saccharomyces pastorianus* (lager yeast), with the latter species producing approximately 90% of the global product (Canadean, 2011). Over the course of 4-20 days, the fermentable sugars within the brewing media (wort) are consumed and fermentation products (predominantly ethanol and CO$_2$) are produced. Figure 2.1 details the trends observed over fermentation for several commonly measured parameters as presented in the handbook of brewing (Priest and Stewart, 2006). The density of the media (commonly expressed as specific gravity or apparent extract) is often used as an
easily measured analog for the concentration of sugar within the media (although this must be corrected for alcohol concentration).

2.2.1 Sugar Consumption During Fermentation

Highly dependent upon the malt and mashing style, every wort will be comprised of a different configuration of fermentable and non-fermentable sugars. The sugars present in wort (and typical concentrations) are listed in Table 2.1. While different strains are able to metabolize different sugars, Table 2.1 highlights those most commonly found and metabolized within brewers wort as identified by Stewart (2006). During brewing operations, the uptake of fermentable sugars by yeast is a highly ordered process; glucose and fructose are consumed first with any sucrose present being hydrolyzed extracellularly via the enzyme β-fructosidase (invertase) excreted by yeast (Briggs et al., 2004a). The presence of glucose in sufficient quantities has been shown to inhibit respiration and the
uptake of maltose in brewing yeast. Once the concentration of glucose is sufficiently low, maltose is sequentially utilized by the yeast, followed by maltotriose (Stewart, 2006). Both maltose and maltotriose are hydrolyzed into glucose via the enzyme α-glucosidase (maltase) intracellularly (Briggs et al., 2004a). Most brewing strains, including the stain used in this study, cannot metabolize longer chain sugars (Stewart and Russell, 1998).

The ordered consumption of sugars over a typical fermentation is detailed in Figure 2.2 (Priest and Stewart, 2006).

Table 2.1 Typical sugar components of brewing wort

<table>
<thead>
<tr>
<th>Saccharide:</th>
<th>Chemical formula</th>
<th>Typical Percent Composition¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>C₆H₁₂O₆</td>
<td>10-15</td>
</tr>
<tr>
<td>Fructose</td>
<td>C₆H₁₂O₆</td>
<td>1-2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>C₁₂H₂₂O₁₁</td>
<td>1-2</td>
</tr>
<tr>
<td>Maltose</td>
<td>C₁₂H₂₂O₁₁</td>
<td>50-60</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>C₁₈H₃₄O₁₆</td>
<td>15-20</td>
</tr>
<tr>
<td>Higher Saccharides</td>
<td>H₂O+(C₆H₁₀O₅)ₙ</td>
<td>20-30</td>
</tr>
</tbody>
</table>

¹Typical composition as a percent of total sugars (Stewart, 2006).

Figure 2.2 Overview of the consumption of common fermentable sugars during fermentation (Priest and Stewart, 2006).
2.2.2 The Products of Fermentation

The primary products of fermentation are ethanol and CO₂. Once the various fermentable sugars have been hydrolyzed to glucose, it will be intracellularly converted into two ethanol and two CO₂ molecules. Specifically, one glucose molecule will result in two pyruvate molecules with the energy released used to form two adenosine triphosphate (ATP) molecules. This results in the conversion of two nicotinamide adenine dinucleotide (NAD+) ions into two NADH (NAD+ with an attached hydrogen ion) molecules. Each individual pyruvate molecule is subsequently converted into acetaldehyde releasing one molecule of CO₂. Finally, the two acetaldehydes are converted to ethanol using the acidic hydrogen of the NADH, converting those back to NAD⁺ (Lallemand, 2007). This process is illustrated in Figure 2.3.

Figure 2.3 An overview of the conversion of glucose into ethanol and CO₂ (Lallemand, 2007), where ADP stands for Adenosine Diphosphate and Pᵢ stands for inorganic phosphate.

According to Briggs et al. (2004a), the conversion of sugar to ethanol during brewing operation typically reaches only 85% (approximately) of theoretical yield with
the shortfall due to yeast biomass formation and other metabolite products. Of the many additional products of fermentation, some are beneficial to the end product (i.e., glycerol), while others can be detrimental (i.e., diacetyl) and are considered defects. After ethanol and CO₂, the most abundant fermentation product (by 2-3 orders of magnitude) is glycerol, which has been detected in commercial beers between the concentrations of 436-3971 mg·L⁻¹ (Briggs et al., 2004b). The production of glycerol is necessary as glycerol both protects the cells from osmotic pressure (especially important during “high gravity” brewing) and helps to maintain NAD⁺/NADH ratios during biomass production, shown in Figure 2.3 (Lallemand, 2007).

Figure 2.4 An overview of glucose conversion into both ethanol and glycerol, adapted from Lallemand (2007).

### 2.2.3 Carl Balling’s formula

In 1865, the chemist Carl Balling analyzed brewing operations focusing on the products of fermentation. Using beer with original wort extract of 10–14 degrees Plato (°P – a measure of density), Balling reported that from 2.0665 g of fermented
extract, the following products were generated: 1.000 g alcohol, 0.9565 g CO₂ and 0.11 g dry yeast matter (Balling, 1845-1865). Upon analysis it appears as though this formula is a combination of the theoretical conversion of glucose to ethanol and CO₂, combined with empirical assessments of yeast mass generation measured at the end of fermentation. This formula, and associated calculation of original extract (OE), are utilized worldwide and endorsed by both the European Brewing Convention (EBC Method 9.4) and the American Society of Brewing Chemists (ASBC Beer-6B). However, that is not to say the formula has remained unchallenged. In the ensuing years since its derivation, the formula has been disputed on multiple grounds (as will be discussed in Chapter 5). Subsequent researchers have noted that while not perfect, the formula is a good approximation that is well known and widely utilized (Neilson et al., 2007). Additionally, several issues with Balling’s formula can be corrected for as summarized by Neilson et al. (2007). The aforementioned studies have assessed the accuracy of Balling’s formula used to model fermentations, specifically the relationship between final values and OE. However, the ratio of fermentation products is known to vary throughout the fermentation. For example, the majority of yeast propagation is completed during the first half of fermentation whereas the initial CO₂ produced is dissolved within the wort and does not evolve. Modern methods of analysis now allow researchers to follow the parameters of Balling’s formula over the entire fermentation and examine how the product ratios change with time.
2.3 \textbf{CO}_2 \textbf{IN BEER DURING AND AFTER FERMENTATION}

Large modern breweries often capture the CO\textsubscript{2} released from fermentors during fermentation. In 1976 it was shown by Alford (1976), that the partial pressure of CO\textsubscript{2} gas released could be used to assess the amount of dissolved CO\textsubscript{2} within the fermentor, thus providing basis for the use of CO\textsubscript{2} release as a measure of fermentation progress. However, problems with implementation, and the need for correction factors illustrate that the relationship between CO\textsubscript{2} release and fermentation progress is not yet fully understood (Corrieu et al., 2000). While much work has been completed on the theoretical formation of CO\textsubscript{2} during brewing operations, less work has been completed on CO\textsubscript{2} as related to other parameters. It is well known that the generation of bubbles and subsequent agitation within the brewing fermentor is essential to the fermentation process. However, the degree of supersaturation typically achieved during fermentation, or when CO\textsubscript{2} release begins to generate appropriate shear to keep yeast in suspension (or allow flocculation) are little-known and understudied. Using measurements of CO\textsubscript{2} released and dissolved within wort (along with the aforementioned theories of saturation) in conjunction with models of CO\textsubscript{2} generation, this thesis aims to provide the tools to answer these questions and provide greater understanding of the brewing process.

In contrast to the lack of studies examining CO\textsubscript{2} solubility during fermentation, there are many reports examining the solubility of CO\textsubscript{2} in finished product. The concentration of carbon dioxide within finished beer is commonly measured by brewers for quality assurance and packaging purposes; as the level within beer is known to influence the processability, mouth feel, and stability of beer. The methods currently available to assess the solubility of CO\textsubscript{2} within beer are based upon a combination of
theoretical knowledge and empirical data. The phenomenon of gas solubility in water is well understood. Work by William Henry formulated Henry’s law in 1803 describing changes in gas solubility within water (Battino and Clever, 1965). This work established a coefficient (also known as Henry’s constant) that describes the solubility of the gas with respect to pressure. Henry’s coefficient is known to vary considerably with temperature. There are many equations describing the relationship between temperature and gas solubility (Battino and Clever, 1965), such the Jacobus Henricus Van ’t Hoff’s equation (Van’t Hoff, 1885). Using the combination of Henry’s law and the Van ’t Hoff relationship between Henry’s constant and temperature, the solubility of gas in pure water can be determined for a given temperature and pressure. However, the physical composition of beer is more complex than water, additional parameters such as ethanol content (Postigo and Katz, 1987), and sugars (Descoins et al., 2006) should be considered in any calculation of CO2 the solubility within beer. The evolution and accuracy of current methods for determination of CO2 solubility in beer are discussed in Chapter 6.

2.3.1 Carbon Dioxide Terminology

The dynamics of dissolved gases within aqueous solutions is well understood, however, some of the terminology may imply various meanings dependent upon the scientific field. The term “CO2 solubility”, as used in the brewing industry usually refers to the amount of gas that will dissolve within a product at equilibrium with the partial pressure of CO2 in the headspace. Unless otherwise stated, it is assumed the headspace is composed of only CO2 at 1 atm. Therefore, when comparing the published “solubility” to real world dynamics, it is important to remember that most fermentations begin with a headspace with very little CO2. The wort quickly becomes highly supersaturated (defined
within this thesis as having a concentration of $\text{CO}_2 > \text{equilibrium concentration}$ with the
actual partial pressure of $\text{CO}_2$ in the headspace) due to the higher rate of $\text{CO}_2$ generation
versus release. The supersaturation will eventually facilitate bubble generation. The
formation and characteristics of bubbles within fermented beverages was explored in

2.4 MINIATURE FERMENTATION

Several experiments detailed within this thesis utilize a miniature fermentation
that was designed by Lake et al. (2008) to examine wort for evidence of Premature Yeast
Flocculation (PYF). PYF is a phenomenon in brewing fermentations, characterized by
early and/or excessive yeast settling, prior to exhaustion of fermentable sugars. Dr.
Lake’s method has been adopted as a standard method by the ASBC, listed within their
official methods of analysis as ASBC Yeast-14. This miniature assay provides an
excellent method to create multiple, carefully controlled fermentations allowing for the
isolation and examination of specific brewing parameters over the course of fermentation.
However, as the method was designed to accommodate malt producers and the brewing
industry, the method includes malt grinding specifics and utilizes a sampling frequency
designed around a typical work day (as opposed to a fermentation progression). Thus, the
official method has been modified for use in this thesis to increase accuracy and allow for
additional data collection. Specifically, in Chapter 3 the method was modified to use wort
from participating industrial breweries, while in Chapters 4-5 the sampling frequency was
greatly increased and alternative (more accurate) instrumentation was used.
2.4.1 The Mathematical Modeling of Fermentation

Since Balling’s work, many studies have established mathematical relationships between fermentation products and examined the effect of fermentation deviations. Unfortunately, not every fermentation has each variable monitored in real-time; in industrial settings measurements are usually taken intermittently and when convenient for scheduling purposes. With a limited number of data points, important trends can be missed and small errors in measurement can greatly affect alcohol and extract calculations. With the development of computer aided modeling, scientists have applied nonlinear fitting techniques to model and more precisely determine interpolated values of variables (Speers et al., 2003). Since then, advances in other scientific fields have introduced novel models that may be more adept at modeling the patterns observed during fermentation. Chapter 4 examines several common models used inside and outside of the brewing industry for use in modeling the sugar consumption during brewing. The findings of this study are used in later chapters. Please note that the term “modeling” used throughout this thesis refers to the fitting of equations (empirical and theoretical) to experimental data. This is consistent with terminology used in the brewing industry. Therefore the equations introduced in subsequent chapters are referred to as “models”.

2.4.2 SMA Yeast

The brewing yeast strain utilized in this study is SMA. This is an industrial lager strain available from VLB, (Berlin DEU) or WYeast (Odell, USA). Unfortunately the author has been unable to discern the origin of the name “SMA”, if it is an acronym or a designation. This strain is often utilized as a standard and is a requested test strain by the Japanese (personal communication, R.A. Speers, 2013) and is commonly used by
Canadian malting companies. The SMA yeast strain is described as “a moderately flocculent yeast strain” by Lake et al. (2008), and as a “highly flocculent” strain by Panteloglou et al. (2010).

2.4.3 Barley and Malt

The malted barley utilized for the experiments detailed in Chapters 4-7 was provided by the Canadian Grain Commission (CGC). Analysis conducted by the CGC associated Grain Research Laboratory indicated that a congress wort (Malt-4) produced with this grain has a Free Amino Nitrogen (FAN) content of 170 mg·L⁻¹ using the ASBC method ASBC Wort-12B.

2.4.4 Methods of the Miniature Fermentation Assay

Malted barley samples fermented according to Yeast-14 are fermented with two replicates at each sampling period. A total of one hundred and fifty grams (150.0 ± 0.03 g) of each sample were ground using a grist mill (Bühler Universal, Braunschweig, DEU) set to the ASBC “fine” standard (ASBC Malt-4). Samples were mashed according to the ASBC Congress standard mashing regime (ASBC Malt-4) using a mash bath (International Equipment and Control Pty. Ltd. Melbourne, AUS). This mashing regime is designed to extract as much fermentable sugar from the malt as possible, thereby preserving consistency between assays. This amount of ground malt typically yields sufficient liquid (450 mL) to complete one miniature fermentation assay (30 test-tube “fermentors” each containing 15 mL of wort). After completion of the mash cycle, the liquid was filtered through coarse fluted filter paper (Reeve Angel 802, Whatman Inc. Florham Park, NJ) to remove solids and was autoclaved for 20 min at 121 °C. The
subsequent wort was cooled and refrigerated for 12-24 hr at 4 °C. The wort was then centrifuged at $3.31 \cdot 10^3$ g, for 15 minutes to remove trub. Prior to fermentation, the wort was adjusted to a final density of 16.1 °P with D-glucose so as to achieve a rapid rate of fermentation and sustain sufficient agitation within the miniature test-tube fermentors. Finally, the wort was oxygenated at 20 °C for 5 minutes by bubbling medical grade, compressed oxygen through the adjusted wort.

Forty-eight hours prior to pitching, cultures of the industrial SMA yeast strain were removed from agar and aseptically transferred into four 125 mL flasks containing 50 mL of Yeast Extract Peptone Dextrose (YEPD) broth, consisting of 20 g·L$^{-1}$ dextrose (Difco, Detroit, MI), 20 g·L$^{-1}$ peptone (Difco, Detroit, MI) and 10 g·L$^{-1}$ yeast extract (Difco, Detroit, MI). Cultures were aerobically incubated with an orbital shaker at 100 rpm for 24 hr at 30 °C. The resulting slurry was centrifuged ($3 \cdot 10^3$ g for 3 min) and yeast pellets re-suspended in sterile, reagent grade water (~ 20 mL per 50 mL centrifuge tube). The centrifugation and re-suspension of the resulting yeast/water slurry was repeated twice more as described above for a total of three “washes”. After the final washing step, the re-suspended yeast pellets were combined in a single tube. The resulting yeast slurry was used to pitch five 250 mL flasks, each containing 100 mL YEPD broth at $1.5 \times 10^7$ cells·mL$^{-1}$. Cultures were incubated as before for an additional 24 hr before being washed (as above) and combined for use as the fermentor inoculum. This process generated sufficient yeast to pitch 30 test tube fermentors at $1.5 \cdot 10^7$ cells·mL$^{-1}$. The cells were enumerated according to ASBC Yeast-4: a small aliquot of the water-washed yeast slurry was diluted with 0.1 N sodium acetate buffer with 10 mM (pH 4.4)
ethylenediaminetetraacetic acid, and the number of cells assessed using a haemocytometer.

One sterile polytetrafluoroethylene boiling stone (Sigma-Aldrich St. Louis, MO) was transferred to each sterile test-tube fermentor and the thoroughly mixed and pitched wort was aseptically distributed to the 30 sterile fermentation tubes (15.0 mL each). Each tube was stoppered with a sponge bung and the tubes set to ferment (in a water bath) at 21 °C until sampling.

According to ASBC Yeast-14, samples should be taken at 0, 1, 6, 22, 26, 30, 46, 50, 54, 70, 74 and 78 hr, or as close to these times as practical. However, throughout much of this thesis (as noted), the scheduling frequency has been increased in an attempt to better characterize the fermentations. At each reading three tube fermentors were emptied for density and absorbance measurements. Absorbance was measured (UV-Visible system 8453, Hewlett Packard Palo Alto, CA) at 600 nm from the top 3.5 mL of each of 3-20 mL tubes transferred to clear-sided cuvettes. Care was taken not to dislodge excessive bubbles from the fermenting fluid. After the reading, the balance was filtered through Whatman #4 filter paper into a clean test tube until the filtrate was at a depth of approximately 2 cm, necessary for density measurement with an Anton Paar, DMA 35 portable densitometer (Anton Paar Canada, Saint Laurent, PQ).


Siebel Institute of Technology 2012. *Yeast Nutrition* [course material]. World Brewing Academy North American Head Office, Chicago, IL.


CHAPTER 3  SUITABILITY OF THE MINIATURE FERMENTABILITY METHOD TO MONITOR INDUSTRIAL FERMENTATIONS

Materials in this chapter have been published in the *Journal of the American Society of Brewing Chemists* and are being reproduced in this thesis with permission from the publisher, the American Society of Brewing Chemists. Minor changes have been made to the original manuscript.


3.1 ABSTRACT

Malt barley breeders and maltsters often strive to improve the quality of their product by improving fermentability. Small-scale assays are often used to assess the fermentability of wort produced from malt under standardized mashing techniques. However, anecdotal reports suggest that these assays have poor correlation with industrial fermentations in addition to inconsistency between assays. There are several factors that are likely to contribute to this behavior such as yeast strain, pitching rate, the mashing regime, fermentation temperature, barley modification, and batch size. This aim of this study was to isolate and examine the effect of fermentor size on wort fermentability through the use of miniature-scale (15 mL) assays fermented in parallel to industrial sized operations. These miniature fermentations were conducted at identical temperatures to their industrial scale counterparts and used oxygenated wort mashed and pitched by local craft breweries. Wort density was measured throughout the fermentations using a portable densitometer while the turbidity was assessed via spectrophotometer at 600 nm. It was found that fermentation vessel geometry had a significant effect upon the apparent degree of fermentation, however observed disparities were consistent between the assay

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and fermentor dimensions. For example, a difference in final density of 1.1 °P ± 0.2 °P, was observed between the final density of a 19.6 hL craft brewery and a miniature fermentation assay over three consecutive experiments. However, when the wort from an 8.5 hL brew-pub was tested using this lab assay, no significant differences in final attenuation were found (p>0.05). The shear generated through consumption of sugar and subsequent production of carbon dioxide was theoretically estimated for each fermentation, the maximum shear calculated was ~ 46 s⁻¹, and the magnitude varied with fermentation rate.

A reduced shear generated within the shorter (miniature scale) fermentors likely influenced the yeast floc distributions and suspended yeast levels, subsequently affecting the final density of the assay fermentation.

3.2 INTRODUCTION

A key malt quality parameter is fermentability, which is defined as the extent subsequent wort solids can be converted to alcohol and other metabolites under specific mashing regimes. High gravity and larger volume fermentations are now widely conducted by craft breweries, therefore the impact of fermentability on production efficiency and profit continues to grow. The brewing industry can now select from many malting barley varieties due to the success of barley breeding programs (Edney, 2005). These varieties produce wort with varying degrees of fermentability. Differences in fermentability amounting to fractions of a percent can have a substantial impact on the brewer’s (and thus the maltster’s) profitability. With the increase in popularity of “light” (low calorie) beer, the production of malt with potentially high and consistent fermentability is even more desirable. In recognition of the importance of this quality
trait, the Brewing and Malting Barley Research Institute (BMBRI) lists fermentability as a trait and breeding target urgently requiring further understanding and research (Brewing and Malting Barley Research Institute, 2010).

Fermentability is partially determined by the concentration of fermentable sugars within wort (Boulton and Quain, 2006). Fermentable sugar levels are, in turn, dependent upon the amount of carbohydrates and starch degrading enzymes in malted grain and the thermal stability of these enzymes. These factors are determined by barley malt variety (Edney, 2005) and growing conditions. Other processing parameters after harvest such as the malting and mashing regimes (Evans et al., 2002), yeast pitching level and strain (Evans and Hamet, 2005; Hsu et al., 2001; Lake et al., 2008) can influence fermentability. Additional evidence has indicated that nutrients such as amino acids and minerals affect the rate and extent of fermentation (Edney, 2005). According to Edney (2009), “...factors that effect fermentability, and how these vary among barley varieties, are poorly understood”. Further reports by our group (Jin and Speers, 1998; Lake et al., 2008; Speers, 2012; Speers et al., 2003) have noted (not surprisingly), that yeast flocculation influences fermentability. Even fermenter size or the number of brews fermented can influence final Apparent Degree of Fermentation (ADF) values (Speers and Stokes, 2009). Thus, in order to maximize yield, an accurate assessment of malting barley fermentability is of great importance to barley breeders, maltsters and brewers. As a result, barley breeders have begun utilizing small-scale fermentation assays to provide feedback on malting barley varieties.

These fermentations that assess the fermentability of wort are normally prepared by mashing barley using ASBC standard techniques to produce a ‘Congress’ wort (ASBC
Malt-4) while requiring only limited amounts of grain. However, industry has reported poor correlation with industrial scale fermentations in addition to inconsistency between assays (Lake et al., 2008). While stirred assays are employed because they are rapid and convenient, the use of existing (agitated) fermentation procedures are unsuitable to monitor the effect of factors affecting yeast flocculation. The mechanical agitation supplied to stirred assays does not reflect normal brewing operations where fermentor mixing is driven primarily through CO₂ release that varies during the fermentation (Boulton et al., 2005). The concentration of yeast cells in suspension was found to be related to CO₂ evolution rates by Lake et al., (2008). This trait ultimately influenced the final extract of the assays by up to (approximately) 10 % (Lake et al., 2008). It is noteworthy that mechanical agitation used in many current fermentability procedures artificially maintains the yeast in suspension and thus cannot detect premature yeast flocculation factors (Lake and Speers, 2008; Lake et al., 2008). The new ASBC standard mini-fermentation assay (ASBC Yeast-14; Lake et al., 2008; Speers et al., 2010; Speers et al., 2011) is designed to mimic the extract and absorbance patterns observed within industrial fermentors and is hypothesized to more closely replicate yeast in suspension values found during large-scale fermentations.

Wort is subjected to velocity gradients (i.e., sheared) throughout the brewing process when transferred, stirred fermented or otherwise agitated (Speers et al., 2004). The “average root mean squared (RMS) velocity gradient”, or “average shear rate” within a fermentor is defined as a function of the power dissipated per unit volume and the viscosity of the medium with units of s⁻¹ (Droste, 1997). Throughout normal fermenting operations, carbon dioxide is generated as sugars are metabolized. As wort becomes
supersaturated with CO2, bubbles form at the bottom of the fermentor at nucleation sites primarily located on the yeast bed, trub particles (Boulton and Quain, 2006) or the fermentor wall. As the bubbles form and rise through the liquid, energy is dissipated into the medium. The amount of energy dissipated by the gas is dependent on the rate of CO2 evolution, pressures involved, and the distance travelled by the bubble (Delente et al., 1969). Gas driven agitation within cylindroconical fermentors and the associated currents has been described in detail by Boulton and Quain (2006). This work highlighted the effect of fermentor geometry (particularly the height/width aspect ratio) on the associated wort currents generated during fermentation.

When utilizing “small scale” fermentation assays, it is important to consider the possible effects of test fermentor geometry. Work conducted by Lake et al. (2008), confirmed that fluid flow is necessary to maintain a sufficient number of yeast cells in suspension in order to complete the fermentation. However, a lack of appreciable CO2 driven agitation during the first ~ 8-12 hours of fermentation contributes to relatively low shear environments in static fermentations. This contrasts with mechanically agitated assays where turbulent shear environments are maintained. Moreover, fermentation temperatures employed in previous lab assays have not been standardized and vary with each report of this technique. These factors make interpretation and comparison of published reports difficult.

Work by our research group (Speers et al., 2006) emphasized the dependence of yeast in suspension to fermenter shear rate. Specifically, the extent of yeast flocculation decreased with higher agitation resulting in smaller flocs that require additional time to settle (note that this is only one of many parameters that affect yeast flocculation). A
method to estimate the average shear rate within a fermentor has been described (Lake et al., 2008; Speers et al., 2004) where non-linear regression techniques were utilized to model CO₂ evolution and calculate the energy dissipated per unit volume.

3.3 EXPERIMENTAL

As previously discussed, there are many factors that may affect the potential fermentability of malt (under specific mashing regime). To isolate and study the effect of a single factor (fermentor size) upon wort fermentability, identically oxygenated and pitched wort was used in a series of miniature fermentation assays. The wort was prepared by participating breweries and fermented in parallel at the industrial and laboratory scales. The fermentors used were of cylindroconical design with similar aspect ratio (height > twice the diameter) to the mini-fermentation assay (ASBC Yeast-14). Two local breweries participated in the experiment, providing aliquots of 450 mL oxygenated, pitched wort for each trial (three trials were completed at each brewery). The first brewery (a local brew-pub) provided wort from an 8.5 hL fermentor. The second participating brewery was a larger, craft brewery that provided wort from a 19.6 hL fermentor. Care was taken to ensure that for each trial, the yeast strain, mashing techniques, pitching rate, et cetera was identical between the 15 mL and industrial scale fermentations (note that neither fermentation was mechanically stirred). The wort for each trial was pitched with the commercial breweries yeast strain. While details concerning the strain used by each brewery are proprietary it was observed that they exhibited a typical ale phenotype. The wort was fermented in parallel at the 15 mL and either the 8.5 or 19.6 hL scales. Aliquots for the miniature assays were taken immediately after the brewery fermentors had been filled, thereby ensuring a representative sample of
the (temporarily) homogeneous (Boulton and Quain, 2006) mixture. To minimize loss of dissolved gases, the sample was transported in a sealed flask with minimal headspace. The sample was agitated prior to use to restore homogeneity and was divided into miniature fermentors within ~ 15 min of taking the sample.

The miniature fermentation assay used throughout this experiment has been accepted as an ASBC standard method (ASBC yeast-14) with slight modifications. The method describes a miniature assay using thirty 15 mL fermentors and is generally completed within 72 hr. Each fermentor has an aspect ratio similar to a cylindroconical vessel and contains a boiling chip as an artificial nucleation site. In deviation from the standard method, steps describing the formulation of wort (including oxygenation and sugar addition) were not completed as the wort for these experiments was obtained from the participating breweries. This miniature fermentation method was chosen, as the assay was previously shown to have high consistency (Lake et al., 2008) and the sample size required is very small (450 mL per assay). As per the method, the initial sample was partitioned into the 30 fermentation vessels each containing 15 mL of wort and a boiling chip. In an additional exception to the ASBC method, the assay temperature was held at the brewery fermentation temperature through the use of a temperature controlled water bath. Within the craft brewery the wort temperature was controlled at 21 ºC, however, the brew-pub temperature ranged from 16 – 20 ºC over the course of the fermentation. The temperature of the assay was adjusted accordingly at each sampling period.

Samples were taken from the brewery fermentation and during the laboratory miniature scale assay at regular intervals throughout the experiment (nominally at 1, 6, 22, 26, 30, 46, 50, 54, 70, 74 and 78 hr). These samples were taken as close to the
scheduled time as possible, however due to industrial scheduling variation, some samples were unavailable. The density of each sample was measured using an Anton Paar, DMA 35 portable densitometer (Anton Paar Canada, Saint Laurent, QC). When sampling the miniature scale assay, fermentors were destructively tested in triplicate (once the wort within a 15 mL fermentor was tested, it was discarded). During the final trial, the absorbance of each sample was also determined spectrophotometrically at 600 nm.

The change in apparent extract was described with the logistic model, Equation 3.1 (Figure 3.1) which has been successfully shown to predict the change with extract with time (ASBC Yeat-14; Lake et al., 2008; Speers et al., 2003; Speers et al., 2010; Speers et al., 2011), while the modeled data from both the craft brewery and brew-pub trials are shown in Figure 3.2.

\[ P_t = P_e + \frac{P_i-P_e}{1+e^{-B(t-M)}} \]  

(3.1)

where \( P_i \) is the initial asymptotic density value for the density attenuation regression, \( B \) is a function of the slope at the inflection point, \( M \) is the time at point \( B \) and \( P_e \) is the equilibrium asymptotic density value.

Figure 3.1 Representation of the logistic model (Equation 3.1) used to model the density attenuation during each fermentation.
Figure 3.2  Density attenuation during each fermentation, modeled using Equation 3.1.
The absorbance of the wort at 600 nm is known to correlate well with the number of yeast cells in suspension (Davis and Hunt, 1986). Absorbance measurements of the wort were taken at each sampling period during the final brew-pub trial (Figure 3.3). The absorbance data was described with a tilted Gaussian equation defined by Equation 3.1b:

\[
Abs_t = R \cdot t + A \cdot e^{-\frac{(t-\mu)^2}{2\sigma^2}}
\]  

(3.1b)

where “A” describes the absolute amplitude (height - AU) of the Gaussian portion of the curve while, “\(\mu\)” defines the mean (midpoint - hr), and “\(\sigma\)” describes the standard deviation (width - hr). The term R sets the rotation of the Gaussian curve (AU/hr). This equation is a low parameter empirical fit for absorbance data that our laboratory has used on multiple occasions to help compare fermentation absorbance profiles. It was replaced in Chapter 6 with the tilted normal equation, as the latter was found to provide a superior fit for cell counts.

The trend in absorbance for each fermentation was very similar, the number of cells in suspension increased until peak sugar consumption (“M” in Equation 3.1) and subsequently declined. While the initial measurements and trends were identical, the absorbance of the assay remained (slightly) lower over the entire assay as compared to the Brew-pub fermentation. As the number of yeast in suspension is related (at least in part) to the shear rate within a fermentor (Lake et al., 2008), the shear rate within each fermentor was calculated.
Figure 3.3 Absorbance measurements taken over the final brew-pub trial (3), and the associated attenuation.

Within a fermentor that is not mechanically mixed, agitation is primarily driven through CO₂ evolution (Delente et al., 1969). As the shear rate within the fermentor has been previously shown to influence the number of yeast in suspension, the average shear rate in the miniature fermentation assay and the industrial fermentations were assessed. Techniques developed by several researchers were employed to determine the shear within each fermentor. Work by Delente et al. (1969), showed that the energy dissipated through CO₂ evolution could be calculated from the rate of sugar consumption. The extract measurements (as °P) taken during each fermentation were modeled (Table 3.1) by the modified logistic model (Equation 3.1) using non-linear regression techniques (Speers et al., 2003).

The wort density (Pₜ, measured as °P) during each fermentation followed a sigmoidal pattern and was modeled using a generalized logistic function (Equation 3.1) as described by Speers et al., (2003). The model was fitted to the data using a generalized reduction gradient non-linear regression algorithm (With Excel® for Mac 2011 using the
“Solver for Excel 2011 for Mac” add-in, Frontline Solvers, Incline Village, NV.). The regression parameters derived for each trial are presented in Table 3.1 in addition to the results of an F-test used to determine the probability of a single model representing both the industrial fermentation and the assay. The F-test was completed using a statistical analysis package (Prism 5c for Mac OS X, Graphpad Software, Inc. San Diego, CA) where the datasets were compared using a “Global Fit” option that shared all regression parameters.

Table 3.1 Extract attenuation parameters fit to the logistic model for each fermentation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Batch Size (L)</th>
<th>P_i (Std.Err.) (°P)</th>
<th>P_e (Std.Err.) (°P)</th>
<th>B (Std.Err.) (hr atm)</th>
<th>M (Std.Err.) (hr)</th>
<th>Coefficient of Determination (r^2)</th>
<th>F Test P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craft Brewery 1</td>
<td>1960</td>
<td>12.4 (0.39)</td>
<td>2.17 (0.12)</td>
<td>-0.082 (0.007)</td>
<td>25.6 (1.17)</td>
<td>0.998</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>13.7 (0.75)</td>
<td>3.13 (0.14)</td>
<td>-0.072 (0.008)</td>
<td>21.7 (2.26)</td>
<td>0.988</td>
<td></td>
</tr>
<tr>
<td>Craft Brewery 2</td>
<td>1960</td>
<td>11.3 (0.08)</td>
<td>2.31 (0.09)</td>
<td>-0.120 (0.004)</td>
<td>35.5 (0.36)</td>
<td>0.999</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>11.7 (0.59)</td>
<td>3.24 (0.36)</td>
<td>-0.088 (0.013)</td>
<td>33.7 (2.11)</td>
<td>0.978</td>
<td></td>
</tr>
<tr>
<td>Craft Brewery 3</td>
<td>1960</td>
<td>12.1 (0.05)</td>
<td>2.90 (0.56)</td>
<td>-0.150 (0.006)</td>
<td>25.1 (0.17)</td>
<td>0.998</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>12.2 (0.39)</td>
<td>3.90 (0.24)</td>
<td>-0.090 (0.009)</td>
<td>29.5 (1.39)</td>
<td>0.981</td>
<td></td>
</tr>
<tr>
<td>Brew-Pub 1</td>
<td>850</td>
<td>10.7 (0.07)</td>
<td>1.51 (0.18)</td>
<td>-0.070 (0.003)</td>
<td>41.7 (0.63)</td>
<td>0.999</td>
<td>0.4645</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>10.7 (0.43)</td>
<td>1.51 (1.12)</td>
<td>-0.068 (0.012)</td>
<td>43.7 (3.47)</td>
<td>0.979</td>
<td></td>
</tr>
<tr>
<td>Brew-Pub 2</td>
<td>850</td>
<td>11.1 (0.33)</td>
<td>2.90 (0.19)</td>
<td>-0.155 (0.04)</td>
<td>27.5 (1.74)</td>
<td>0.999</td>
<td>0.5851</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>11.1 (0.33)</td>
<td>3.22 (0.14)</td>
<td>-0.152 (0.02)</td>
<td>28.9 (1.17)</td>
<td>0.991</td>
<td></td>
</tr>
<tr>
<td>Brew-Pub 3</td>
<td>850</td>
<td>13.7 (0.59)</td>
<td>2.35 (0.29)</td>
<td>-0.086 (0.01)</td>
<td>33.3 (1.63)</td>
<td>0.997</td>
<td>0.0048</td>
</tr>
<tr>
<td>Assay</td>
<td>0.015</td>
<td>13.7 (0.53)</td>
<td>2.30 (0.25)</td>
<td>-0.08 (0.01)</td>
<td>38.6 (1.55)</td>
<td>0.992</td>
<td></td>
</tr>
</tbody>
</table>

† The asymptotic standard error of each parameter was determined by taking the square of the product of the Sy.x (standard deviation of the residuals) and the parameter’s diagonal element of the covariance matrix (dispersion matrix).

A multivariate polynomial (Equation 3.2, below) described by Cutaia et al. (2009) was used to calculate the actual percent solids or Real Extract (RE) at time (t) and was modeled using the same modified logistic equation as the original measurements with RE{(t)} in replace of P{(t)} (Equation 3.3). Finally, the rate of sugar consumption was
determined by taking the 1st derivative with respect to time of the logistic model (Equation 3.4) and inserting the best-fit parameters from the real extract regression.

\[ RE = (0.49681569 \cdot A_{w/w}) + (1.0015341 \cdot AE) - (0.00059105 \cdot A_{w/w} \cdot AE) - (0.00029431 \cdot AE^2) - (0.00847470 \cdot A_{w/w}^2) + (0.00018356 \cdot A_{w/w}^3) + (0.00001115 \cdot AE^3) + (0.00000245 \cdot A_{w/w}^2 \cdot AE^2) \]  

(3.2)

\[ RE(t) = P_e' + \frac{P_i' - P_e'}{1 + e^{-b'(t-M')}} \]  

(3.3)

Where \( A_{w/w} \) is the alcohol content, \( AE \) is the apparent extract, \( P_i' \) is the initial asymptotic density value for the real extract regression, \( b' \) is a function of the slope at the inflection point of the change of the real extract with time, \( M' \) is the time at the inflection point of the change of real extract with time and \( P_e' \) is the equilibrium asymptotic density value for the real extract regression.

\[ \frac{dRE}{dt} = -b'(P_i' - P_e')e^{(b't + b'm')} \]  

(3.4)

The rate of sugar consumption (as determined using Equation 3.4) was used to estimate the rate of \( CO_2 \) generation, as the products of maltose metabolism by \textit{Saccharomyces cerevisiae} are known. The stoichiometric relationship below relates the mass of consumed maltose to the mass of \( CO_2 \) produced (Holle, 2003):

\[ \text{Maltose} + \text{amino acid} = \text{yeast} + \text{ethanol} + \text{CO}_2 + \text{energy} \]

\[ 100g + 0.5g = 5g + 48.8g + 46.8g + 209\text{ KJ} \]  

(3.5)

A method described by Delente et al. (1969) was employed to determine the power generated within each fermentor, considering the rate of bubble formation and the differences in energy between the atmospheric and hydrostatic pressure on bubble
evolution (Equation 3.6). Delente describes the equation for power generated per unit volume as proportional to the rate of CO₂ flow and as a function of fermentor height:

\[ P = B_{vol}Q_{CO2}P_a \left( \frac{P_a + P_b}{P_b} \ln \frac{P_a + P_b}{P_a} - 1 \right) \]  

(3.6)

where \( P \) is power released, \( P_a \) is the atmospheric pressure, \( P_b \) is the hydrostatic pressure in the fermentor, \( Q_{CO2} \) is the CO₂ evolution rate and \( B_{vol} \) is the wort volume.

Once the amount of energy dissipated within a liquid is known, the average shear rate can be calculated (Equation 3.7) as described by Lake et al. (2008) provided the physical properties of the liquid are available. This method was not used to determine the shear within the first ~ 12 hours of fermentation as both our experiments and Boulton et al., (2005) observed a “…lack of appreciable CO₂ and heat evolution during this early period (that) would produce little or no natural mixing action” in industrial scale fermentations. While CO₂ was likely produced during this initial period, the wort must become supersaturated prior to appreciable CO₂ evolution (Boulton et al., 2005).

The RMS velocity gradient originally described by Camp and Stein (1943) was calculated using Equation 3.7:

\[ \dot{\gamma} = \left( \frac{p}{\eta B_{vol}} \right)^{1/2} \]  

(3.7)

where \( \dot{\gamma} \) is the average turbulent shear rate (or RMS velocity gradient) and \( \eta \) is the viscosity.

The average peak shear rate for the 3.56 m (19.6 hL) craft brewery fermentor and assay were calculated as 44.1 and 7.4 s⁻¹ respectively. Values of 35.3 and 7.8 s⁻¹ correspond to the 1.9 m (8.5 hL) brew-pub fermentor and assay respectively (Figure 3.4).
Over each fermentation the shear rate was calculated after ~12 hr (the estimated saturation point). Once wort saturation was reached, the shear increased with CO₂ until peak sugar consumption and subsequently diminished. The calculated shear rates are comparable to previous studies that have examined the amount of shear within industrial fermentations (Speers and Ritcey, 1995).

Figure 3.4 Comparison between 19.6 and 8.5 hL fermentors and assay

From Figure 3.2, it is evident that the fermentations within each brewery are not consistent. This variation was expected as the experiments were conducted over several months and can be explained by differences in pitching rate, mashing conditions and other brewery procedures characteristic of smaller brewing operations. However, the trials completed at the brew-pub scale brewery were similar to the assay completed using identical wort. It was notable that the trials using wort from the craft brewery consistently showed a lower final density (resulting in a higher alcohol yield) than the assay, as shown in Figure 3.2. This difference between the 19.6 hL fermentor and the miniature
fermentation assay was significant (p < 0.05) and consistent (1.1 ± 0.2 °P) over the three fermentations conducted.

The observed difference between the apparent extract values of the assay and craft brewery is likely related to the scale of the assay as this was the only identified disparity between the fermentations. The F-test, comparing the change in extract with time (Equation 3.1) of the assay to industrial fermentation indicated that every craft brewery fermentation was significantly different from the miniature fermentation assay (Table 3.1). Between the brew-pub and the assay, no significant difference (P < 0.05) in the fermentation curves were detected for two of three trials (Figure 3.1, brew-pub 1 and 2) yet the midpoint value was always higher during the assay fermentation. As seen in Table 3.1, the third brew-pub fermentation was significantly (P < 0.05) but not substantially different (determined through visual inspection) from the miniature fermentation assay.

3.4 CONCLUSION

The attenuation data modeled using Equation 3.1 (as shown in Figure 3.2) indicates that each Craft Brewery fermentation was different (P < 0.05) from its corresponding assay. Therefore, fermentor size had a significant effect on the final density of larger craft fermentors, but not for smaller brew-pub operations. While the difference between the brew-pub and assay was not significant, it is noteworthy that the density at the midpoint of the assay was in each case higher than the brew-pub. Additionally, the discrepancy within larger craft brewery fermentors (Figure 3.2) was consistent to the assessed brewery. For example, a large difference of 1.1 °P ± 0.2 °P was observed between the final densities of the 19.6 hL brewery and the miniature
fermentation assay over three consecutive experiments, while the differences between an 8.5 hL brew-pub and the small assay were not significant.

The shear rates within each fermentor were mathematically modeled utilizing the theoretical energy released through the evolution of CO₂ within the system at any given time (t). While the theoretical CO₂ generation rates per unit volume of wort were found to be very similar between each fermentation and the miniature fermentation assay, the theoretical average shear rates within the wort were found to vary with the height of the fermentor. As the agitation within each fermentor is not driven through impeller action, much of the traditional work on scale up ratios (such as described by Geankoplis, 1983) cannot be directly applied. However, as previously discussed in the introduction, earlier research (Delente, et al., 1969) has shown that the height of a fermentor affects the amount of energy imparted to the medium by rising CO₂ bubbles. The additional energy per unit volume in higher fermentors results in larger shear rates within the wort. It was hypothesized that this increase maintained the yeast in suspension longer than within the 15 mL assay. This would result in reduced yeast activity during the latter portion of the miniature fermentation assay affecting the final density of the fermentation and thus the calculated “fermentability” of the wort. This factor may have contributed to the differences observed between the miniature fermentation assay and larger craft fermentations.

Another possibility is that the reduced height of the assay may have facilitated additional yeast settling at the beginning of the fermentation as a much larger proportion of yeast is located within settling distance of the bottom of the lab fermentor than in the craft vessel. Settled yeast cells are not easily re-suspended once the wort became
saturated with CO₂ and agitated. Thus, in a lab assay the total number of yeast cells in
suspension over the entire fermentation would be reduced in comparison to its industrial
scale counterpart. This factor may have also affected the final apparent extract of the
miniature fermentation.

In summary, it was found that the type and scale of fermentation vessel affected
the fermentation. Possible mechanisms for the observed effects include additional yeast
settling at the beginning of fermentation in miniature fermentation assay and the reduced
shear generated in the assay which influences yeast floc distributions near the end of the
fermentation. By correlating the assay to specific fermentor geometry and brewing
techniques, the miniature fermentation assay will likely be able to consistently predict
wort fermentability. As the miniature fermentation (ASBC Yeast-14) is an inexpensive
and simple assay, this may be a useful tool for smaller breweries to assess wort
fermentability and quality of malt prior to full scale fermentation using a standard
mashing procedure. While the power generated through CO₂ evolution can be calculated,
further research is necessary to understand the effect of agitation on yeast in suspension
and how this, and other factors affect the final density (and thus fermentability) of wort.
If maintaining or forcefully re-suspending yeast results in a more complete fermentation,
this may have very important repercussions on how brewers view not only miniature
scale assays, but also variable sized fermentors and fermentor agitation.
3.5 REFERENCES


Evans, D., E., Ma, Y., Eglinton, J., K., Langridge, P., Louge, S., and Barr, A. 2002. The Relationship Between Malt Performance, β-amylase, Diastatic Power and
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CHAPTER 3  CARBON DIOXIDE SOLUBILITY IN BEER

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3.1 ABSTRACT

The amount of CO2 within a beer is a partial function of CO2 solubility, which in turn is affected by temperature, containing pressure and beer composition. Historically, this variable was assessed through empirically derived pressure/temperature charts with the first appearing about 1939. Modern methods often involve empirical or semi-empirical formulas that yield close approximations to the aforementioned charts (at typical storage conditions). Other methods to determine CO2 solubility incorporate additional variables such as extract and alcohol content. Unfortunately the origin of various pressure-temperature solubility charts contained in ASBC’s Methods of Analysis or MBAA’s Beer Packaging: A Manual for the Brewing and Beverage Industries, are largely unknown, as are the composition of the beer used to create these charts. This discrepancy results in potentially inaccurate CO2 values for differing beer compositions and is especially problematic when assessing modern methods that incorporate additional beer properties. This paper attempts to compare and contrast modern and historical methods while considering the limited solubility reports for CO2 in beer, sugar and ethanol solutions. In this paper the accuracy of CO2 solubility charts and formulas are discussed while considering assumptions reported by the original authors. Finally,
modern formulae are used with non-linear optimization techniques to generate the likely composition of the “standard beer” used to construct the original ASBC solubility chart. It appears that a “standard beer” of yesteryear is stronger than an average modern beer with an alcohol content of 4.22 % (w/w) and a RE of 5.78 °P.

3.2 INTRODUCTION

During the investigation of CO₂ driven fermenter circulation, a question arose as to how one could estimate the point at which fermenting wort become saturated with CO₂. This investigation lead to an inquiry as to the basis and validity of the well-known CO₂ solubility charts such as is provided in the ASBC “Methods of Analysis” (ASBC Beer-13).

While other methods are now in use, the well known Zahm-Nagel technique (illustrated in Zahm and Nagel Co., 1964) calculates CO₂ levels within beer using measurements of headspace, partial pressure and beer temperature. The ASBC Beer-13 chart (ASBC Beer-13) indicates the volumes of CO₂ at Standard Pressure and Temperature (STP defined as 273.15 K and 101.325 kPa or 14.696 psi) that will dissolve in a “standard beer” (Grey and Stone, 1936) as function of beer temperature and CO₂ partial pressure (at equilibrium). This chart was apparently developed at the Wallerstein Laboratories in New York where the authors Grey and Stone were employed and may be based on values for water, modified for beer, however, the exact origin of the Beer-15 (ASBC Beer-15) chart, now the Beer-13 chart (ASBC Beer-15), is unclear.

Additional charts and algorithms are available and were developed empirically or using unknown assumptions. For example, Wallerstein Laboratories developed an
algorithm and slide rule “brew computer” to calculate the volumes of CO₂ (at STP) dissolved in a “standard beer” (Breyer, 1969). This slide rule was later marketed by The Seibel Institute of Technology (Chicago, IL) and is reported to conform closely to the ASBC chart (Breyer, 1969).

3.3 LITERATURE REVIEW

Reports of CO₂ solubility in beer are scarce, some data were recorded by Findlay and Shen (1911) who in 1911 observed that, “it is somewhat remarkable that the solubility of this gas has been studied so little”. In the ensuing century the authors have been able to find only two more reports in English regarding CO₂ solubility within beer. Unfortunately, a report by Hartung (1934) as cited by Gray and Stone (1936) could not be found despite an appeal to the ASBC community.

In contrast to the paucity of information concerning CO₂ within beer, there is an overwhelming collection of data concerning CO₂ solubility in water. Web-based data is available from the US National Institute of Standards and Technology (2011). A newer paper by Rammert and Pahl (1991) reports the effect of temperature, ethanol and “sugar” on Henry’s coefficient. This paper reinforces the concept that the solubility of CO₂ dissolved in water or solutions of alcohol and carbohydrates (such as wort and beer) is governed by Henry’s law. The law may be defined at the temperatures and pressures of interest as:

\[ k_{HT} = \frac{x}{p} \]  

(3.1)

where \( k_{HT} \) is Henry’s coefficient at a defined temperature \( (T) \), this term is often reported as Henry’s “constant”, however, as it is variable with temperature, it is not technically a
constant. \( X \) is normally expressed as the CO\(_2\) mole fraction and \( P \) is the absolute pressure. However, ratio “\( X \)” can also be expressed in a myriad of combinations such as the mole or volume fractions at a defined pressure and temperature, \( (\text{vol}_{\text{CO}_2}/\text{vol}_{\text{solv}}) \) or combinations such as the number of moles per defined volume \( (\text{mol}_{\text{CO}_2}/\text{vol}_{\text{solv}}) \) et cetera. Unfortunately, (and confusingly) the inverse of Henry’s coefficient as defined above, is also reported as Henry’s coefficient in the literature. There appears to be consensus among brewing scientists that within normal brewing temperature and pressure ranges, Henry’s law (Equation 3.1) holds for equilibrium conditions. That is, the volume of CO\(_2\) dissolved at equilibrium is directly proportional to the partial pressure of CO\(_2\) within the headspace.

The tradition in the carbonated beverage industry is to report gas solubility as a ratio of volume of dissolved CO\(_2\) at STP per volume of beer \( (\text{vol}_{\text{CO}_2}/\text{vol}_{\text{solv}}) \) as shown in the ASBC solubility chart (Beer-13) and hereafter reported as v/v. Zahm and Nagel Co. provides a conversion to weight percentage for the ASBC chart which is available from their website (2013). The value at one atmosphere and a specified temperature is known as the Bunsen coefficient since it was first proposed in 1855. These solubility coefficients are very temperature dependent and can be affected by wort and beer constituents. As an alternative to using coefficients and equations, charts for CO\(_2\) solubility as a function of temperature and pressure have been published (i.e., ASBC Beer-13 and a solubility chart released by Zahm and Nagel Co. 1964). A portion of the Zahm and Nagel chart was also republished by the Master Brewers Association of the Americas (MBAA) and is hereafter referred to as the “MBAA” chart (Broderick, 1982). A third CO\(_2\) solubility chart using units of Celsius and Bar is available from the German “Agency for brewing culture”
website (Agentur für Braukultur, 2009). In these charts and this paper, the pressure is
assumed to be due to CO2 and free of any oxygen or nitrogen impurities. While the charts
have different units and ranges, they also follow unique trends.

As previously mentioned, there exists little CO2 solubility data for beer - at least
in the English literature. Only three research groups appear to have measured and
reported on CO2 solubility in beer (Findlay and Creighton, 1910; Gray and Stone, 1936;
Hartung, 1934).

The ASBC-13 solubility chart was apparently developed by Grey and Stone et al.,
at the Wallerstein Laboratories in the 1940’s. Readers might be surprised to find that
despite an extensive literature search and an appeal to ASBC, MBAA and IBD members,
little information is available on how the ASBC solubility chart was constructed other
than it was apparently based upon a “standard beer” (ASBC 1). This chart does seem to
be in general use by the Society by the 1940’s and is listed in Beer-15 of the Fifth Edition
of the Methods of the Society (ASBC Beer-15). The nature of the “standard beer” used to
construct this chart is also unknown although its specific gravity was reported as 1.015.
The current Beer-13 chart (ASBC Beer-13) is unchanged from ASBC Beer-15 except that
the current method seems to have dropped a decimal place and reports the chart as from a
“standard beer” of specific gravity of 1.01!

A second MBAA chart similar to Beer-15 is presented by Broderick (1982);
however no further details on the development of this “MBAA” chart seem to be
available either.

As mentioned, reports on the solubility of CO2 in sugar solutions (von Loesecke,
1949) and in ethanol (Dalmolin et al., 2006) for the temperature and pressure ranges
encountered in beer are more widespread. Information and techniques concerning the solubility of CO₂ in water are very common. For example, the US National Institute of Standards and Technology (2011) corrects Henry’s coefficient (k_H_T) for temperature using the Van ‘t Hoff expression:

\[
k_{H_T} = k_{H^0} \cdot e^{\left(\frac{C - \frac{1}{273.15}}{T}\right)}
\]  

(3.2a)

where \( k_{H^0} \) is a reference value of Henry’s coefficient at 25 °C, and \( T \) is the absolute temperature in Kelvin (K). It is noteworthy that the Van ‘t Hoff expression is algebraically equivalent to the Arrhenius equation developed in 1884 (Arrhenius, 1889):

\[
k_{H_T} = a \cdot e^{\frac{b}{T}}
\]  

(3.2b)

where \( a \) and \( b \) are variables when this equation is fit empirically to predict the effect of temperature on \( k_{H_T} \).

In addition to the ASBC and MBAA charts, various algorithms have been proposed to estimate the Henry’s law as a function of temperature. One of the earliest formulas was reported by Beyer (1969):

\[
CO_{2w} = 5.093 \cdot P \cdot SG \cdot 0.00965 + 1.612 \cdot 10^{-10} \cdot (914000 \cdot (77 - T_F) + 10100 \cdot (77 - T_F)^2 + 40 \cdot (77 - T_F)^3 + (77 - T_F)^4)
\]  

(3.3)

where \( SG \) is the specific gravity and \( T_F \) is the temperature in °Fahrenheit (°F). As Beyer was on the Wallerstein Laboratories staff with Stone and Grey and since Equation 3 is a rather complex polynomial, it would appear the ASBC chart was experimentally derived.

A more common expression used to predict the solubility of CO₂ with temperature and pressure has been cited by Holle (2003):
where $P_{\text{psig}}$ is the headspace pressure in units of psig value and $T_F$ is the temperature in °F. Determining the origin of this function has been a challenge and a mystery. The earliest reference to this function the authors could find is a paper by Rohner and Thompkins (1970). The function appears to be a purely empirical relation of temperature and pressure to carbon dioxide solubility, however no explanation or justification was given for the relationships. It is also unclear how a further modification to this expression published online by VitalSensors Technologies LLC (O’Leary R. 2008) and included as a calculator in Beer-13, was developed:

$$CO_{2v/v} = 4.85 \cdot \left(\frac{P_{\text{psig}} + 14.7}{T_F + 12.4}\right)$$

(3.4a)

where $CO_{2v/v}$ is the volume of CO$_2$ at STP dissolved in 1 volume of beer, $SG$ is the specific gravity and $A_{w/w}$ is the percent alcohol level by weight. The formula can be expressed using percent alcohol by volume (Cutaia et al., 2009):

$$CO_{2v/v} = 5.16 \cdot \left(\frac{P_{\text{psig}} - 14.7}{T_F + 12.4}\right) \cdot SG \cdot (1 + \frac{1}{0.789} \cdot A_{w/w})$$

(3.4b)

Another expression relating CO$_2$ solubility to pressure and temperature was released in a recent paper by Trelea et al., (2004) attributed to the Institut Français de Brasserie et Malterie (IFBM):

$$CO_{2v/v} = 2.83 \cdot 10^{-2} \cdot P_{\text{Bar}} \cdot \left(\frac{T}{273.15}\right) \cdot e^{-0.0335 \cdot T}$$

(3.5)
where $P_{m\text{Bar}}$ is the absolute pressure in mBar and $T$ is the temperature in °C. While this expression was reportedly used in-house by IFBM, they are apparently no longer aware of the algorithm (private communication to Speers, from Patrick Boivin October, 2012).

Finally, there exists a non-reviewed report (deLange, 2011), that models the ASBC Beer-13 solubility chart in detail. In this paper, deLange developed the following expression to predict CO$_2$ solubility:

$$ CO_{2v/v} = P \cdot (0.01821 + 0.090115 \cdot e^{\frac{(T-32)}{43.11}} ) - 0.003342 \quad (3.6) $$

With the exception of the Arrhenius/Van ‘t Hoff models, the aforementioned equations are empirically derived. While these expressions will be discussed later, it should be noted that empirical relations by their nature are unreliable to extrapolate and inherently less desirable to use when compared to a theoretical, or in the case of a chemically undefined solution such as beer, a semi-empirical model.

The majority of the literature predicts the change in Henry’s coefficient with temperature and has not considered the effect of ethanol or sugar concentration on the solubility of CO$_2$ in beer. The only exception found to date (as previously mentioned) is the formula reported by Rammert and Pahl (1991) which predicts the absorption coefficient ($\zeta$CO$_2$) in beer and fruit juice as a function of oxygen, temperature, extract, NaCl, alcohol and fruit juice. The expression below is a simplified version of their formula assuming the absence of oxygen and fruit juice within the beer:

$$ \zeta \text{CO}_2 = 3.36764 - 0.12723 \cdot T_c + 2.8256 \cdot 10^{-3} \cdot T_c^2 - 3.3597 \cdot 10^{-5} \cdot T_c^3 + 1.5933 \cdot 10^{-7} \cdot T_c^4 - (0.4723 - 2.988 \cdot 10^{-2} \cdot T_c + 1.1605 \cdot 10^{-3} \cdot T_c^2 - 2.251 \cdot 10^{-5} \cdot T_c^3 + 1.5933 \cdot 10^{-7} \cdot T_c^4) \cdot \left( \frac{E}{128} + \frac{A_{v/v}}{43} + \frac{N_{aCl}}{27} \right) \quad (3.7) $$
where $\zeta_{CO_2}$ is dissolved CO$_2$ expressed in g·L$^{-1}$·bar$^{-1}$, $T_C$ is the temperature in °C, $E$ is the real extract expressed in g·L$^{-1}$, and NaCl is sodium chloride in g·L$^{-1}$. $A_{v/v}$ is the volume of alcohol per volume of solution. This equation (Equation 4.7) is reported to be valid through the following parameter ranges:

$$0.7 \leq \zeta_{CO_2} \leq 3.4 \text{ g·L}^{-1}·\text{bar}^{-1}$$
$$0 \leq T_C \leq 60 \, \text{°C}$$
$$0 \leq E \leq 300 \, \text{g·L}^{-1}$$
$$0 \leq \text{NaCl} \leq 50 \, \text{g·L}^{-1}.$$ 

Equation 6.7 has been adapted by Anton Paar for calculating CO$_2$ with their CarboQC™ instrument. The expression has also been employed by Schöck et al. (2012), in sound velocity measurements for carbonated liquids. There are concerns with this function, however, as it is not clear that each variable was assessed independently. When we compare the predicted variation of CO$_2$ solubility with temperature (Dalmolin et al., 2006) to that of water (which is governed by the Van ‘t Hoff expression) this polynomial function seems unlikely to reflect the theoretical trend, as a fourth degree polynomial describes a curve that changes direction 3 times (a phenomenon not observed in other related relationships).
3.4 Analysis of Literature Data

With present day computing and the advent of non-linear regression techniques, it is relatively easy to examine the statistically derived fit of the models discussed earlier to ASBC and MBAA solubility charts as well as to CO\textsubscript{2} solubility data in beer, water and sugar and ethanol solutions (ASBC Beer-13; Dalmolin et al., 2006; Findlay and Creighton, 1910; Findlay and Shen, 1911; Von Loesecke, 1949; National Institute of Standards and Technology, 2011).

Not surprisingly, Henry’s law is adhered to in all the substrates examined; ranging from water (National Institute of Standards and Technology, 2011) to wort, beer, and ethanol (Trelea et al., 2004; Findlay and Shen, 1911). Figure 3.1 shows the trend at 25 °C. As one might predict, the ASBC and MBAA charts also follow this trend. Additionally, the data shows that CO\textsubscript{2} is less soluble in strong beers.
Figure 3.1 Carbon dioxide solubility data from the brewing literature with that of water and ethanol at 25 °C (*ASBC, Beer-15; **Findlay and Creighton, 1910; ***Findlay and Shen, 1911), where Beer A, B, and C have an A v/v of 4.17, 5.17, and 7.13 respectively.

We also examined how the various models that predict Henry’s coefficient ($kH_T$, calculated as $\frac{CO_{2v/v}}{p}$) perform. These models (Equations 4.2-4.6) were used to predict CO$_2$ solubility as a function of pressure and temperature as reported in the ASBC-13 and MBAA charts. The non-linear regression module of Systat-11 (Systat Software, Inc Chicago, IL) was used to minimize the residual sum of squares (RSS) between the
model and charts using the Gauss-Newton estimation technique. This method was employed to examine the fit of the models to both the ASBC and MBAA charts. The fits are presented in Table 4.1. It is important to note that the “solutions” portrayed in Table 4.1 are the best estimate of parameters. Non-linear regression provides a “best guess” rather than an exact answer (this is also the case for linear regression).
Table 3.1  Algorithms for CO$_2$ solubility compared to the ASBC Beer-13 and MBAA charts.

<table>
<thead>
<tr>
<th>Data Set &amp; model</th>
<th>RSS (v/v/psia)</th>
<th>$r^2$</th>
<th>Estimated Parameter Value</th>
<th>ASE of Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A= $1.092\cdot10^{-5}$</td>
<td>$5.829\cdot10^{-8}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B= $2.511\cdot10^{3}$</td>
<td>1.526</td>
</tr>
<tr>
<td>ASBC-13 (Arrhenius$^1$)</td>
<td>4.010$\cdot10^{-1}$</td>
<td>0.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A= $5.800\cdot10^{-10}$</td>
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</tr>
<tr>
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<td></td>
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<td>$1.248\cdot10^4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C= $3.061\cdot10^{3}$</td>
<td>$1.142\cdot10^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D= $2.296\cdot10^3$</td>
<td>2.139</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>B= $4.938\cdot10^{-3}$</td>
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<tr>
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<td></td>
<td>A= $5.800\cdot10^{-10}$</td>
<td>$2.868\cdot10^{-11}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B= $2.536\cdot10^6$</td>
<td>$1.248\cdot10^4$</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>C= $3.061\cdot10^{3}$</td>
<td>$1.142\cdot10^2$</td>
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<td></td>
<td>D= $2.296\cdot10^3$</td>
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</tr>
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<td>B= $4.633\cdot10^{-5}$</td>
<td>$3.061\cdot10^{-4}$</td>
</tr>
<tr>
<td>ASBC-13 (Rohner &amp; Thomkins$^3$)</td>
<td>5.615$\cdot10^{-4}$</td>
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</tr>
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<td>B= $9.155\cdot10^{-2}$</td>
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<td>C= $3.061\cdot10^{3}$</td>
<td>$1.142\cdot10^2$</td>
</tr>
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<td></td>
<td></td>
<td>D= $1.142\cdot10^{-2}$</td>
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<td></td>
<td></td>
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<td>$7.604\cdot10^{-5}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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</tr>
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</tr>
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<td></td>
<td></td>
<td>B= $-5.702\cdot10^{-8}$</td>
<td>N.C.</td>
</tr>
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<td></td>
<td></td>
<td>C= $3.220\cdot10^{-3}$</td>
<td>N.C.</td>
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<tr>
<td>MBAA (Beyer$^2$)</td>
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<tr>
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<td></td>
<td>A= $4.705$</td>
<td>$2.126\cdot10^{-2}$</td>
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<td>B= $1.098\cdot10^{-1}$</td>
<td>$2.150\cdot10^{-1}$</td>
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<tr>
<td>MBAA (Rohner &amp; Thomkins$^3$)</td>
<td>1.500$\cdot10^{-4}$</td>
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<td>A= $7.244$</td>
<td>$4.328\cdot10^{-2}$</td>
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<td></td>
<td></td>
<td></td>
<td>B= $-7.135$</td>
<td>$4.319\cdot10^{-2}$</td>
</tr>
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<td>MBAA (Trelea et al.$^4$)</td>
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</tr>
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<td>A= $3.630$</td>
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<td>B= $6.419\cdot10^{-3}$</td>
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<td>MBAA (deLange$^5$)</td>
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<td>0.988</td>
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</tr>
</tbody>
</table>

Note: Mean corrected $r^2$ reported, RSS is the Residual Sum of Squares, ASE is the Asymptotic Standard Error and N.C. stands for Not Computed.

1Where CO$_2$/v/v/P=A$\cdot$e$^{(B/T)}$
2Where CO$_2$/v/v/P=5.093$\cdot$9.65$\cdot$10$^{-3}$+1.612$\cdot$10$^{-10}\cdot$(A(77-T$_F$)+B(77-T$_F$)$^2$+C(77-T$_F$)$^3$+D(77-T$_F$)$^4$)
3Where CO$_2$/v/v/P=A/(T$_F$+B)
4Where CO$_2$/v/v/P=A(T/273.15)$\cdot$e$^{-(B\cdot Tc)}$
5Where CO$_2$/v/v/P=(A+B$\cdot$e$^{(T/323.15)})$-D/P

Examination of Table 3.1 indicates that all of the five models fit the ASBC and MBAA data reasonably well. However, when examining the residual data there are definite (and undesirable) trends in that of Rohner and Thomkins (1970), Trelea et al. (2004) and deLange (2011), specifically that the difference between the model and data
showed pattern deviation. In the case of the Arrhenius fit, the variation of actual and predicted data was less than 0.01 v/v in most cases with a maximum ± 0.04 v/v in a few cases. Given the theoretical basis of the Arrhenius equation, it is probably the preferred model to predict the solubility of CO₂ solely as a basis of temperature and pressure.

Aside from the Rammert and Pahl equation for CO₂ solubility as a function of temperature, pressure, solids and alcohol levels (Rammert and Pahl, 1991), no other studies have tested calculations of CO₂ solubility as a function of all these variables. As mentioned earlier, this expression is used by Anton Parr to estimate CO₂ concentration. It also has been employed by Schöck et al. (2012) in studies of sound velocity measurements in carbonated liquids and is reported to be accurate to ± 2 % (Rammert and Pahl, 1991). The formula was compared to literature data by comparing the Bunsen Coefficient calculated using Equation 4.7 to published Bunsen Coefficients. The literature data is portrayed in Table 3.2 with the temperature, RE, and alcohol values. Figure 3.2 shows the predicted Bunsen coefficients calculated from Equation 3.7 (with pressure set to a standard atmosphere and salt content assumed to be 0.1 g·L⁻¹) contrasted to the measured Bunsen coefficients shown in Table 3.2. As can be seen, Equation 3.7 does not perfectly predict all data recorded in Table 3.2, although the trends are accurately reflected. This indicates that further data is needed to either confirm or dispute Equation 3.7.
Table 3.2  Bunsen coefficient values for CO₂ in beer, water, ethanol and sugar solutions.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Alcohol (v/v)</th>
<th>Real Extract (°P)</th>
<th>Bunsen Coeff. (v/v)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>25.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.750</td>
<td>Findlay and Creighton, 1910</td>
</tr>
<tr>
<td>25.0</td>
<td>2.97</td>
<td>0.00</td>
<td>0.743</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>3.03</td>
<td>0.00</td>
<td>0.744</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>8.97</td>
<td>0.00</td>
<td>0.720</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>0.00</td>
<td>12.4</td>
<td>0.678</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>4.17</td>
<td>4.32*</td>
<td>0.721</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>6.10**</td>
<td></td>
<td>0.696</td>
<td>Findlay and Shen, 1911</td>
</tr>
<tr>
<td>25.0</td>
<td>7.13**</td>
<td>11.1***</td>
<td>0.660</td>
<td>Findlay and Shen, 1911</td>
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<td>0.00</td>
<td>0.00</td>
<td>1.534</td>
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<tr>
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<td>0.00</td>
<td>1.310</td>
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<td>0.00</td>
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<tr>
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<td>0.00</td>
<td>0.985</td>
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<td>0.00</td>
<td>0.755</td>
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<tr>
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<td>0.00</td>
<td>0.687</td>
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<td>0.620</td>
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<td>1.022</td>
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<td>0.798</td>
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<td>0.685</td>
<td>Dalmolin et al., 2006</td>
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<td>0.00</td>
<td>0.578</td>
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</tr>
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<td>0.00</td>
<td>1.00</td>
<td>0.995</td>
<td>von Loesecke, 1949</td>
</tr>
<tr>
<td>15.6</td>
<td>0.00</td>
<td>2.02</td>
<td>0.989</td>
<td>von Loesecke, 1949</td>
</tr>
<tr>
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<td>0.00</td>
<td>3.04</td>
<td>0.982</td>
<td>von Loesecke, 1949</td>
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<td>0.00</td>
<td>4.07</td>
<td>0.975</td>
<td>von Loesecke, 1949</td>
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<td>15.6</td>
<td>0.00</td>
<td>5.10</td>
<td>0.967</td>
<td>von Loesecke, 1949</td>
</tr>
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<td>0.959</td>
<td>von Loesecke, 1949</td>
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<td>0.907</td>
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<tr>
<td>15.6</td>
<td>0.00</td>
<td>13.68</td>
<td>0.902</td>
<td>von Loesecke, 1949</td>
</tr>
</tbody>
</table>

Beers A*, B** and C*** and Real Extract expressed as g/100mL. NIST – National Institute of Standards and Technology. Using this method, the RSS was minimized by varying the original extract estimate.
Figure 3.2  A comparison of reported and calculated Bunsen coefficients using literature data (Table 3.2).

Following this analysis, Equation 3.7 was used to approximate the characteristic of the “standard beer” used to construct the ASBC - 13 chart. With knowledge of the AE of the “standard beer” (3.63 ºP) and by making the OE, alcohol, and RE values variable (with reasonable constraints), Equation 3.7 was then fit to the ASBC CO₂ solubility data using Systat-11 (Systat Software, Inc Chicago, IL). The best fit for each parameter was determined using a RSS optimized for all data points. The minimum RSS occurred at an
OE of 13.84 °P, $A_{w/w}$ value of 4.22 % and a RE of 5.78 °P. This is a likely estimate for the characteristics of the “standard beer” used to construct the ASBC solubility chart.

### 3.5 CONCLUSION

Brewers are increasingly pushing the bounds of beer composition from low sugar “dry” ales to alcohol free “beers”. These variables have been shown to affect CO$_2$ solubility within beer, which in turn will affect the stability, packaging choices and sensory aspects of these beers. From Figure 3.1 it appears evident that the pressure/temperature charts are no longer suitable for the range of styles encountered in modern beers. Unfortunately there is not as yet a “best” formulaic approach either, as simplistic, theoretical models do not account for the complexity of beer and empirical models have (to date) been constructed using specific “typical” beers. Presently brewers accept the inaccuracy of published solubility charts, or utilize one of the empirical models discussed in this paper.

By knowing the underlying assumptions and methods used to determine of CO$_2$ solubility, brewers and brewing researchers can estimate CO$_2$ solubility more effectively. Ideally, as CO$_2$ solubility within a range of beer styles is documented, a more accurate approach to determine CO$_2$ saturation in beer will become standard.
3.6 References


CHAPTER 4  MODELING THE ATTENUATION OF FERMENTABLE SUGARS DURING BREWING OPERATIONS

Materials in this chapter are drawn from work that has been presented at the European Brewing Convention.


4.1 ABSTRACT

Throughout the brewing process, sugars are metabolized into alcohol and carbon dioxide resulting in density attenuation of the wort. When mapped with respect to time, this decline follows a sigmoidal (s-shaped) curve, from an initial sugar concentration of anywhere from 10-20 % to 2-4 % over the course of a typical fermentation. Mathematical models can be fit to this data allowing brewers to predict, assess and more accurately compare fermentations. Within the brewing industry there are several models that can be applied, each with advantages and disadvantages. Some models are theoretically derived while others are fully or semi-empirical. In modeling sugar attenuation, brewing researchers utilize simpler models; however these may not accurately characterize real world fermentations (particularly at the onset and latter half of fermentation). This paper utilizes several common sigmoidal models (including the logistic, incomplete beta-function and Gaussian) to model the consumption of each fermentable sugar over an entire fermentation. The results of this study show how the yeast strain “SMA” consumed glucose preferentially over other sugar types. However, the utilization of every other type of fermentable sugar was initiated well before the complete consumption of glucose. Additionally, it was found that the fermentable sugar attenuation during brewing
fermentations follows a non-symmetrical sigmoidal distribution and should be modeled accordingly when sufficient data is available. Of the models assessed, a five parameter logistic model most accurately described the data and should be considered by brewing researchers and those studying the attenuation of extract post fermentation. The advantages and disadvantages of other common models are also discussed.

4.2 INTRODUCTION

Brewing wort is a mixture high in fermentable sugars generated during the mashing process. Wort density is often used as a measure of fermentation progress as the consumption of sugar and subsequent production of alcohol results in density attenuation. This decline in density (commonly measured in units of degree Plato (°P) or specific gravity) observed in brewery fermentations characteristically follows a sigmoidal or s-shaped curve (Corrieu et al., 2000 Trelea et al., 2001 and Speers, et al., 2003). Similarly, each individual fermentable sugar follows a sigmoidal decline. However, these consumption curves are influenced by a variety of factors such as yeast state, species, sugar type, et cetera. Thus, the consumption of total sugar (as shown in Figure 4.1), as well as individual sugar attenuation, is often lagged prior to consumption and may be symmetrical or asymmetrical. Modeling total sugar consumption has many advantages such as predicting the final density/sugar content (Defernez et al., 2007), approximating the time until completion (Speers, 2003) and phenotyping the yeast strain. Non-linear models are already promoted for use in various analytical methods within the brewing industry such as nearest neighbor and predictive modeling techniques (Trelea et al., 2001b), where easily measured parameters are related to others. The most common functions used to predict density decline in brewing fermentations are the logistic model,
(Speers et al, 2001; ASBC Yeast-14). Others, such as the regularized incomplete beta-function (IBF) (Trelea et al., 2001), and the modified Gompertz function (Gibson et al., 1988) may also be used. When these models are fit to fermentation data, they can produce variable results. Differences in reported and predicted density can significantly influence the decision making process in large breweries and can make comparing metrics (such as fermentability of grain) very problematic.

Figure 4.1 Experimental data detailing the attenuation of extract during brewing fermentation using the standard ASBC method yeast-14 (ASBC, 2013). The data presented is the mean of three replicate trials where the standard deviation was too small to be accurately represented.

This study reviews and compares these aforementioned commonly applied non-linear equations with respect to modeling the attenuation of fermentable sugar during brewing operations. This work was necessitated by difficulty our laboratory encountered when attempting to model the initial hours of fermentation where estimates generated using common models deviated significantly from observed behaviour. The next step involved examining many models (including several found outside the field of brewing science) to determine which most accurately described the fermentation data. We were able to ascertain the most appropriate model through the use of Akaike’s (corrected)
Information Criterion (AICc), as well as comparison of the coefficients of determination ($r^2$) and absolute RSS. Ideally, the data would adhere to a simplistic theoretically derived formula such as the four parameter (4P) symmetric logistic model (Speers et al., 2003). Unfortunately, the variability in both shape and lag time for each individual sugar necessitated a more flexible model. Of the three models outlined below, the first – Gompertz’s model – is an empirical model that is widely used in microbiology (Gibson, 1988) to describe growth curves (analogous to consumption curves). The second – the incomplete beta distribution – is an applied empirical distribution reported in the brewing literature (Trelea et al., 2001). The final - Richard’s model - is based upon theoretical principles (Richards, 1959) but is not (to the authors knowledge) used in the brewing industry. Each model is described in detail as follows:

### 4.2.1 Logistic Models

The four parameter logistic function (4P logistic model) is a sigmoidal curve often used to describe changes in population as it effectively models autocatalytic behaviour (Equation 4.4). This curve is commonly used in the brewing industry to model the decline in apparent extract. The 4P model is the basis of ASBC Yeast-14 used to assess malt for premature yeast flocculation behavior and to compare the fermentability of yeast strains. The generalized logistic model is a five parameter variant of the logistic model (Equation 4.5) that expands the theoretical basis to an asymmetrical curve (Richards, 1958) required for modeling sugar attenuation. The Five Parameter (5P) logistic model has not (to the authors knowledge) previously appeared within brewing literature, however it is commonly used in other fields for such applications as modeling population growth and
dosage calculations (Gottschalk and Dunn, 2005). The generalized logistic curve is equal to the symmetrical 4P logistic when the parameter $s = 1$:

\[
P_{(t)} = P_e + \frac{P_l - P_e}{(1 + e^{-B(t-M)})^{1/s}} \quad (4.4)
\]

\[
P_{(t)} = P_e + \frac{P_l - P_e}{(1 + se^{-B(t-M)})^{1/s}} \quad (4.5)
\]

where $s$ is a variable which modifies the point of inflection ($M$).

4.2.2 Gompertz Model

As shown in figure 5.1 the consumption of sugar follows an asymmetrical sigmoidal curve. As the consumption of sugar is analogous to yeast growth, the use of a common biological growth curve should work well for this application. The Gomertz model (GM) is an empirical model named after Benjamin Gompertz (1825), and is widely used in the field of microbiology to predict the growth curves of bacteria (Buchanan 1997). This model is a special case of the generalized logistic formula and describes a sigmoidal curve where the latter half of the curve approaches the asymptote more slowly than the initial. This model is often used when one expects an asymmetrical curve when working with microorganisms and is a special case of the generalized logistic function. A modified version of the Gompertz curve mentioned in the brewing literature to describe density attenuation is described in Equation 4.1 (Speers et al., 2001):

\[
P_t = P_e + (P_l - P_e) \cdot e^{(-B(t-M))} \quad (4.1)
\]

where $P_l$ and $P_e$ are the upper and lower asymptotes respectively, $M$ is the time of the inflection point of the curve, $B$ is the consumption rate factor and $t$ is the time at $P_{(t)}$. 


This version of the Gompertz model is an empirical model not derived from theory (Speers et al., 2003). An advantage of this model is the low number of parameters required to fit the model (four) while still allowing for an asymmetrical shape; this is particularly advantageous with a limited number of data points. However in testing of data from over 50 industrial brewing fermentations, Speers et al. (2003) showed the 4P logistic model fit the data superior to the modified Gompertz model.

### 4.2.3 IBF Model

The incomplete beta function (IBF) can be utilized to describe an asymmetric curve as described by Equation 4.2. The full name for this equation is the regularized incomplete beta-function, however the name is often shortened in literature to the incomplete beta-function. The IBF has been modified for describing the attenuation of extract by Treala et al. (2001) and has been used by several researchers (i.e., Defernez et al., 2007) to model and predict the end parameters of fermentation. Equation 4.2 is the aforementioned modified version of the IBF with two additional terms ($P_l$ and $P_e$) added to fit experimental data (describing the upper and lower boundaries of the sugar consumption curve). With the additional variables, the IBF can be used to describe brewing fermentations quite well, however, as with the modified Gompertz model, the fit is purely empirical and the shape parameters do not describe biological functions.

\[
IBF = \frac{\beta(x; \alpha, \beta)}{\beta(\alpha, \beta)} = \frac{\int_0^\alpha u^{\alpha-1}(1-u)^{\beta-1}du}{\int_0^1 u^{\alpha-1}(1-u)^{\beta-1}du}
\]

(4.2)

where $\beta$ and $\alpha$ are shape factors and:

\[
P_t = P_l - (P_l - P_e) \cdot IBF(x \cdot t; \alpha, \beta)
\]

(4.3)
4.2.4 Additional Models

While the 4P logistic, IBF and Gompertz models are all discussed within brewing literature, there are many additional models designed to describe sigmoidal curves outside of this area. The fields of predictive microbiology, medical science and biology all offer many models that may be useful in describing sugar attenuation. Prior to the publication of this work, many additional models were also considered for this investigation (Table 4.1). Each model was determined to be less robust than the 5P logistic for this application through the same statistical methods described below. It is noteworthy that a review of the literature will reveal many unequal versions of the logistic model that all that effectively describe sigmoidal curves.

Table 4.1 Additional sigmoidal models assessed for suitability in modeling sugar attenuation data.

<table>
<thead>
<tr>
<th>Model Name</th>
<th>Equation</th>
<th>P.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigmoidal Dose</td>
<td>$P_t = P_1 + \frac{(P_L - P_e)}{1 + 10^{M-x}}$</td>
<td>3</td>
<td>GraphPad, 2008</td>
</tr>
<tr>
<td>Sigmoidal Dose (variable slope)</td>
<td>$P_t = P_1 + \frac{(P_L - P_e)}{1 + 10^{(M-x)B}}$</td>
<td>4</td>
<td>GraphPad, 2008</td>
</tr>
<tr>
<td>4P logistic</td>
<td>$P_t = \frac{(P_L - P_e)}{1 + \left(\frac{t}{M}\right)^{-B}} + P_e$</td>
<td>4</td>
<td>Speers, 2003</td>
</tr>
<tr>
<td>Variant of 4P logistic</td>
<td>$P_t = \frac{(P_L - P_e)}{1 + \left(\frac{t}{M}\right)^{-B}} + P_e$</td>
<td>4</td>
<td>Gottschalk and Dunn, 2005</td>
</tr>
<tr>
<td>Variant of 5P logistic</td>
<td>$P_t = \frac{(P_L - P_e)}{1 + \left(\frac{t}{M}\right)^{-B}} + P_e$</td>
<td>5</td>
<td>Gottschalk and Dunn, 2005</td>
</tr>
</tbody>
</table>

Where P. is number of parameters

4.3 EXPERIMENTAL

To amass sufficient data with which to accurately apply each model, a controlled fermentation was completed in triplicate using an ASBC standard method Yeast-14
(ASBC, Yeast-14). This is a miniature scale assay that utilizes wort created via a congress mash (ASBC Malt-4) and that is fermented in numerous test-tube fermentors. The sampling regime (and corresponding number of test-tubes) was increased from that described in Yeast-14 to better detail the consumption of each sugar by sampling during anticipated reductions in density (Figure 2). Samples were scheduled using the rate (calculated by taking the 1st derivative of the density curve modeled with the 4P logistic model) of attenuation decline from a previously completed trial not reported here. The resulting schedule utilized 81 samples (i.e. fermentors) over 27 sampling times.

Figure 4.2 Sampling schedule, a sample was taken at each vertical line yielding a sample at roughly equal density increments.

Each sample was assessed using High-Pressure Liquid Chromatography (HPLC) via a “Waters” separation system (Waters 2695 Separations Module, Waters Corporation, Milford, Massachusetts) with attached refractive index detector (Waters 2414 Refractive Index Detector, Waters Corporation, Massachusetts, USA). The column utilized was a Benson Polymeric Ag+ form column (806 BP-100 Ag+ Carbohydrate Column, Benson
Polymeric Inc., Sparks, Nevada) at the reported optimal temperature (90 °C). In this manner the consumption of each sugar was assessed in addition to the total sugars (Figure 4.3). Once collected, each model (The Modified Gompertz – MG; Incomplete Beta Function - IBF; and the 5P Logistic - 5P) was applied to the fermentation data (Figure 4.4-4.7). The Prism software package Version 5.00 (GraphPad Software Inc., La Jolla, CA) was used to apply each model and compare using AICc. Table 5.2 details the fit of each model through examination of the residuals, coefficients of determination and absolute residual sums of squares while Table 4.3 shows the results of comparisons using AICc.

Figure 4.3  Raw sugar attenuation values taken throughout the triplicate experiments: a) total fermentable sugar b) each individual sugar.
Figure 4.4 Modeled maltose attenuation data (MG - Modified Gompertz; IBF - Incomplete Beta Function; 5P – 5 Parameter Logistic), the residuals for each model are depicted on the right.

Figure 4.5 Modeled glucose attenuation data (MG - Modified Gompertz; IBF - Incomplete Beta Function; 5P – 5 Parameter Logistic), the residuals for each model are depicted on the right.
Figure 4.6 Modeled maltotriose attenuation data (MG - Modified Gompertz; IBF - Incomplete Beta Function; 5P – 5 Parameter Logistic), the residuals for each model are depicted on the right.

Figure 4.7 Modeled fructose attenuation data (MG - Modified Gompertz; IBF - Incomplete Beta Function; 5P – 5 Parameter Logistic), the residuals for each model are depicted on the right.
Table 4.2  Residual analysis for each sugar attenuation modeled using the Modified Gompertz, IBF and 5P logistic models.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Modified Gompertz Residual pattern</th>
<th>IBF Residual pattern</th>
<th>5P logistic Residual pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Random$^2$</td>
<td>Pattern</td>
<td>Random$^2$</td>
</tr>
<tr>
<td></td>
<td>0.998-0.308</td>
<td>0.996-0.678</td>
<td>0.998-0.299</td>
</tr>
<tr>
<td>Fructose</td>
<td>Random$^2$</td>
<td>Random$^2$</td>
<td>Random$^2$</td>
</tr>
<tr>
<td></td>
<td>0.987-0.015</td>
<td>0.989-0.013</td>
<td>0.988-0.014</td>
</tr>
<tr>
<td>Maltose</td>
<td>Pattern</td>
<td>Pattern</td>
<td>Random$^2$</td>
</tr>
<tr>
<td></td>
<td>0.979-3.473</td>
<td>0.991-1.436</td>
<td>0.996-0.660</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>Pattern</td>
<td>Pattern</td>
<td>Random$^2$</td>
</tr>
<tr>
<td></td>
<td>0.986-0.210</td>
<td>0.994-0.084</td>
<td>0.996-0.061</td>
</tr>
</tbody>
</table>

$^2$The heteroscedacity caused by known variance inherent to the assay was common to all models.

Table 4.3  A comparison of each model to the sugar attenuation data using Akaike’s corrected Information Criterion (AICc).

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Preferred Model</th>
<th>Preferred Model</th>
<th>Preferred Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Ambiguous</td>
<td>5P</td>
<td>5P</td>
</tr>
<tr>
<td>Fructose</td>
<td>Ambiguous</td>
<td>5P</td>
<td>5P</td>
</tr>
<tr>
<td>Maltose</td>
<td>MG</td>
<td>5P</td>
<td>5P</td>
</tr>
<tr>
<td>Maltotriose</td>
<td>MG</td>
<td>5P</td>
<td>5P</td>
</tr>
</tbody>
</table>

4.4  DISCUSSION

As shown in Figure 4.3, each sugar was found (not surprisingly) to follow a sigmoidal attenuation. Noteworthy is that while the attenuation of glucose was immediate, all other sugars (including fructose) were delayed (lagged) to varying degrees. The attenuation of each sugar appeared to follow the uptake and metabolism patterns as described by Priest and Stewart (2006).

As shown in Table 4.3, the model most likely to accurately describe the attenuation of brewing sugars is the 5P logistic model as determined using AICc. Additionally, the absence of pattern in the residual (compared to other models) supports this conclusion. The Gompertz model, while being widely used for modeling the growth of many organisms (Buchanan and Cygnarowicz, 1990), has limited potential in
modeling brewing fermentations. This model fits sugar attenuation well, provided the sugar does not experience consumption “lag”. However, as we can see with maltose and maltotriose (Figures 4.4 and 4.6 respectively), this model deviates from the data near the beginning of fermentation creating a trend in the residual error. Therefore, care should be used when utilizing this model as it may fail to adequately describe brewing data. The IBF describes a versatile sigmoidal curve, however, it is an expanded mathematical distribution not designed to model biological behaviour. The limits of this approach are apparent when modeling consumption data for sugars without a lag period. This is especially evident with glucose attenuation data (Figure 4.5), where the derivative of the curve (the rate of consumption) at time zero will be zero by definition.

The theoretical basis behind the logistic model is that the primary variable (sugar concentration or density) will have an autocatalytic effect upon the rate of change. While this is shown to likely be true (as attested to by the sigmoidal shape of the sugar curves), the nonsymmetrical nature of the data alludes to additional factors beyond substrate consumption that slow attenuation during the second half of fermentation (such as alcohol concentration). Therefore, the semi-empirical logistic model which allows for asymmetry within the curve (i.e. 5P logistic) produces the most accurate fit conforming to the actual shape of the attenuation curves. Additionally, the biological significance of the parameters described by Richard’s curve provides a means of comparison between trials. As evidenced by the lack of pattern in every residual chart, this model can be used to accurately describe sugar attenuation in brewing operations. That said, the 5P logistic model may suffer from “overparameterization” should the number of data points fall sufficiently low (The number depends upon when the samples are taken, however
“overparameterization” with 10 samples or fewer has been encountered within our laboratory). This can be assessed by comparing the 4P logistic to the nested 5P using an F-test.

4.5 CONCLUSION

In summary, the IBF and Gompertz models each show residual patterns when applied to glucose, maltose and maltotriose attenuation data (Figure 4.4-4.7), while the 5P logistic model shows none. Furthermore, the 5P logistic model consistently shows the lowest sum of residual squares and is chosen using AICc in every example with the exception of the fructose curve (where extremely low sugar amounts increase the error to the point where the model showed no advantage to the simpler Gompertz model). As concluded by researchers, non-linear regression is a powerful tool that is easily utilized by modern brewers. This study shows that for brewery application the 5P logistic formula is superior in modeling the attenuation of sugar to previously published methods. The additional accuracy resulting from the use of this formula should help to improve existing methods while allowing brewers to accurately model the initial hours of fermentation.


GraphPad Software, Inc. 2008. Prism 5 Help: Sigmoidal dose-response (variable slope). GraphPad Prism version 5.00 for MacIntosh, GraphPad Software, San Diego CA


CHAPTER 5  CONSUMPTION OF SUGARS AND GENERATION OF FERMENTATION PRODUCTS DURING BREWING OPERATIONS

Materials in this chapter are drawn from work that has been presented at the American Society of Brewing Chemists Annual Meeting.


5.1 ABSTRACT

Brewers monitor the attenuation of density during fermentation for a variety of reasons, such as to detect process deviations, approximate other fermentation parameters and to predict time remaining. Of the tools available to relate density attenuation (analogous to sugar consumption) to fermentation products, the most common is Balling’s equation. Despite widespread use and near global recognition, it has been argued that the assumptions used by Balling in the formation of his formula do not reflect conditions commonly found within modern brewing operations. This study explores the theoretical link between sugar consumed during fermentation and the resulting products throughout the entire fermentation. This was accomplished through the design of a mass balance completed on a series of assay fermentations with a high sampling frequency. Each parameter in the fermentation was assessed to examine how closely it followed both Balling’s theorem, and modern theory to give the reader an understanding of how and why Balling’s theory may yield misleading results (especially when used out of context). Information concerning how product ratios change over a fermentation are reported allowing brewers to make informed
decisions concerning the original gravity of partially fermented beer and to more accurately estimate alcohol, yeast and sugar content of their final product. Finally, the mass balance allowed several other aspects of fermentation, such as yeast dynamics and sugar consumption, to be studied in detail.
5.2 INTRODUCTION

Modern breweries almost invariably use some form of parameter estimation (such as relating alcohol to OE), either through formula or instrumentation. Until such time as reliable, inexpensive sensors are available for each measurement, fermentation parameters will be monitored and related to other important fermentation parameters/indicators with variable degrees of accuracy. The consumption of sugars during brewing fermentations has been extensively studied and is generally well understood. Many researchers, such as Daoud and Searle (1990), have performed complex material balances at the end of industrial fermentation. However, in practice many brewing operations and researchers utilize simpler empirically derived equations (such as developed by Olearly 2008) or the semi-empirical (but very dated) Balling’s theorem (Balling, 1845-65). These are often employed within instrumentation or formulae that are used everyday. Researchers, such as Parcunev et al. (2012), outline Balling’s theorem as a basis for online monitoring models, and according to Neilson et al. (2007) major breweries use this theorem to calculate the original density of the wort (equivalent to OE). This can cause problems and propagate error, as most empirical formulae are specific to beer brand/fermentor geometry, and there are numerous reported criticisms of Balling’s theorem (Neilson et al., 2007). In an attempt to see how these problems can propagate, and assess the accuracy of modern theory, this study looks at the theoretical conversion of sugars into fermentation products throughout fermentation. Further, a novel approach to modeling brewing operations is proposed with applications in anticipating product ratios and advancing modeling techniques. Experiments were conducted at assay scale to monitor both sugar consumption and the
formation of products throughout the fermentation. The accuracy of both the theoretical product formation and Balling’s equation are assessed. The measurement of each parameter is scrutinized and several noteworthy trends exposed and characterized.

5.2.1 Balling

Published in a series of books from 1846-65, Balling’s theorem (Equation 5.1) relates the ethanol, yeast and CO₂ generated during fermentation to the loss of sugar (Neilson et al., 2007). This formula combines the theoretical conversion of glucose into ethanol and CO₂ (Equation 5.2) with empirical measurements of yeast generation:

\[
2.0665 \text{ g (lost extract) } = 1.000 \text{ g EtOH, } 0.9565 \text{ g CO}_2 \text{ and } 0.11 \text{ g yeast} \quad 5.1
\]

\[
C_6H_{12}O_6 \text{ (glucose)} = 2 \cdot C_2H_5OH \text{ (ethanol)} + 2 \cdot CO_2 \quad 5.2
\]

where EtOH is ethanol, and yeast is the amount of dried yeast generated.

As noted by Cutaia (2007), Balling has made several assumptions in the formation of this theory, notably, that to maintain this balance all the sugar would have to be composed of monosaccharaides (e.g. glucose), as sugar with a higher degree polymerization will be hydrolyzed, thus acquiring mass. The use by Balling of an (supposedly) empirically derived constant to represent yeast generation is also unlikely to be accurate. Since Balling’s time, much work has been completed upon yeast growth. It is now known that many factors influence the growth and reproduction of yeast during brewing operations (such as FAN concentration, strain, wort density, etc.), making accurate prediction of growth very difficult (Stewart and Russell, 1998).
Further critiques of this approach have been compiled by Neilson et al. (2007). However, when assessed by Carlsberg’s modern brewing operations in 1943, 1973 and 2007, the formula was apparently successful at predicting OE to within 0.1 °P for a “major international brand” of beer (Neilson et al., 2007). However, as noted during this study, the formula was only accurate at the end of fermentation (with the predicted OE variable throughout the fermentation), and the predicted yeast growth was highly suspect. Regardless of probable theoretical inaccuracies, the formula has likely endured due to successful prediction of OE and ease of use. Balling’s formula is cited in the EBC and ASBC for determination of OE (EBC Method 9.4; ASBC Beer-6B)

While the apparent utility of Balling’s formula has suppressed the need for a more accurate formula, modern understanding of fermentation and analytical tools have improved to a point where these are now possible. While unlikely to be accepted as a replacement, a more accurate theoretical understanding of the relationship between sugar consumption and fermentation products will have many uses in the brewing industry. Building on the work of Cutaia (2007), Daoud and Searle (1990), and others, this study attempts to show how each parameter is related during brewing operations while demonstrating the inaccuracies of Balling’s approach so that brewers and researchers are able to make informed decisions. A more detailed understanding of these relationships will also aid in the modeling and prediction of fermentation parameters.

### 5.2.2 Sugar Consumption

The major carbohydrate source for brewer’s yeast are various fermentable sugars, each affecting the density of the solution. Brewers yeast also require FAN, however the amount utilized is typically only 0.01 g·100 mL⁻¹ (Lekkas et al., 2005),
and is usually ignored in mass balances. The sugars within wort are typically glucose, fructose, maltose, sucrose, and maltotriose, in addition to longer chain saccharides not typically fermented by industrial yeast strains. The consumption of sugars during fermentations is highly ordered. Any sucrose within the wort is first hydrolyzed resulting in a corresponding increase in glucose and fructose, followed by the uptake and utilization of glucose stoichiometrically represented by Equation 5.2. The presence of high glucose concentrations within malt represses both respiration and maltose consumption in most brewing yeast (Stewart and Russell, 1998). Respiration is inhibited within the first few minutes of contact with glucose concentrations by the short-term Crabtree effect and in the presence of >0.2 % w·w⁻¹ glucose due to the (different) long-term Crabtree effect (Briggs et al., 2004). According to Stewart (2006), the consumption of maltose will only occur once 40-50 % of the glucose has been consumed with the expression of the maltase and maltose permease related genes (MALS and MALT) being repressed at concentrations >1 % w·v⁻¹. Once the level of glucose has sufficiently attenuated, yeast will bring maltose and maltotriose into the cell where they will be hydrolyzed into glucose (Stewart, 2006). Most of the sugar consumed will be converted into ethanol and CO₂ yielding 2 ATP per monosaccharide molecule; however some will also be utilized for the formation of biomass and other metabolites. The metabolite produced in the next highest quantity during brewing operations is glycerol, with concentration measured up to ~ 4 g·L⁻¹ (Briggs et al., 2004b). Glycerol is produced by yeast to resist osmotic pressure (especially during high gravity brewing) and to balance reduction-oxidation reactions.
A major assumption made by Balling in the formulation of his equation was that glucose would be the only sugar consumed (Cutaia, 2007). In the consumption of glucose via ethanolic fermentation, the mass balance described by Balling’s theorem is correct. However, when maltose is hydrolyzed intracellularly by *Saccharomyces*, a water molecule is added to the mass of the products (two for trisaccharides etc.). Therefore, as the amount of higher polymerized saccharides (i.e. maltose and maltotriose) within wort varies, so does the accuracy of a mass balance based upon glucose.

### 5.2.3 Hypothesis

Despite its popularity, the Balling theorem is based upon several flawed assumptions that do not represent industrial brewing operations (Cutaia, 2007). While this may not have been a problem for typical brewing operations of Balling’s day, the variability of modern production techniques (from high adjunct to high gravity fermentations) necessitate an accurate understanding of how parameters change over fermentation. The aim of this study was to explore how sugars and products vary over the fermentation process, and to improve the accuracy of modeling through the application of modern theory. It was believed at the onset of this study that additional parameters to those used by Balling would be necessary to accurately reflect real-world brewing operations, and that product ratios would change over the course of the fermentation. To accomplish these goals a mass balance was developed according to accepted brewing theory (incorporating work completed by various researchers) that would account for changes in mass during consumption and include all significant products. The products used in this balance include those estimated by Balling, with the inclusion of glycerol, as this metabolite has been shown to often be produced at levels high enough to
significantly (>0.1 g·100 mL⁻¹) affect the mass balance (Daoud and Searl, 1990; Briggs et al., 2004b). The final proposed equation is shown as Equation 5.3:

\[ I + 1.0536 \cdot II + 1.0714 \cdot III + FAN = EtOH + gly + CO_2 + yeast + other \]  

5.3

where “I”, “II”, and “III” represent the mass of monosaccharide, disaccharide and trisaccharide sugars consumed (respectively), FAN is the amount of free amino nitrogen consumed, “EtOH”, “gly”, “CO_2”, and “yeast” are the mass of ethanol, glycerol, CO₂, and dried yeast generated, while “other” represents other products of fermentation such as fusel alcohols.

This mass balance differs from previous attempts such as Trelea et al., (2001) through the absence of empirical constants. However, as done in previous mass balances, this equation does not measure the many minor metabolites that are produced during fermentation or FAN as the concentration of these are typically very low. The most abundant non-volatile products are higher polyols, found at concentrations less than 28 mg·100 mL⁻¹, while the most abundant volatiles are higher alcohols, found at concentrations typically less than 8 mg·100 mL⁻¹ (Briggs et al., 2004b). Individually these fall short of 0.1 g·100 mL⁻¹. However, all combined, these may have an effect upon the mass balance. Another source of error is the failure of this experiment to include the utilization of oxygen and FAN within the mass balance. However, as with “other” metabolites, these are generally very small; typical FAN consumption is in the range of 0.01 g·100 mL⁻¹ (Lekkas et al., 2005) and initial oxygen concentrations are measured in parts per million.
5.3 EXPERIMENTAL

The experimental methods described below are the culmination of several years of study where each measurement technique was independently assessed. As each measurement was subject to considerable time constraints (due to a high sampling frequency), the results and methods have been refined over several attempts to improve the mass balance; each previous experiment identified flaws in experimental procedure (specifically quantifying fermentation products) that were subsequently corrected. Throughout each of these trials the trends observed did not change, the refined methods are described below:

To assess both the consumption of sugars and the generation of fermentation products, the miniature fermentation assay ASBC Yeast-14 was chosen due to high consistency between trials, and suitable scale for our laboratory. Several modifications were made to the mini fermentation to address problems with scheduling and measurement, as will be discussed.

5.3.1 Sampling Schedule

The number of samples taken during the miniature fermentation were increased from the 10 described in ASBC Yeast-14, to 27, to better detail the consumption of each sugar. The sampling times were scheduled to coincide with anticipated reductions in density (Figure 5.1). These were scheduled using the rate of density attenuation from a previously completed trial (calculated by taking the 1st derivative of the density model). The resulting schedule utilized a total of 81 individual fermentors (as three replicate measurements were taken at each sampling time), yielding a sample at roughly equal
density increments. The trial ended after 121.25 h (increased from 78 h as per Yeast-14) to ensure the fermentation had completed).

Figure 5.1 (A) Sampling schedule for ASBC Yeast-14, (B) The sampling schedule for this experiment. Sampling points were taken at each vertical line.

5.3.2 Sugar Attenuation

The concentration of each sugar and alcohol was determined throughout the fermentation using HPLC. Samples taken throughout the mini fermentation were immediately frozen until after the fermentation at which point all samples were processed simultaneously. Samples were filtered through 0.2 µm (pore size) syringe filter paper (13mm disposable filter device, Whatman Inc. Florham Park, NJ). Each sample was assessed using HPLC via a “Waters” separation system (Waters 2695 Separations Module, Waters Corporation, Milford, MA) with attached refractive index detector (Waters 2414 Refractive Index Detector, Waters Corporation, Milford, MA). The column utilized was a Benson Polymeric Ag⁺ form column (806 BP-100 Ag⁺ Carbohydrate Column, Benson Polymeric Inc., Sparks, NV) at optimal temperature (90 °C). This column resolves oligosaccharides up to DP-11 (degrees of polymerization) using size exclusion as the primary separation mechanism (Benson Polymeric, 2013).
technique, also known as gel-permeation or gel-filtration chromatography, separates particles on the basis of molecular size. This resulted in larger molecules passing through the column (and resolving) more quickly than smaller molecules. Although this column could resolve fructose and glucose as separate peaks, maltose and sucrose co-resolved. While not ideal, this did not pose a problem for this experiment, as there is no difference in mass between these two molecules. Additionally, the amount of sucrose present in malt is characteristically low (1-2 % of total sugars – Stewart, 2006) and is typically hydrolyzed immediately into glucose and fructose (which is measured). The peaks were integrated using Millennium\textsuperscript{32} software (Millennium\textsuperscript{32} Software v3.20, Waters Corporation, Milford, MA) and calibrated using standards (including blanks, individual samples at variable concentrations, and combined standards). The final values were corrected for non-linear drift between calibrations, evaporation, and dilution. The calibration standards were measured with an error of ± 0.01 g. The concentration of fermentable sugars measured at each sampling time are detailed in Figure 5.2A. Figure 5.2B details the total fermentable sugar concentrations created through the summation of each individual sugar.
Figure 5.2 The concentration of fermentable sugars at each sampling point as determined using HPLC.

The change in each sugar and fermentation product throughout the fermentation was modeled using a 5P logistic equation (Richards, 1959) as shown in Equation 5.4. As discussed by Speers (2003), modeling fermentations allows for more accurate comparisons between fermentations. This is especially important when working with fermentations that have a high amount of variability (such as is seen in ASBC Yeast-14). The 5P logistic model fit the sugar attenuation quite well as detailed in Figure 5.3A. Additionally, as demonstrated in Figure 5.3B, the sum of each fermentable sugar also followed a sigmoidal attenuation over the course of the fermentation.
where \( P_i \) and \( P_e \) are the upper and lower asymptotes respectively, \( M \) is the time of the inflection point of the curve, \( B \) is the consumption rate factor, \( t \) is the time at \( P(t) \) and \( s \) is a variable that modifies the point of inflection.

\[
P(t) = P_e + \frac{P_i - P_e}{(1 + s e^{-B(t-M)})^{1/s}}
\] (5.4)

Figure 5.3 Modeled sugar attenuation for (A) each fermentable sugar and (B) the total fermentable sugar.

The individual sugar attenuation rates are a result of yeast consuming sugars through ethanolic fermentation. In typical brewing scenarios, glucose is consumed initially, followed by increasing fructose, maltose and maltotriose consumption (sequentially – Briggs, 2004a). To calculate the consumption rate of each sugar, the 1st derivative of the consumption model (with respect to time, Equation 5.4) was taken and is described as Equation 5.5. The best fit parameters for each sugar were applied resulting in the Figure 5.4.
The sugar consumption by SMA yeast during this experiment was typical of brewing yeast as described by Stewart (2006), with more overlap between sugar consumption than expected. Stewart predicted that maltose would not be consumed until 40-50% of glucose was consumed. Examining Figure 5.4, this statement appears roughly accurate; however the onset of maltose consumption did not match the concentration also

\[
\frac{\partial}{\partial t} \left( P_e + \frac{P_i - P_e}{(1 + s e^{-B(M-t)})^{1/s}} \right) = B (P_i - P_e) e^{(B(M-t))} \left( S \cdot e^{(B(M-t))} + 1 \right)^{(-1/s^{-1})} \quad (5.5)
\]
reported by Stewart (>1 g·100 mL⁻¹), likely influenced by their experiment using a lower initial glucose concentration (~ 2 vs 6 g·100 mL⁻¹). In light of the discrepancies between the reported concentrations of glucose required for repression of maltose consumption between this and previous studies, it is possible that the repression of maltose is not due to a specific glucose concentration. Alternatively, repression may instead be related to the slowing of glucose consumption. As shown in Figure 5.4, maltose consumption is initiated at the first inflection point of the glucose consumption rate curve. To illustrate how the repression of maltose is unlikely to be due to a specific glucose concentration, the consumption rate of the total fermentable sugar was calculated through the summation of each consumption rate model, as illustrated in Figure 5.5. This model is smooth and typical of brewing operations, whereas any model (containing significant glucose) that required a specific glucose concentration prior to the consumption of maltose would likely not be. This suggests that the consumption rates shown in Figure 5.4, are an accurate representation of fermentation.

Figure 5.5 Total fermentable sugar data and the total sugar consumption rate as calculated through the summation of each individual sugar consumption rate.
The findings from this study seemingly challenge several published notions (Briggs et al., 2004a; Stewart, 2006) that glucose must reach specific concentrations (i.e. > 1 g·100 mL⁻¹) prior to easement of maltose repression. To adhere closely to concentration related repression constraints, malts high in glucose concentrations would have to nearly cease fermentation at the transition between glucose and maltose, a phenomenon that is not observed.

5.3.3 Alcohol Production

As with fermentable sugars, ethanol and glycerol concentrations were determined through the use of the Waters HPLC. As shown in Figure 5.6, the formation of both fermentation products followed a sigmoidal growth and were modeled appropriately using Equation 5.4. The concentrations of other alcohols (such as “fusel” alcohols) were not tracked, as the concentrations were not expected to be significant (Briggs, 2004b). As shown in Figure 5.6 the formation of glycerol during fermentation is small but significant.
Figure 5.6 The concentration of measured alcohols at each sampling point as determined using HPLC.

5.3.4 CO₂ production

At the onset of wort fermentation, CO₂ is generated through the metabolism of fermentable sugars. The gas is dissolved within the media until the saturation limit is reached (as determined by media composition and partial pressure differences between the wort and headspace). Common convention dictates that the wort will reach saturation and the carbon dioxide will subsequently evolve into the headspace through surface diffusion, and through gas bubbles when the wort becomes sufficiently over-saturated. Many attempts have been made to relate the solubility of CO₂ based upon temperature and pressure for beer (as discussed in Speers and MacIntosh, 2013). Rammert and Pahl (1991) describe a formula that incorporates temperature, pressure, alcohol, and beer solids as described by Equation 6.6.
\[
\zeta_{CO_2} = 3.36764 \cdot 0.12723 \cdot T_C + 2.8256 \cdot 10^{-3} \cdot T_C^2 - 3.3597 \cdot 10^{-5} \cdot T_C^3 + 1.5933 \cdot 10^{-7} \cdot T_C^4 - (0.4723 - 2.988 \cdot 10^{-2} \cdot T_C + 1.1605 \cdot 10^{-3} \cdot T_C^2 - 2.251 \cdot 10^{-5} \cdot T_C^3 + 1.5933 \cdot 10^{-7} \cdot T_C^4) \cdot \left( \frac{E}{128} + \frac{A_{w/v}}{43} + \frac{Salt}{27} \right)
\]

where \(\zeta_{CO_2}\) is dissolved CO\(_2\) expressed in g\(\cdot\)L\(^{-1}\)\(\cdot\)bar\(^{-1}\), \(T_C\) is the temperature in °C, \(E\) is the real extract expressed in g\(\cdot\)L\(^{-1}\), \(A_{w/v}\) is the alcohol concentration volume per volume, and \(Salt\) is the salt concentration in g\(\cdot\)L\(^{-1}\). This equation (Equation 6.6) is reported to be valid and accurate to within 2 percent when applied within the following parameter ranges:

\[
0.7 \leq \zeta_{CO_2} \leq 3.4 \text{ g}\cdot\text{L}^{-1}\cdot\text{bar}^{-1},
\]
\[
0 \leq T_C \leq 60 \degree\text{C},
\]
\[
0 \leq E \leq 300 \text{ g}\cdot\text{L}^{-1},
\]
\[
0 \leq NaCl \leq 50 \text{ g}\cdot\text{L}^{-1}.
\]

Using Equation 6.6, the solubility of the wort over the fermentation was assessed and reported in units of g\(\cdot\)100 mL\(^{-1}\) atm\(^{-1}\) (Figure 6.7) (salt content was assumed to be 0.1 g\(\cdot\)L\(^{-1}\)). Note that the actual amount of CO\(_2\) (g\(\cdot\)L\(^{-1}\)) is still very dependent upon the CO\(_2\) partial pressure within the headspace, which during this experiment was initially very low (~ 400 ppm is the global atmospheric average). While the calculated CO\(_2\) solubility was found to be initially lower than water (~ 0.15 g\(\cdot\)100 mL\(^{-1}\) atm\(^{-1}\) vs ~ 0.16 g\(\cdot\)100 mL\(^{-1}\) atm\(^{-1}\) for water - NIST, 2011), the value increased over the fermentation.
The total CO₂ generated from the consumption of sugars was calculated from the combined evolved (released) and dissolved gases. The measurement of each form of CO₂ was accomplished gravimetrically. Each fermentor containing pitched wort was weighed (to 0.01 g) at the beginning of fermentation. As the fermentation progressed, CO₂ gas escaped and the loss in mass was assessed by the measured difference in mass. The dissolved CO₂ within each fermentor was then assessed through decarbonation of the wort and measurement of the subsequent loss of mass. The decarbonation was accomplished through the use of a custom apparatus that utilized two techniques (in conjunction) outlined by the ASBC recommended beer degassing methods and alternatives matrix (ASBC Lab Basics). Each sample was held under partial vacuum (60 KPa) and sonification for a period of 15 min to bring the dissolved CO₂ within the wort to < 0.01 g·15 mL⁻¹ (ASBC Lab Basics) using a custom apparatus. For this procedure, the bubbles formed were assumed to be composed entirely of CO₂, however, there may have been other losses if the bubbles were saturated with other volatiles. The amount of water
vapor and ethanol potentially lost through this procedure was calculated using Raoult’s law. It was found that under the worst case scenario (highest ethanol level, and lowest achieved operating pressure), potentially up to 4% of the mass lost through this method (and assumed to be dissolved carbon dioxide) could have been composed of water vapor, with the ethanol loss unsubstantial. The dissolved and total generated (summation of released and dissolved) CO₂ throughout the fermentation was modeled using a skewed normal distribution (Equation 6.7) and the 5P logistic (Equation 6.4) respectively, and are presented in Figure 5.8.

\[
\text{dissolved } CO_2(t) = A \cdot \left( \frac{e^{\frac{-(t-\mu)^2}{2\sigma^2}} \text{Erfc}\left(\frac{\alpha(t-\mu)}{\sqrt{2\sigma}}\right)}{\sqrt{2\pi}\sigma} \right)
\]

where \( t \) is time expressed in hours, \( A \) represents the amplitude (g ml⁻¹) of a skewed-normal distribution with shape parameter \( \alpha \), location parameter \( \mu \) and scale parameter \( \sigma \), and \( \text{Erfc} \) is the complementary error function.

Figure 5.8 The total and dissolved CO₂ as measured gravimetrically at each sampling point.
It was observed that initial CO₂ evolution was below that predicted, given the known rate of sugar consumption and the equilibrium saturation level extrapolated from ASBC (saturation table). As shown in Figure 5.8 the amount of CO₂ within the fermentors increased sharply at the beginning of fermentation near the peak rate of sugar consumption. As the production of bubbles were noted during the first two days, and the amount of CO₂ within the fermentor declined dramatically after peak fermentation, it is very likely that the fermentors became supersaturated with CO₂ during fermentation. It was determined that under normal brewing conditions, the rate of carbon dioxide production exceeds the rate of evolution (driven by differences in CO₂ partial pressure between the wort and headspace) resulting in the supersaturation of the wort. The degree of supersaturation is dependent upon many conditions, such as the shape of the fermentor, temperature, headspace composition, et cetera (Scardina, 2000). Post peak fermentation, the release of supersaturated CO₂ resulted in a period of CO₂ evolution in excess of generation as the level of supersaturation within the fermentor declined. While the level of supersaturation within an unpressurized laboratory setting was ultimately low, high levels of supersaturation may explain an often observed and unexplained “bump” in CO₂ evolution rates (Figure 5.9). Even low amounts of CO₂ leaving supersaturation would be sufficient to influence the trend of CO₂ release (MacIntosh and Speers, 2012).
Figure 5.9 Overview of a “typical fermentation” as prepared by J. Munroe, Handbook of Brewing (Priest and Stewart, 2006) with the “bump” in CO2 release noted.

Also of note is how the amount of dissolved CO2 within the fermentor increased sharply at the beginning of fermentation accounting for virtually all of the initial CO2 generation (Figure 5.10). This phenomenon resulted in a delay between the onset of CO2 generation and measured gas release from the fermentor as illustrated in Figure 5.8. This phenomenon is often (although not always) ignored during the implementation of online monitoring based upon CO2 measurements, often resulting in error most prominent at the beginning and near the end of fermentations.
5.3.5 Yeast Generation

Both the number of Yeast In Suspension (YIS) and Total number of Yeast within the fermentor (TY) were enumerated using the methods of the ASBC (Yeast-4), and also gravimetrically assessed. At each sampling period 10 µL of wort was removed from the fermentor for YIS enumeration (using the methods of ASBC, Yeast-4) and was replaced with synthetic antifoam (Dow Corning® 1520-US antifoam, Dow Corning Corporation, Midland, MI). After gravimetric CO₂ measurements were taken, the fermentor was agitated until all yeast were suspended and a TY count was taken (using the methods of ASBC, Yeast-4). The contents were subsequently added to a pre-dried/weighed centrifuge tube and centrifuged at 3.31·10³ g, for 15 min. The resulting pellet was dried according the methods described by Briggs et al., (2004c) at 110 °C for 3 days in the presence of desiccant. The resulting dried yeast were homogenized and sent for carbon analysis by Canadian Microanalytical (Canadian Microanalytical Service, Ltd., Delta, BC). The carbon content (percentage) of the yeast did not significantly change over the
fermentation (approximately 42 %). Possible errors due to the presence of trub (as described in the method – Briggs et al., 2004c) were not expected as the trub had been removed from this fermentation as per the methodology of ASBC, Yeast 14. The mass increase and yeast cell counts throughout the experiment are shown in Figure 5.11. The total mass and TY counts were modeled using the 5P Logistic equation (5.4), while the YIS was modeled using a skewed normal distribution (Equation 5.8):

\[
YIS = A \cdot \left( \frac{e^{\frac{(x-\mu)^2}{2\sigma^2}} - \text{Erfc}(\frac{\alpha(x-\mu)}{\sqrt{2} \alpha})}{\sqrt{2\pi} \sigma} \right) + \text{Residual Yeast}
\]

where \( A \) represents the amplitude (g ml\(^{-1} \)) of a skew-normal distribution with shape parameter \( \alpha \), location parameter \( \mu \), and scale parameter \( \sigma \), and Residual Yeast is the number of yeast cells remaining in suspension at the end of the experiment.

Figure 5.11 A: The dried mass of generated yeast (total yeast – initial yeast) and B: The total number of cells and cells in suspension, as measured throughout the experiment.
As the total mass and number of yeast cells was known, the average cell mass was determined throughout the experiment and illustrated in Figure 5.12. The mass of the yeast changed over the course of the fermentation, increasing within the first half of fermentation (likely due to a large ratio of daughter cells) settling upon an average mass of \( \sim 40 \text{ pg} \).

![Figure 5.12](image)

Figure 5.12 The dried mass of generated yeast (total mass - mass at time zero) and the total number of generated cells (TY - TY at time zero) was used to calculate the average mass of generated cells throughout the experiment. The trendline was constructed using the models of TY generated and dried mass from Figures 6.11A & B.

Using the number of cells in suspension, and the consumption rate from the total fermentable sugars, the consumption of each cell in suspension was determined (under the assumption that settled cells were no longer significantly fermenting). This relationship (illustrated in Figure 5.13) indicated that the yeast cells within this experiment were fairly consistent in fermenting at \( 50 \text{ pg} \cdot \text{h}^{-1} \) (slightly over their own mass per hour) with no large changes or trends over the course of fermentation. This rate of
sugar consumption is comparable to reports that yeast will “ferment approximately (their) own weight of glucose per hour” (Schneiter, 2004).

Figure 5.13 A: The number of YIS appears to correlate well with the rate of fermentable sugar consumption. B: The rate of sugar consumption per cell was calculated throughout the experiment using raw YIS counts and modeled sugar consumption.

Note that the rise the end of Figure 5.13B is likely due to low YIS counts in some of the fermentors (Figure 5.11A), compounded by the fact that YIS samples were taken from the very top of the fermentor as not to disturb dissolved gas within the media.
5.3.6 Mass Balance

As shown in Figure 5.14, as the sugars are consumed (5.14a), fermentation products are produced (5.14b). The largest differences between Balling’s theorem and the proposed equation are an accurate calculation of yeast mass, glycerol production and changes in sugar mass due to hydrolysis. The difference in sugar mass due to hydrolysis as described by Cutaia (2007) is illustrated in Figure 5.15A, while differences between yeast growth and glycerol production are illustrated in Figure 5.15B.

Figure 5.14 A: Total consumed sugars and B: Total produced products as measured throughout this experiment.
To assess the effectiveness of Balling’s (Equation 5.1) theorem and the theoretical mass balance (Equation 5.3) in the calculation of OE, each formulae was rearranged to calculate OE as Equation 5.9 and 5.10 respectively. The rearranged proposed theorem (Equation 5.10) calculated an OE of 11.95, slightly lower than the actual OE of 12.26, the minor discrepancy possibly due to unmeasured metabolites. However it appears that Balling’s theorem produced nearly identical results with a OE calculation of 11.99. Unexpectedly it appears as though there is little disparity between the two calculations of OE despite large differences in theoretical background. This is likely due to the fact that the differences in sugar weight, glycerol production and yeast generation all roughly cancel during our fermentation. This finding likely contributes to the many reports of inconsistency concerning Balling’s theorem. The inaccuracies are likely compounded in
fermentations with unusually high glycerol or other secondary parameters as these would result in higher error during OE calculation:

\[
OE = \frac{EtOH + CO_2}{1.9565} \cdot (0.11) + EtOH + CO_2 + res\ sugar
\]

\[
OE = EtOH + gly + CO_2 + yeast - hydro\ mass + res\ sugar
\]

where \(OE\) is the original extract expressed in g\(\cdot\)100 ml\(^{-1}\), \(EtOH\) is ethanol concentration in g\(\cdot\)100 ml\(^{-1}\), \(CO_2\) is the \(CO_2\) concentration in g\(\cdot\)100 ml\(^{-1}\), \(res\ sugar\) is the residual (unfermented) sugar in g\(\cdot\)100 ml\(^{-1}\), \(gly\) is the glycerol concentration in g\(\cdot\)100 ml\(^{-1}\), and \(hydro\ mass\) is the additional mass of water added during the hydrolysis of polymer sugars in g\(\cdot\)100 ml\(^{-1}\).

### 5.4 Conclusions

For calculation of fermentation parameters, Balling’s theorem has several advantages over other methods, notably, the low number of easily measured parameters and global recognition. However, as has been widely reported upon (Cutaia, 2007, Neilson, 2007; etc.), there is high potential for inaccuracy, especially in high density wort. A more detailed mass balance (Equation 5.3) was hypothesized to alleviate this inaccuracy, however as shown in this study, both methods were about equally accurate at predicting OE. While Balling’s theorem appears to be built upon unsound theory, either by coincidence or design it is surprisingly accurate at predicting OE. However, Balling’s theorem was also shown to be very poor at modeling parameters such as yeast growth and the production of other metabolites (i.e. glycerol). The application of modern theory through the proposed equation 5.3 gives brewers and researchers the ability to accurately predict and model other fermentation parameters such as glycerol and yeast generation.
At the very least, one or more formulae gives brewers the opportunity to observe where and how their own fermentations deviate from Balling’s theorem. An advantage of using a mass balance such as Equation 5.3, is that as more terms included, the accuracy of the balance improves (i.e. the inclusion of FAN consumption), conversely, some accuracy can be sacrificed if some measurements cannot be taken (through the use of approximations and assumptions similar to Balling’s method).

Additional notes from this study show how the modeling techniques demonstrated in Chapter 5 can be utilized to track sugar consumption and product generation throughout fermentation. The consumption rates for each sugar were determined and from the consumption rates of glucose and maltose it was found that maltose repression is likely more complicated than has been previously reported. While SMA yeast showed evidence of maltose repression (Figure 5.3), significant (seemingly unrepressed) consumption of maltose was observed well before the concentrations of $>1 \text{ g}\cdot\text{100 mL}^{-1}$ was achieved. This has large potential implications for brewers as the usage of adjunct sugars is popular in Canada and in many parts of the work that do not adhere to the German purity law (Reinheitsgebot); the source (and composition) of these adjunct sugars are country dependent with corn, sugar, sorghum and other cereals all widely used (Hardwick 1994). Proper knowledge of how sugars are preferentially consumed will assist brewers in creating or adjusting wort formulations. There exists potential for future work examining the mechanism for this phenomenon and how fermentations with variable concentrations of each sugar are thusly affected.

Concerning the performance of the SMA yeast strain during this experiment, it was found that on average yeast consumed sugar at an average rate of $\sim 50 \text{ pg}\cdot\text{h}^{-1}$ (under
the assumption that only YIS consumed sugars). The average cell mass was \( \sim 40 \text{ pg\cdot cell}^{-1} \) (dry weight) over the experiment, however the average size increased over the fermentation as shown in Figure 6.12 (likely due to the higher concentration of daughter cells in the early fermentation period). Yeast replication was observed only in the first half of fermentation, after peak sugar consumption, cell division was seemingly non-existent (Figure 5.11). This said, the average cell mass did continue to grow after peak fermentation (Figure 6.12). Insofar as the author is aware the yeast growth, replication and consumption patterns were typical of industrial brewing yeast strains.

Under the close scrutiny of this study, brewing becomes less of a “black box” operation and several under-explained phenomenon are better understood. This work examined the consumption of sugar and generation of products allowing insight into Balling’s theorem and how this formula deviates from theory. Additionally, the generation of other significant metabolites were measured allowing yeast growth/consumption dynamics and phenomenon such as glucose repression of maltose consumption, \( \text{CO}_2 \) saturation/release and product generation to be modeled over the entire fermentation. It is the hope of the author that this knowledge will be applied in many ways, the most obvious of which are applications in online monitoring. The trends observed concerning \( \text{CO}_2 \) saturation and release will likely help correct online monitoring techniques that rely on the release of \( \text{CO}_2 \) to predict fermentation progress. Additionally, the modeling techniques explored in this Chapter will provide brewers and researchers with a more accurate method with which to model fermentations.
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CHAPTER 6 CONCLUSIONS

This thesis presented results from several studies involving CO₂ production and use in industrial and assay brewing operations. The tools used to examine CO₂ production were scrutinized from miniature assays, to modeling techniques and the applications thereof. The data spanned three years, over which the objectives detailed in Chapter 1 were completed, these were to:

I. Assess the miniature fermentation method (Detailed in Chapter 2.4) and its use in characterizing industrial fermentations,

II. Estimate CO₂ generation during fermentation using density attenuation,

III. Estimate the shear within fermentations using a model of CO₂ generation,

IV. Investigate the origin and utility of CO₂ solubility charts,

V. Scrutinize techniques, and model sugar consumption,

VI. Model the production of ethanol, glycerol, and CO₂ during fermentation,

VII. Measure and model the release of CO₂ from the fermentor.

The methods used in early studies were scrutinized and improved upon allowing for a more detailed analysis of brewing operations. This insight has allowed us to investigate several unexplained (or under-explained) phenomena and to make several recommendations. The results and conclusions from each study are summarized as follows:

Detailed methods of the ASBC miniature fermentation assay (ASBC Yeast-14) are described in Chapter 2. In Chapter 3 the method (with slight modification to accommodate brewery wort) was assessed for use monitoring industrial brewing operations (Objective I). During these studies, the attenuation of wort density was modeled and the resulting CO₂ generation was calculated using equations that are based upon Balling’s theorem (Objective II). The shear was determined using the approximated
rate of CO₂ generation (Objective III), this variable was believed to be a contributor to
differences observed between the industrial scale fermentation and the results of the
miniature fermentation assay (ASBC Yeast-14). Based upon the results of Chapter 3, the
miniature assay was further refined (in order to enhance accuracy) with the modified
assay used to examine many aspects of barley fermentation, as described in Chapters 5-6.

The study described in Chapter 3 raised several questions concerning the
solubility of CO₂ within brewing wort, and at which point the saturation of wort was
achieved. This lead to a literature review of CO₂ solubility in aqueous sugar and ethanol
solutions as well as an investigation into the accuracy and history of empirically derived
CO₂ solubility charts (Objective IV). The results of this review were surprising, as it
appears likely that these charts are likely inaccurate for beers that deviate from the beers
used to design them. During this research, several formulae to determine CO₂ solubility
were assessed, it was found that a formula utilizing solids and alcohol content in addition
to CO₂ partial pressure and temperature was the most flexible at assessing solubility and
most likely to accurately describe real world solubility levels. This formula was later used
in Chapter 6 to determine at what CO₂ concentration wort would achieve saturation.

In Chapter 5, the models and methods used in Chapter 3 were scrutinized in an
attempt to increase accuracy (Objective V). In previously published work, the density of
brewing wort was modeled using a 4P Logistic model (Speers, 2003). In Chapter 5, the
consumption of individual sugars (and generation of fermentation products) was
assessed, and it was found that some sugars exhibit asymmetric sigmoidal attenuation
that could not be effectively modeled with the 4P logistic model. This model was
compared to several other asymmetric sigmoidal models and it found that when sufficient
data points were available (dependent upon when the samples were taken), a five parameter logistic was superior in modeling the consumption of sugar during brewing operations. However, it was further determined that the number of data points taken during the study described in Chapter 3 were insufficient to justify (statistically) the use of the 5P Logistic model. Based upon these findings, the samples taken during the miniature fermentation assay were increased and rescheduled in subsequent studies to better describe the anticipated consumption of sugar during barley fermentation.

Combining the methods developed and refined though Chapter 3-5 with novel measurement techniques for the assessment of carbon dioxide at the laboratory scale (Objective VII); Chapter 6 examines the sugar consumption and subsequent generation of products while assessing the accuracy of Balling’s theorem (Objective VI). It was found that while theoretically problematic, Balling’s theorem provides a good estimation of OE, but should not be used to predict other parameters based solely upon ethanol or CO₂. From this study several other phenomena were assessed with several unexpected results such as the inhibition of maltose consumption and yeast consumption dynamics deviating from previously described reports. This said, many aspects of the fermentation including product generation (carbon dioxide, yeast biomass generation, glycerol etc.) and the majority of sugar consumption curves were shown to follow trends individually described either in previous reports or earlier studies within this thesis.

6.1 BROADER PERSPECTIVE AND APPLICATIONS

Of most concern to the brewing industry is the application of this thesis. This section attempts to demonstrate how the studies described in this thesis can be applied.
Miniature fermentation assays are necessary tools for assessing fermentation potential, however, of the many different methods described in brewing literature, each has unique limitations inherent to their design. The method used throughout this thesis (Yeast-14) has been shown to be convenient and reliable for several applications. However, it is not reliable at predicting the fermentability of malt at industrial scale, likely due in part to differences in shear that result from fermentor geometry. With a detailed understanding of CO₂ generation and relationship to shear, it is now theoretically possible to produce a stirred assay that mimics the natural shear generation within industrial scale fermentation.

With respect to CO₂ solubility, there does not appear to be a “best” formulaic approach as simplistic, theoretical models do not account for the complexity of beer, empirical models must be constructed using specific beers and advanced theoretical models require the accurate measurement of numerous parameters. Brewers can either accept the inaccuracy of published solubility charts, utilize one of the models discussed in this paper, or simply measure the dissolved CO₂ for various beers under specific temperature and pressure control. With knowledge of the underlying assumptions and methods used to determine CO₂ solubility, brewers and brewing researchers can now estimate CO₂ solubility of wort and beer with a higher degree of certainty.

There are several advantages to modeling the attenuation of density or the consumption of fermentable sugars (Speers et al., 2003). Brewers have multiple techniques from which to choose, however, as shown in Chapter 4, a 5P logistic model is provides a superior fit when sufficient data is available. This is especially important when modeling a parameter (such as CO₂) for use in calculating other parameters, as small
deviations will be magnified greatly impacting the analysis. As with any form of modeling, the data should be visually assessed the data to determine the basic shape of the graph. Fortunately, if this is ambiguous an F-test can be used to assess nested models, i.e. a symmetric model (such as the 4P logistic) or an asymmetric model (such as the 5P logistic).

As stated at the end of Chapter 6, for calculation of OE, Balling’s theorem has several advantages over other methods. (the low number of easily measured parameters and global recognition). A more detailed mass balance proposed in Chapter 6 (Equation 6.3) alleviates many of the inaccuracies of Balling’s theorem, however it requires the measurement of many additional parameters to be effective. The application of Equation 6.3 gives brewers and researchers the ability to accurately predict and model other fermentation parameters such as glycerol and yeast generation, however it is unlikely to be adopted as a routine procedure due to the difficulty measuring some of the parameters. However, this formulae gives brewers the opportunity to observe where and how their own fermentations deviate from Balling’s theorem and to plan future fermentations accordingly.

There have been many attempts by brewing researchers to describe and predict a fermentation using only the CO₂ collected throughout the fermentation (i.e. Kobayashi et al., 2005; Corrieu et al., 2000; Pandiella, et al., 1995). These have had moderate success, however often employ constants and factors to describe behaviour that does not conform to the theories applied. One potentially large source of error with these methods appears to be dissolved CO₂, while some researchers (such as Kobayashi et al., 2005) account for dissolved CO₂ using a solubility equation, others use constant values (Pandiella, et al.,
1995), or ignore this variable completely (requiring correction factors). No research groups apply the full dynamics of supersaturation and release as described in this thesis. This is problematic as the supersaturation causes a notable delay in the release of CO₂, and a subsequent period of off-gassing after fermentation is complete. Thus, relying on CO₂ release alone will overestimate the time required to complete a fermentation and consistently misrepresent the fermentation progress. While it is now possible to create a more accurate representation of how CO₂ release relates to a fermentation using the methods and theory described in this thesis and earlier papers, it may be equally beneficial for many breweries to simply understand the limitations of such systems.

6.2 Recommendations for Future Work

As tools and equipment become available to more continuously monitor industrial sized brewing operations, researchers will have the opportunity to further refine the findings of this thesis such as calculation of shear in Chapter 3 and the mass balance described in Chapter 6. Chapter 3 identified that shear likely played a role in observed differences between industrial and assay scale fermentations, therefore this variable should be controlled. If the type of shear can replicated, such as through a bubble column, it will likely lead to a more accurate assay and understanding of the fermentation process. This will eventually lead to a greater understanding of scaled-up fermentation for the brewing industry.

As discussed in Chapter 6, the consumption of maltose and inhibition thereof is an aspect of fermentation that should be investigated further. Both the behaviour at different sugar concentration and the mechanism of inhibition can be assessed using miniature scale fermentations offering potential insight into fermentation dynamics, with potential
application in adjuncts selection and usage. Finally, there remains much work to be completed examining the relationship between CO₂ solubility and other parameters within beer.

6.3 **CONTRIBUTIONS TO THE ADVANCEMENT OF KNOWLEDGE**

Much of the groundwork for this thesis was laid by previous researchers who have studied modeled and utilized CO₂ within brewing operations. Building on this work with modern instrumentation and theory, this study provided insight into several aspects of brewing through studies carried out over several years. Individually each of these studies have contributed to diverse brewing fields, however combined, they enabled a closer examination of the fermentation process. Using an improved understanding of CO₂ saturation, agitation and release gained from Chapters 3-4, the amount of CO₂ generation was able to be accurately tracked in Chapter 6 with application in online monitoring. With the modeling techniques refined in Chapter 5, it was possible to accurately model the consumption of sugars and subsequent production of products such as CO₂. This enabled an assessment of a fermentation using a theoretical mass balance, facilitating understanding of several previously unexplained or understudied phenomenon.

Whereas many previous studies have tracked the consumption of sugar and several products, this study was unique in accounting for both the consumption of individual fermentable sugars, and the generation of all major fermentation products while providing a theoretical basis and explaining the limitations of assumptions commonly employed. The high rate of sampling also allowed for more accurate models to be fit to the data allowing for a greater understanding of the fermentation process.
Beyond the experimental contributions, this thesis advanced the understanding of CO$_2$ solubility and saturation through a literature review and analysis. Understanding the historical context behind common CO$_2$ solubility charts and the advantages and disadvantages of various solubility equations gives brewers and researchers the option to select an appropriate methods and the knowledge to understand the limitations.

Overall the results from this thesis are useful to brewers and brewing researchers alike. It is the author’s hope that this thesis provides a small step forward in improving the understanding of the brewing fermentation process while providing opportunities for brewers to improve their operations.
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