FATE OF ALPHA-AMYLASE USED TO DEGRADE STARCH IN WATER-BASED DRILLING FLUIDS

A Thesis

by

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ABSTRACT

The removal of water-based mudcakes (filter cakes) from horizontal sections is a difficult task. The use of the enzyme α-amylase in enzymatic degradation of mudcake has proven conditionally effective in laboratory and in field treatments. Even so, the fate of the enzyme after treatment and the product distribution formed during treatment is unknown. This thesis presents a method of characterizing the mudcake-enzyme (specifically the starch-α-amylase) degradation system using analytical methods adopted from established biochemistry techniques. These methods were used to compare the effectiveness of the enzyme degradation system under various degradation conditions.

The enzyme's thermal tolerance under High Temperature/High Pressure (HPHT) conditions were determined using mud aging cells. Enzyme baseline activity (defined as rate of starch degradation) was established under well-mixed reaction condition. All enzyme treatment tests were performed in HPHT filter press to simulate downhole conditions. Retained permeability was determined for each test and concentrations of enzyme and unreacted starch was determined using UV-Vis spectroscopy. Starch degradation reaction product distribution was determined using high performance liquid chromatography coupled with refractive index detection (HPLC-RID).

Experimental results show that the specific company provided enzyme is not effective in degrading filter cake at any of the tested temperatures and that the analytical methods developed were effective in characterizing the starch-α-amylase reaction system.

DEDICATION

To my family.

ACKNOWLEDGEMENTS

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Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my parents and my sister, for all their love and support.

NOMENCLATURE

HPHT	High Pressure/High Temperature
HPLC	High Performance Liquid Chromatography
OFTIE	OFI Testing Equipment, Inc.
RID	Refractive Index Detector
RPM	Rotations per Minute
TCC	Total Carbohydrate Content
P/N	Part Number

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1. INTRODUCTION

This section outlines the current state enzyme utilization in the oil industry, presents an overview of drilling fluid (mud) filter cake (mudcake) with emphasis on water-based drilling fluid and the role of starch in filter cake formation, an gives a brief treatment on the characteristics of starch and the α-amylase which degrades it.

Following these relevant technical background subsections, the gaps in current knowledge upon the topic of enzyme degradation of starch in water-based filter cake are presented and experimental objectives are set out.

1.1 General Introduction

Enzymes are biological catalysts which accelerate the rate of specific bio-chemical reactions. Since the early 1990s there has been a revival in their use due to introduction of new biotechnologies in the oil and gas industry (Brannon et al. 2003). Initial codification of laboratory procedures by Beall and colleagues (1996) allowed further investigation of the specifics of enzyme treatment in both laboratory and field settings. During his time in industry my adviser, professor Nasr-El-Din, and his team investigated many aspects of enzyme treatment. These included the first dynamic closed-looped study of the entire enzyme treatment process (Siddiqui and Nasr-El-Din 2005), the first extended soaking time for enzyme treatment fluid downhole (Al-Otaibi and Nasr-El-Din 2005), the first extensive laboratory evaluation of a surfactant/enzyme

coupled treatment system (Nasr-El-Din et al. 2007), and the first use of chemical additives to stabilize enzymes for elevated temperature use beyond normal limitations (Samuel et al. 2010). My own proposed work will add to this existing body of knowledge by coupling analytical chemistry to the evaluation of spent enzyme solutions to determine enzyme fate and extent of enzymatic reaction.

1.2 Drilling Fluid Filter Cake

1.2.1 Filter Cake Formation

It is well known in the oil and gas industry that drilling fluids are inherently damaging to rock formation. Due to this, modern drilling fluids are designed to form an impermeable filter cake onto the formation face to prevent hydraulic communication between the wellbore and the formation (Kabir and Gamwo 2011). However, in order to achieve maximal productivity in either vertical (Kabir and Gamwo 2011) or in horizontal wells (Ding et al. 2004) filter cake must be removed prior to production. Filter cakes can be moved either mechanically or chemically (acids, oxidizers, chelating agents, enzymes) (Samuel et al. 2010).

1.2.2 Role of Starch

In older water-based drilling fluid formulations, clays such as bentonite and minerals such as barite were used to impart fluid loss control and viscous transport property. However because of their tendency to produce thick filter cake and their inefficiency in fluid loss control, they have largely been replaced by biopolymers (Simonides et al. 2002). In modern water-based drilling fluids, fluid loss control in imparted by starch, low-shear viscosity is imparted by xanthan gum and bridging is provided by sized calcium carbonate or salt particulates (Hanssen et al. 1999; Simonides et al. 2002). The separation of roles in newer drilling fluid formulations has a critical benefit, laboratory evidence shows (Hanssen et al. 1999) removal of only the bridging material (in my case starch, in the case of the paper, polyanionic cellulose) is necessary for complete degradation of filtercake, thus allowing xanthan gum to be retained to continue to impart needed viscosity to the remaining drilling fluid. Thus, there is a need for a highly reaction specific chemical to degrade only the starch in drilling fluid filter cake.

1.2.3 Case of Horizontal Wells

Over the last decade, the drilling of wells in horizontal or high-angle configurations as well as multi-lateral completions has accelerated primarily due to the ability of these wells to impart greater contact to hydrocarbon-bearing payzones to each

single well than traditional vertical wells and thus increase per well productivity (Levitan et al. 2004). However, this extended reach into the reservoir can also cause significant problems in stimulation. Reservoir heterogeneity and the sheer length of the horizontal leg make acid placement and diversion difficult, in addition, the low drawdown pressures in horizontal wells results in longer lift times for spent acid from the well, increasing the chances for uneven treatment along the well length (Nasr-El-Din et al. 2006). There has been attempts to modify acid and oxidizer systems in order to control rate of release to obtain even removal of filter cake in horizontal sections (Nasr-El-Din et al. 2006) however, laboratory and available field evidence has shown polymer linkage specific enzymes (PLSE) are the best treatment option for filter cake removal in horizontal wells (Al-Otaibi and Nasr-El-Din 2005; Beall et al. 1996; Samuel et al. 2010). The specific strain of enzyme proven to be useful in removal of starch water-based filter cake is α-amylase (Samuel et al. 2010; van der Maarel et al. 2002).

1.3 Starch Degrading Enzymes

1.3.1 Starch

Starch is a polysaccharide that plants synthesize as a product of photosynthesis. The glucose residues form two kinds of biopolymer, (1) amylose and (2) amylopectin. While amylose is a linear polymer consisting up to 6,000 glucose residues, amylopectin consists of short linear chains of 10-60 glucose and side chains of 15-45 glucose units. In

nature, both of these polymers are organized into granules, in which the amorphous regions are amylose and crystalline regions are amylopectin. Critically, amylopectin is soluble in water while amylose and the starch granules are not (van der Maarel et al. 2002). The glucose backbone of the starch is formed through α-1,4 glycosidic bonds, branching molecules are possible with α-1,6 glycosidic bonds. At the end of the starch polymeric chain, a latent aldehyde group called the reducing end is present. The natural 3-D structure of granular starch is complex (**Fig. 1**).

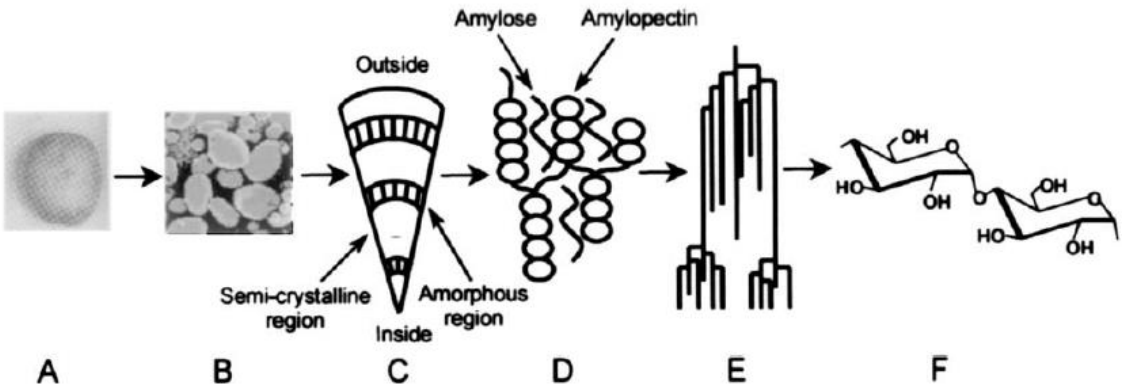

Fig. 1—Successive close-ups of a potato starch tuber (A). In decreasing order of size, electron microscope image of starch granules (B). Cross-section of a starch granule showing the growth rings consisting of semi-crystalline and amorphous regions (C). Detail of the semi-crystalline region (D). Tree-like organization of amylopectin molecule (E). Two glucose molecules with a α-1,4 glycosidic bond (F). It is the highly structured and interbedded nature of the amylose and amylopectin that makes starch granules insoluble in water. The interbedded nature allows starch to take up and store water within its structure and also bind to bridging agents, thus conferring the necessary properties needed for drill-in fluid filtercake formation. Figure from van der Maarel et al. (2002), reprinted with permission.

In order to degrade starch, the glucose backbone must be cleaved by hydrolysis of the α-1,4 glycosidic bond between the glucose. There are many types of enzymes found in nature which can modify starch. α-amylase is the most effective enzyme to cause the liquefaction (the process of turning into a liquid-like state) of starch. The effectiveness of α-amylase is due in part to the fact that it is the most abundant member of the endoamylase group of starch-converting enzymes (**Fig. 2**). Endoamylases are able to cleave α-1,4 glycosidic bonds in the inner part (endo-) of the amylose or amylopectin chain, this allows α-amylase to, by itself, breakdown the starch to soluble glucose, maltose, and maltodextrins, all with α-configurations (van der Maarel et al. 2002). The degradation of starch provided by α-amylase is entirely sufficient to degrade filter cake both in the laboratory and in the field (Al-Otaibi and Nasr-El-Din 2005; Samuel et al. 2010). Thus α-amylase is a good stand-alone treatment option for degrading starch in filter cake.

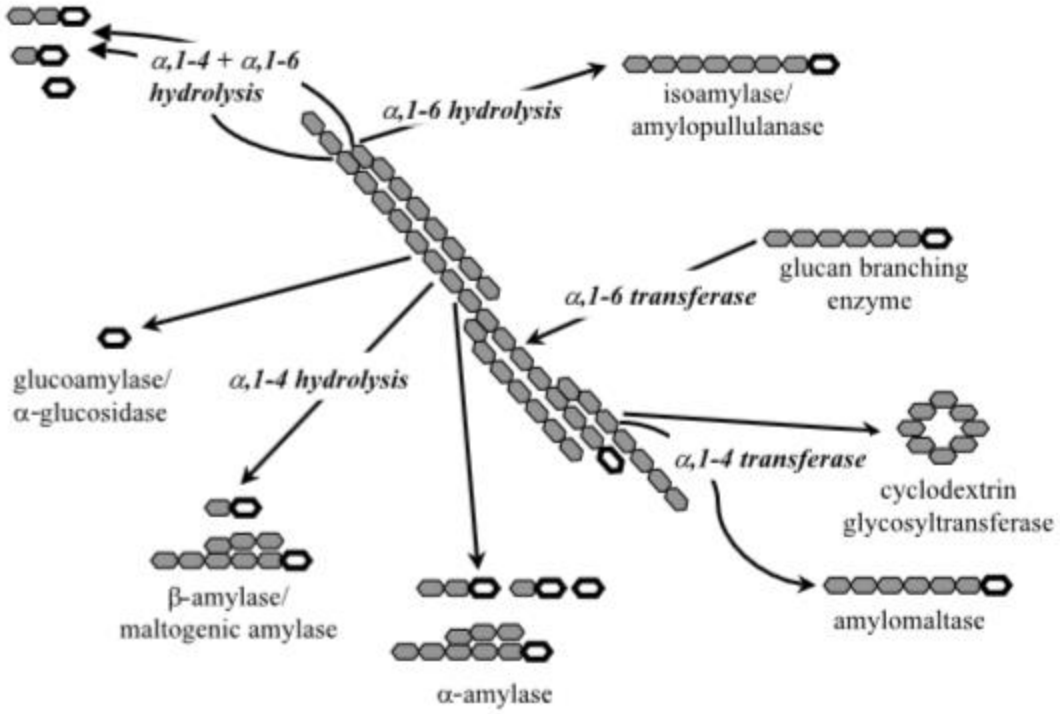

Fig. 2—Schematic of different enzymes involved in starch degradation. The open ring structure symbolizes the reducing end of a polyglucose molecule. Of all the enzymes, α-amylase displays the greatest variety of reducing end combinations, meaning it has the most reaction sites along a starch biopolymer. Figure from van der Maarel et al. (2002) reprinted with permission.

1.3.2 α-amylase

α-amylase is a highly reaction and reactant specific catalyst. This is due to the specific conformation that the $(\beta/\alpha)_8$ or TIM barrel (**Fig. 3**) of the enzyme imposes upon the substrate. The imposition allows the catalytic residues of the enzyme to react in a deliberate manner with only starch (**Fig. 4**). These characteristics make α-amylase an

ideal chemical species to fulfill the requirement of environmentally friendly, substrate-specific degradation of starch in water-based drilling mud.

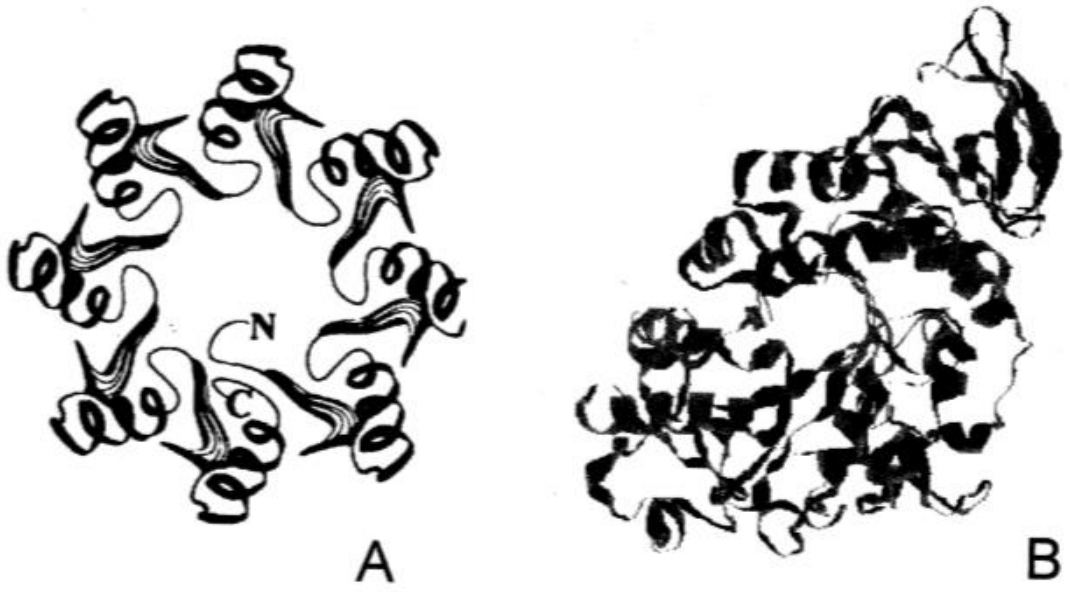

Fig. 3—Schematic representation of the TIM barrel (A) and 3-D structure of α-amylase (B). The confining nature of the TIM barrel forces the substrate to align properly in order to react, thus only specific substrate can be used, this is what confers the specificity of the enzyme. The enzyme shape allows it to isolate the reaction from the surrounding environment, thus conferring chemical robustness to the reaction scheme. Figure from van der Maarel et al. (2002), reprinted with permission.

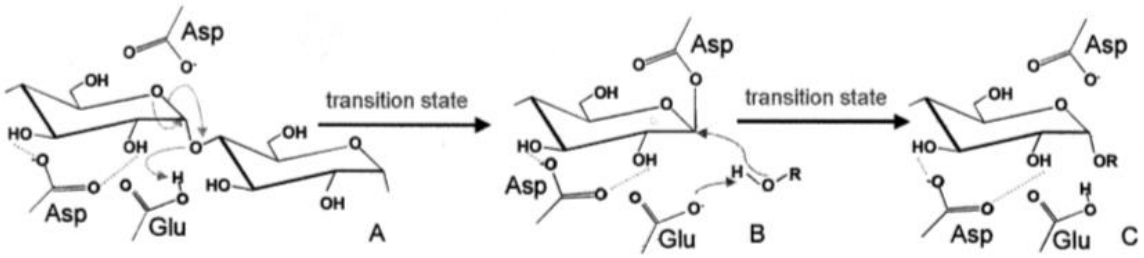

Fig. 4—Reaction mechanism for α-amylase catalyzed starch hydrolysis. This α-retaining double displacement involves two catalytic residues in an active site: a glutamic acid acts as an acid/base catalyst and an aspartate acts as the nucleophile. At the end of the reaction, the active sites are restored to their original states, allowed further reaction, thus fulfilling the definition of a catalyst. Figure from van der Maarel et al. (2002), reprinted with permission.

1.4 Gaps in Current Knowledge

While enzyme treatment has proven to be effective in laboratory evaluations (Beall et al. 1996; Nasr-El-Din et al. 2007; Samuel et al. 2010; Siddiqui and Nasr-El-Din 2005) as well as field treatments (Al-Otaibi and Nasr-El-Din 2005; Beall et al. 1996) the analytical methods applied thus far incomplete in describing the enzyme-filter cake reaction system in its entirety (**Fig. 5**).

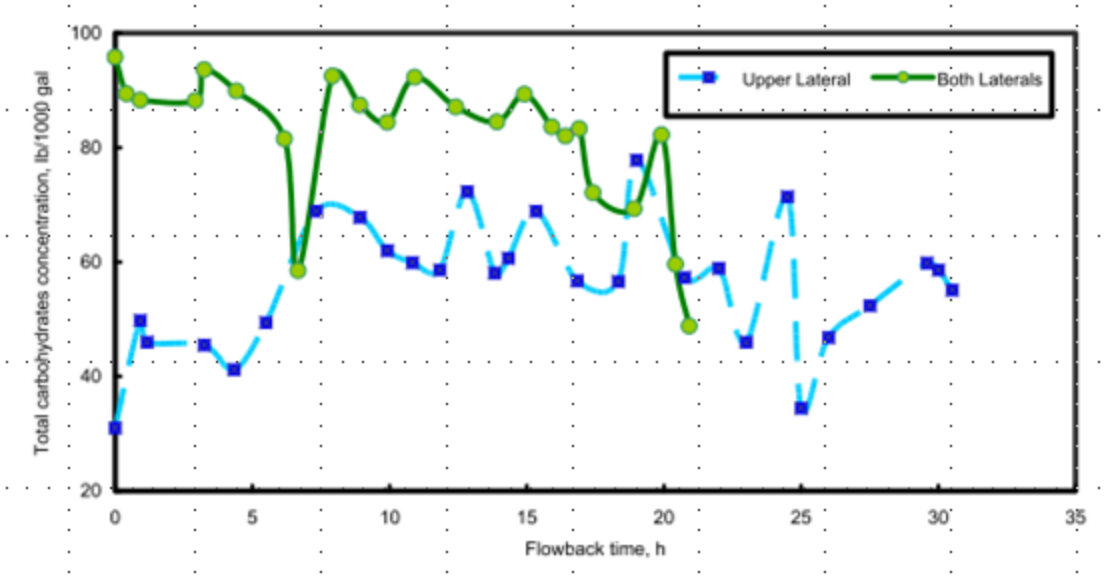

Fig. 5—Anthrone test results obtained by Al-Otaibi and Nasr-El-Din (2005) on flowback fluid from enzyme field treatment. Anthrone analysis is limited to detection of total carbohydrate concentration. Reprinted with permssion.

The most complete attempt in literature which attempted to conduct rigorous analytical chemical analysis on spent treatment fluid by Hanssen et al. (1999) has a significant drawback in that the enzymatic analysis was done radiologically on a trace spike in the enzyme stock and the carbohydrate detection was relegated to a test of enzyme activity rather than a true determination of carbohydrate distribution. Critically for the purpose of my study, the analysis that was done by Hanssen et al. (1999) was on polyanionic cellulose (PAC) and not starch. Therefore, direct analytical determination of α-amylase fate after the degradation of starch and the extent of reaction of α-amylase with starch is needed.

1.5 Research Objectives

1) Develop/adapt methods to determine enzyme concentration, residual starch concentration and starch-enzyme reaction product distribution in solution.
2) Replicate laboratory experiment of Al-Otaibi and Nasr-El-Din (2005) to serve as basis experiment
3) Compare enzymatic degradation of filter cake at varying conditions to optimize reaction system
4) Determine enzyme fate and product distribution for all reaction modes.

2. METHODS

In this section, a general overview of the experiments performed and the means and rational behind them is presented. Brief descriptions of the equipment used follows. The analytical methods are introduced and their procedures briefly described. Finally, the procedure used in the enzyme thermal stability, enzyme activity and filter cake buildup and degradation experiments are described. A summary of the materials and equipment used in the experiments is given in the appendix.

2.1 Experimental Overview

HPHT filter press will be used to build up and degrade the filtercake. The spent solution analyzed with the Bradford method for determination of enzyme concentration, with the Starch-Iodine assay for residual starch concentration, with the Anthrone test for total carbohydrate content, and with high-performance liquid chromatography coupled with refractive index detection (HPLC-RID) for determination of the concentration and distribution of carbohydrate.

The HPHT filter press procedure closely follows the method of Beall et al. (1996) and Samuel et al. (2010) with modifications. The Bradford method uses the method and formulation given by Bradford in his original paper on the matter (1976). The Starch-Iodine assay will be use of a modification of Lugol's Iodine with guidance by the literature review of Yoo et al. (1987) and the work of Xiao et al. (2006). The

Anthrone test will use the methodology of Morris (1948) in his quantification of Dreywood (1946) qualitative test to replicate the work of Al-Otaibi and Nasr-El-Din (2005). The HPLC method shall closely follow that of Chávez-Servín et al. (2004) and the calibration of the method shall conform to the standard set forth by Cuadros-Rodríguez (2007). However as with any HPLC method, the exact run conditions and sample preparation must be determined through trial and error, the trial and error involved in determining HPLC run conditions are partially described in the results section.

2.2 Equipment

2.2.1 HPHT Filter Press

The filter press used in this study is the OFI Testing Equipment, Inc. (OFITE) Dynamic HTHP (sic) Filter Press (P/N:170-50, **Fig. 6**).

Fig. 6—Photo of HPHT filter press, (OFITE 2014). Reprinted with permission.

The apparatus allows for temperature setting of between 100 to 500°F and a maximum pressure setting of 1,250 psi for static mode (no rotation) or 800 psi for dynamic mode. Test fluid capacity is a standard 500 mL and the RPM (rotations per minute) allowed ranges from 1 to 1200 for the dynamic mode.

2.2.2 Mud Aging Cell

The mud aging cell used is the OFTIE Pressurized Aging Cell, 500 mL series (**Fig. 7**). Both the 303 (P/N: 175-30) and the 316 (P/N: 175-50) Stainless styles were

used. In the case of this study there was no difference between the two in terms of experimental outcome. The apparatus allows for a maximum temperature of 500°F and a maximum applied pressure of 2,000 psi.

Fig. 7—Photo of mud aging cell, (OFTIE 2014). Reprinted with permission.

2.2.3 Spectrophotometer

The spectrophotometer used in this study is the Orbeco SP600 pectrophotometer (P/N: L712000) (**Fig. 8**). It is a single beam spectrophotometer with a wavelength range of 330 – 900 nm and a photometric range of -0.3 to 2.5 Abs. The cuvettes used for this study is VWR's Standard Cuvette, PS Grade Polystyrene, 4.5 mL with pathlength of 10 mm (P/N: 58017-880).

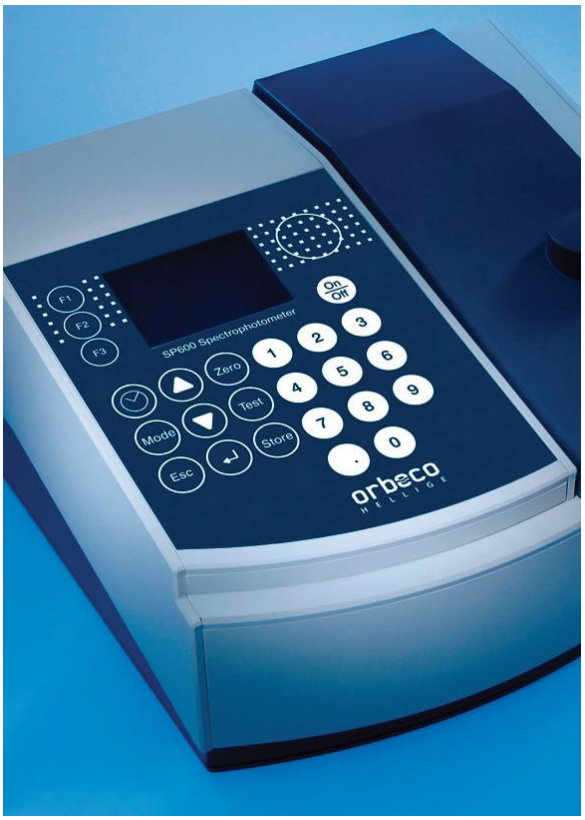

Fig. 8—Orbeco SP600 Spectrophotometer, (Orbeco 2014). Reprinted with permission.

2.2.3 HPLC-RID

The HPLC-RID apparatus used in this study is the Agilent 1100 Series (**Fig. 9**) with the exception of the RID itself which is from the Agilent 1200 Series.

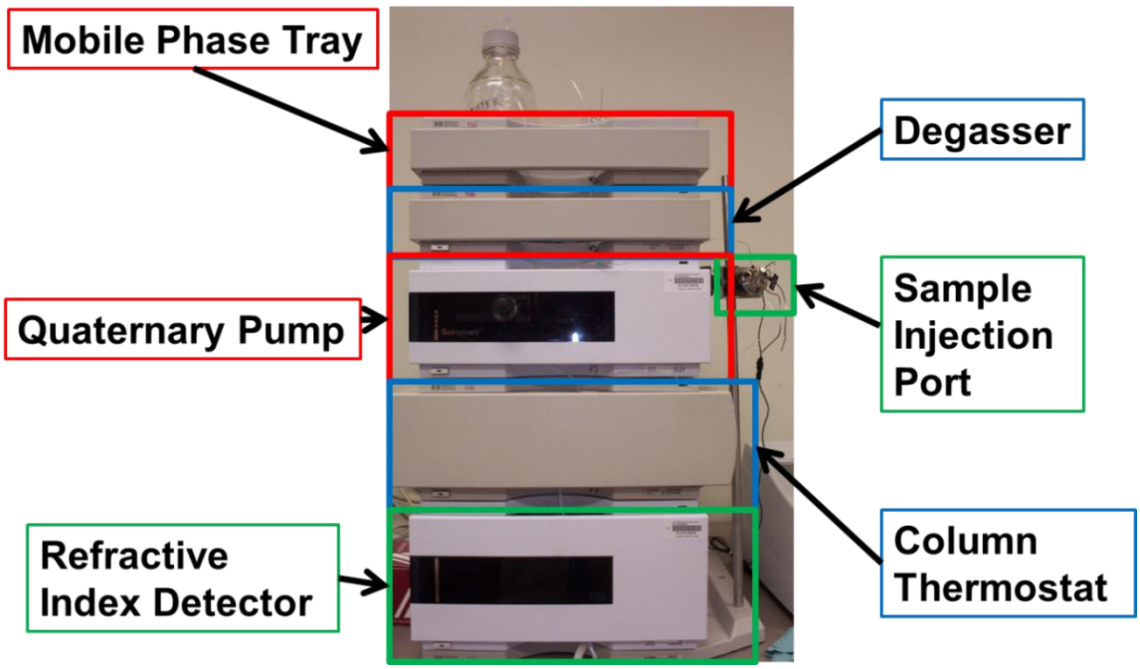

Fig. 9—Photo of HPLC machine, with the modules labeled.

The columns used are the Phenomenex Rezex RSO-Oligosaccharide Ag+ analytical column (P/N: 00P-0133-N0) and its corresponding guard column (03R-0133-N0).

2.3 Analytical Methods

In this section, the operations of the spectrometer and the HPLC-RID are covered. The theoretical basis of the spectrophotometer methods (Bradford, Starch-Iodine, Anthrone) and the HPLC-RID are given brief treatment. The recipes for the reagents used in the spectrophotometric methods are given.

2.3.1 Spectrophotometry

The following theoretical treatment of spectrophotometry is taken largely from Harris (2007). Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. A beam of light that is a single wavelength is said to be monochromatic. When a beam of light is partially or wholly absorbed by a sample, the irradiance of the light beam is reduced. Irradiance, P, is defined as the energy per second per unit area of the light beam. Within a spectrophotometer, the generated monochromatic light, with irradiance of P_0, strikes the sample of length b and emerges on the other side with irradiance of P.

The transmittance, T, of this system is defined as the fraction of original light that passes through the sample. Transmittance is defined mathematically through **Equation 1**:

$$T = \frac{P}{P_0} \qquad \textbf{(Equation 1)}$$

The transmittance is a fraction and has range of 0 to 1. The percent transmittance is between 0 and 100%. Absorbance (sometimes referred to as optical density) is defined through **Equation 2**:

$$A = \log_{10}\left(\frac{P_0}{P}\right) = -\log_{10} T \qquad \textbf{(Equation 2)}$$

Absorbance is analytically important because it is directly proportional to the concentration of the analyte through the Beer-Lambert law (or simply Beer's law), given in **Equation 3**:

$$A = \varepsilon bc \qquad \textbf{(Equation 3)}$$

Where,

ε is the extinction coefficient

b once again is the pathlength

c is the concentration of analyte in the sample.

2.3.1.1 Operating The Orbeco SP600

The only measurements performed with the spectrometer in this study were direct measurements of the sample absorbance/transmittance. Calibrations of the absorbance with the standards and the conversion of sample absorbance to concentration is done with raw data in Excel. The procedure for operating the Orbeco SP600 to obtain absorbance/transmittance may be found in the instruction manual provided on Orbeco's website (Orbeco 2014) in section 2.4.6 Lab Functions under the heading of Absorption/Transmission.

2.3.1.2 Enzyme Quantification: The Bradford Method

The appropriate method for enzyme quantification was selected after a literature survey. The simplest method, direct assay of the protein sample at an optical density of 280 nm (Harris 2007) was discarded due to the possibility of signal interference from compounds with aromaticity. The Bicinchoninic acid (BCA) assay (Smith et al. 1985) is based upon the Biuret reaction (Gornall et al. 1949). The copper reduction reaction is specifically with the peptide bonds and is thus free from interference of free amino acids. However, the relative insensitivity of the assay and the fact that sample preparation takes upwards of 30 min under ideal heated conditions (2 hours for room temperature) as well as the sensitivity of the assay to carbohydrates (the product of the starch-α-amylase) precludes the BCA assay as a viable candidate for enzyme quantification for this study.

The Lowry method (Lowry et al. 1951), incidentally the most cited paper in the history of science (Kresge et al. 2005), consists of the Biuret reaction (cited above) followed by reduction under the alkaline conditions of the Folin-Ciocalteu reagent (Folin and Ciocalteu 1927). The Lowry method is not appropriate for the purposes of this study due to its sensitivity to a variety of contaminants and its exacting requirements of timing and mixing of reagents.

The Bradford method is a fast, high throughput method for enzyme quantification. It relies on the principle of protein-dye bonding. The recipe for making 100 mL of Bradford reagent is given in **Table 1**. The analytical wavelength used is 595 nm. The reagent volume per test is 3 mL and the sample volume is 0.1 mL. The sample

should be added to the cuvette first before reagent addition. According to Bradford (1976), and confirmed by the author's experience, 2 min should elapse after reagent addition before the measurement is taken. Bradford cautions against measurements for sample-reagent solution which are more than 30 min old (this suggestion was followed by the author without attempts to confirm).

Table 1—Recipe for making 100 mL of Bradford reagent.

Material	Amount	Unit	Function
Coomassie Brilliant Blue G-250	10	mg	Active Dye Reagent
85% Phosphoric Acid	10	mL	pH Control
Absolute Ethanol	4.75	mL	Dehydrant
diH_2O	85.25	mL	base

The method was first presented by Bradford (1976). The Coomassie Brilliant Blue G-250 (**Fig. 10**) active ingredient in the Bradford reagent has a maximum absorbance at 470 nm in its cationic state and a maximum absorbance at 595 nm in its anionic state—the dye also has a neutral species which has a maximum absorbance at 650 nm but this species is not relevant for the mechanism of protein binding (Compton and Jones 1985). The anionic form is the form that engages in protein-dye bonding.

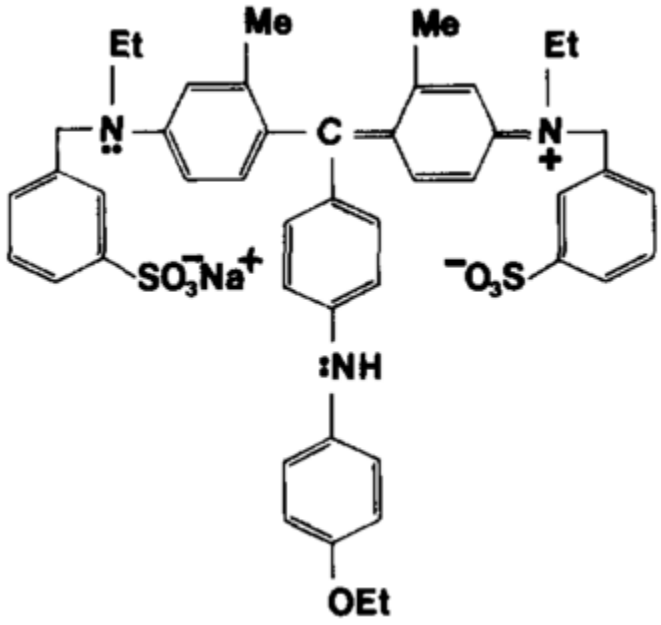

Fig. 10—Molecular structure of Coomassie Brilliant Blue G-250 (CBBG). An example of the molecule as a neutral species. Figure from Compton and Jones (1985), reprinted with permission.

Referring to Fig. 9, the CBBG is doubly protonated (cationic) at pH 0.3 and is doubly unprotonated (anionic) at pH 1.25. The protonation/deprotonation occurs at the two sulfonate groups. With 8.5% phosphoric acid in the reagent, the dominant form is solution is cationic form. As protein substrate is added into the system and anionic CBBG is bond to the protein, Le Chatelier's principle forces the CBBG equilibrium to the right (**Fig. 11**) due to the depletion of the anionic dye from protein binding. It is the increase in the anionic form and the accompanying increase in absorbance at 595 nm that gives the analytically quantifiable response.

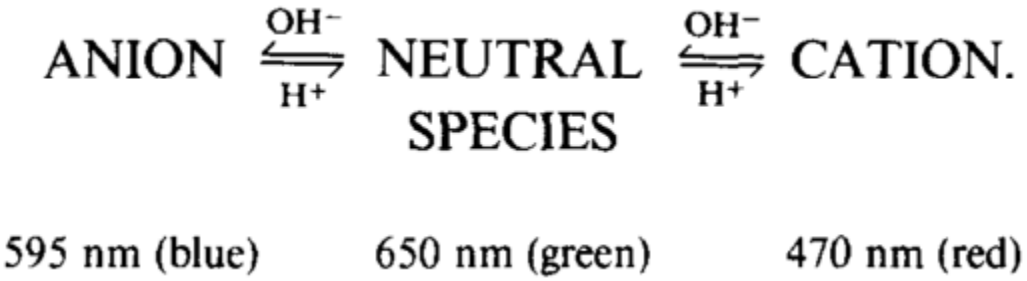

Fig. 11—Equilibrium reaction for CBBG. Figure from Compton and Jones (1985), reprinted with permission.

The Bradford method should only be used for a single enzyme system (or multiple enzyme systems with non-varying fraction of each enzyme used), due to the CBBG's differentiating preference for different amino acids (**Fig. 12**). The color response of the Bradford method is not linear over a large range of protein concentrations, therefore, appropriate ranges of protein concentrations must be determined for each individual protein system (see results sections).

DYE-BINDING RESPONSES TO POLYMERIC L-AMINO ACIDS

Polymeric amino acid	Molecular weight	Mean blank corrected absorbance (595 nm)	Relative response[a]
poly(Arg)[b]	40,000	4.033	36.0
poly(Tyr)[c]	100,000	0.531	4.7
poly(Try)[c]	5,000	0.491	4.4
poly(His)	11,000	0.472	4.2
poly(Phe)	15,000	0.215	1.9
poly(Lys,Ala)			
100, 0	35,000	0.112	1.0
80, 20[d]	38,000	0.028	0.3
46, 54[d]	37,000	0.001	0.0
0, 100[c]	25,000	0.000	0.0
poly(Gly)	6,000	0.000	0.0
poly(Asn)	9,000	0.000	0.0
poly(Asp)	20,000	0.000	0.0

Note. Samples are aqueous, 1.0 mg/ml unless otherwise stated.

[a] Compared to poly(lysine).

[b] Sample was diluted to 125 μg/ml to bring value in range. The value shown is dilution corrected.

[c] Dissolved in DMSO, 1.0 mg/ml.

[d] Random copolymers of L-alanine and L-lysine, by molar ratio.

Fig. 12—Dye-binding Reponses to polymeric L-amino acids. Figure from Compton and Jones (1985), reprinted with permission.

2.3.1.3 Starch-Iodine Assay

The Iodine – Starch Assay is used to quantitatively test the amount of gelatinized starch in solution. The reagent used is a modified version of Lugol's iodine and follows the work of Xiao et al. (2006) and Yoo et al. (1987). In order to obtain gelatinized starch, the company provided granular starch is thermally activated by heating at 95°C in well-mixed solution for 30 min (Juansang et al. 2012). The recipe for 100 mL of Iodine reagent is as given in **Table 2**. The analytical wavelength is 700 nm. The recipe is exactly a solution of 5 mM I_2 and 5 mM KI. The reagent volume per test is 3 mL and the sample volume is 0.1 mL in order to remain consistent with the Bradford method to speed up experimental time. There was disagreement in literature as to the optical density wavelength and the Iodine reagent component concentration (Xiao et al. 2006; Yoo et al. 1987). Values chosen from literature were tested in combination and the values presented in this section (namely optical density wavelength of 700 nm and concentration of 5 mM for both components in the reagent) were chosen. The sample is added before the reagent into the cuvette. In the experience of the author, solution equilibration time is about 1 min.

Table 2—Recipe for 100 mL of Iodine reagent (5 mM I_2 and 5 mM KI)

Material	Amount	Unit	Function
0.951 N I_2	1.05	mL	Iodine reagent component
KI	83	mg	Iodine reagent component
diH_2O	98.95	mL	base

2.3.1.4 Anthrone Test

The Anthrone test is a test to measure to determine total carbohydrate content (TCC). The test calls for polysaccharide hydrolysis by sulfuric acid, followed by mono- and oligosaccharide units to form furfural by further conversion due to the sulfuric acid. The furfural then reacts with anthrone in the colored reaction (Yang and Flippen 1997). This method has a major drawback: it is non-illuminating in terms of the distribution of the sugar product distribution, it is nevertheless incorporated into this study due to this method's utilization in Al-Otaibi and Nasr-El-Din's (2005) paper presenting a coupled enzyme lab and field evaluation. The recipe for the Anthrone reagent and the conditions for the reaction are given by Morris's (1948) quantitative formulation modified from Dreywood's (1946) original qualitative formulation for Dreywood's Antrone test. Morris's (1948) formulation was used because Al-Otaibi and Nasr-El-Din (2005) cited Yang and Flippen (1997) who cited Morris (1948) and neither of the first two papers gave details on their experimental setup for the Anthrone test. The recipe for 100 mL of

Anthrone's reagent is presented in **Table 3**. The test solution is 2 parts reagent and 1 part sample by volume. Optical density is measured at 625 nm, following the value used by Al-Otaibi and Nasr-El-Din (2005). It is important to note that in the original method developed by Morris (1948) used 620 nm or 540 nm as the optical density. The formulation of Al-Otaibi and Nasr-El-Din was chosen in order to establish consistency to aid in comparison of laboratory results. According to Morris (1948), 4 or 5 mL of sample solution is reacted under thorough swirling with 8 or 10 mL of reagent in a test tube of 19- to 25-mm diameter; color development requires reaction of 10 min or more.

Table 3—Recipe for 100 mL of of Anthrone's reagent

Material	Amount	Unit	Function
Anthrone	200	mg	Calorimetric Reaction
H_2SO_4 (100%, theoretical)	95	mL	Carbohydrate dehydration, furfural formation
diH_2O	5	mL	Dilutant

2.3.2 HPLC-RID

The following theoretical treatment is taken largely from Harris (2007). Liquid chromatography is important because most compounds are not sufficiently volatile for gas chromatography. High-performance liquid chromatography (HPLC) uses high pressure to force solvent through closed columns packed with very fine particles giving high-resolution separations. Column separation efficiency, column efficiency for short,

is determined by the rate at which solute equilibrates between the stationary and mobile phases: increasing the rate of equilibration increases the column efficiency. In liquid chromatography, because of the relatively slow rate of solute diffusion (100 times slower when compared to gases in gas chromatography) column packing is utilized to shorten the distance needed for solute diffusion and to increase the surface area available for solute/stationary phase interaction. Particle size is also controls column efficiency, in general, the smaller the particle size used for packing, the higher the column efficiency.

The column used in this study is an ion-exchange column, specifically one with strongly acidic cation-exchange resin, with sulfonated styrene divinyl benzene as the solid support. In any ion-exchange column, retention is based on attraction between charged sites on the samples and charged sites bound to the stationary phase.

The detector used in this study is a refractive index detector (RID). It reads the refractive index signals of the sample components that were separated with the column in the eluted mobile phase

Following are operations sections for the specific HPLC-RID used in this study. The full development process for the HPLC-RID method used in this study may be found in the results section.

2.3.2.1 Normal Operations

The following steps for proper startup and shutdown of HPLC-RID are taken from experience and trial and error. The procedures and constraints outlined the HPLC

(Agilent 2014a), RID (Agilent 2014b), and column (Phenomenex 2014a, 2014b) user manuals and technical specification sheets are followed throughout all HPLC experiments.

1. Ensure that the machine was completely shutdown and the relevant programs are closed.
2. Get fresh diH2O. Check wiring.
3. Turn on modules in order from up to down (degasser, pump, column holder, RID).
4. Turn on Bootp, after proper server connection, turn on Agilent ChemStation.
5. Wait until degasser finishes initializing, set RID temperature to 40°C, run system at 0.1 mL/min for 45 min to stabilize pump operations.
6. Set pump to 0.2 mL/min, set column temperature to 60°C, purge reference cell for 20 min.
7. Set pump to 0.3 mL/min, let run for 45 min to stabilize system to operating conditions.
8. Inject and run samples. (Samples injected must be 7x sample loop volume to ensure reproducibility).
9. For shutdown: Set RID temperature to “not controlled”, let run at 0.3 mL/min flow rate and column temperature at 60°C for 15 min.
10. Set column temperature to “not controlled”, set flowrate for 0.1 mL/min. Let run until temperature returns to room temperature.

11. Turn off pump, wait until pressure is 0 bars.
12. Turn off Agilent ChemStation, turn off Bootp, turn off modules in down to up order (RID, column holder, pump, degasser).
13. Empty out waste water. Check wiring.

For all HPLC tests in this study, normal operating conditions is defined through **Table 4**.

Table 4—Normal operating conditions for HPLC.

Category	Setting
Column Temperature	60°C
RID Temperature	40°C
Flowrate	0.3 mL/min
Backpressure	< 20 bars

2.3.2.2 Sample Preparation

According to the column care guide (Phenomenex 2014a), the sample must be filtered with a 0.2 or 0.45 micron filter in order to prevent column clogging. VWR's Sterile Syringe Filter w/ 0.2 μm Cellulose Acetate Membrane (P/N: 28145-477) is used to syringe filter samples prior to injection. In all runs, both calibration and sample runs, the solution must be spiked with a known quantity of 1,6-hexanediol (the internal

standard in this study, IS for short) before it is filtered and ran. The internal standard was chosen from literature (Dee and Bell 2011) and was confirmed to be appropriate for this study in experimentation.

2.3.2.3 Column Cleaning

Both the analytical column and the guard column are cleaned on a biweekly basis. Cleaning of both follows the user manual (Phenomenex 2014a). Each column must be cleaned individually. The cleaning must be done with the column in reverse flow with the flowrate at 0.2 mL/min and the column temperature at 75°C. The flow must bypass the RID.

2.3.2.4 Connection Tubing Cleaning

Three pieces of connective tubing, namely, the column to column connection and the column to detector connection (also used for column cleanings as column to waste connection) must be cleaned on a biweekly basis or as needed. The nature of the cleaning is such that the sampler to column connection is cleaned along with the other two connections.

The cleaning is done bypassing both the columns and the detector with ~1 m/v% sodium persulfate solution at ambient temperature at a flowrate set to the highest allowable up to 0.3 mL/min which will give a backpressure of less than 40 bars. A photo

of the connection between the sampler to column connection and any of the other connections is given below as **Fig. 13**.

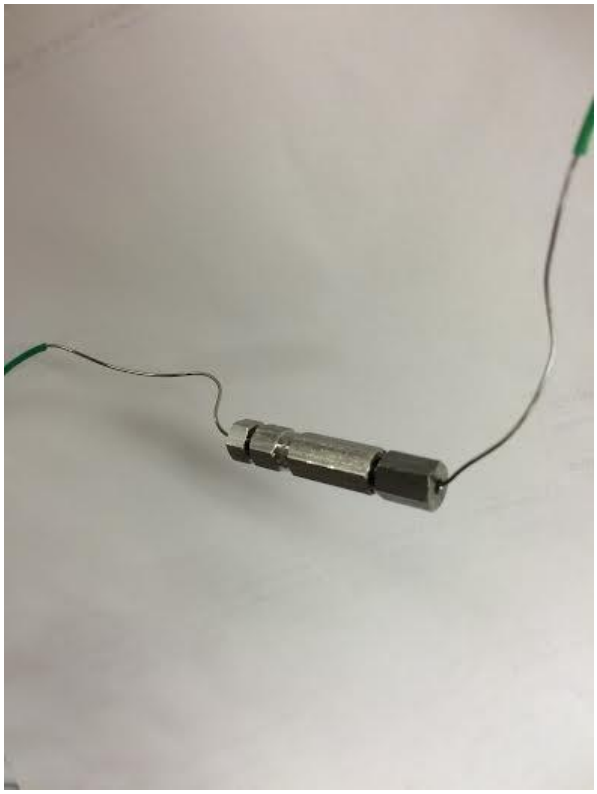

Fig. 13—Photo of connection between two HPLC tubings without using columns. Note the connection part needed.

2.4 Enzyme Characterization Experiments

2.4.1 Enzyme Thermal Stability

Enzymatic thermal stability was tested by proxy with the determination of the time required for enzyme denaturation at various temperatures (150, 175, 200, 225 and 250°F). The applied pressure is isobaric at 300 psi throughout the entire series of experiments. Tests were performed by preparing 35 mL aliquots of 5 vol% enzyme solution, measuring their initial Bradford response, placing them into mud aging cells, heating them at the prescribed temperature for the prescribed amount of time, then measuring the post-heating Bradford response. The thermal stability experiments performed are summarized below in **Table 5**.

Table 5—Summary of conditions for enzyme thermal stability experiments. All tests were performed with 35 mL aliquots of 5 vol% enzyme solution at 300 psi.

	Time (hr)				
Temperature (°F)	0.5	1	2	4	6
150	Test 1	Test 2	Test 3	Test 4	Test 5
175	Test 6	Test 7	Test 8	Test 9	Test 10
200	Test 11	Test 12	Test 13	Test 14	Test 15
225	Test 16	Test 17	Test 18	Test 19	Test 20
250	Test 21	Test 22	Test 23	Test 24	Test 25

2.4.2 Enzyme Activity

Enzyme activity experiments were adapted from the Fuwa experiments presented by Yoo et al. (1987). The experiment described in the paper tests for the amount of amylase (mg) required to decrease a starch solution's 700 nm optical density by 10% in 30 min of reaction under 37°C and 1 atm. A major drawback of this approach is the fact that only one data point is taken for the enzyme-starch reaction system in the end: the amount of amylase. The experiment performed in this study generates what is best described as an activity curve. A solution of 5 g of starch in 99 mL of deionized water is heated under stirring to 95°C under atmospheric pressure and held for 30 min (to thermally activate the starch). Bradford and Starch-Iodine assay measurements of this solution is taken to establish a basis for comparison. The reaction was allowed to proceed for 1 hour and at various time intervals, Bradford and Starch-Iodine assay measurements were taken. The measurements taken are summarized in **Table 6**. This experiment was repeated four times to ensure statistically significant results.

Table 6—Measurements taken for enzyme activity test using 5 wt% of starch as substrate and 1 vol% of enzyme as reagent. Tests were done under atmospheric pressure at 95°C. Note: Anthrone measurements not taken.

Time (min)	Bradford	Starch-Iodine
0	Measurement 1	Measurement 2
0.25	Measurement 3	Measurement 4
0.5	Measurement 5	Measurement 6
0.75	Measurement 7	Measurement 8
1	Measurement 9	Measurement 10
2	Measurement 11	Measurement 12
3	Measurement 13	Measurement 14
4	Measurement 15	Measurement 16
5	Measurement 17	Measurement 18
6	Measurement 19	Measurement 20
7	Measurement 21	Measurement 22
8	Measurement 23	Measurement 24
9	Measurement 25	Measurement 26
10	Measurement 27	Measurement 28
15	Measurement 29	Measurement 30
20	Measurement 31	Measurement 32
30	Measurement 33	Measurement 34
40	Measurement 35	Measurement 36
50	Measurement 37	Measurement 38
60	Measurement 39	Measurement 40

2.5 Filter Press Experiments

2.5.1 Static Filtration

HPHT filter press is chosen to test fluid loss because of its ability to closely approximate downhole conditions. Filtration outcome is determined by temperature, pressure, solid type and quantity, and finally any interactions these solids might have

physically or chemically. The formation face is simulated by a core of known permeability and dimensions of 2.5 in in diameter and 0.25 in in thickness. The core is loaded into the core holding end of the cell and the drilling fluid poured in at the other end (**Fig. 14**) and the cell is loaded into the filtration apparatus (**Fig. 15**).

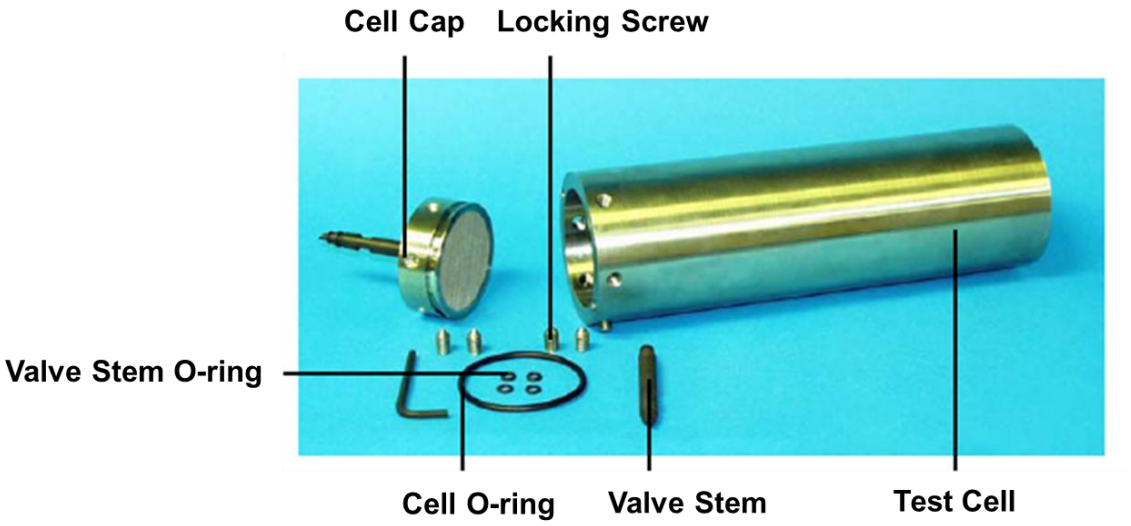

Fig. 14—Photo of HPHT test cell with parts labeled. Source: OFITE website. Reprinted with permission.

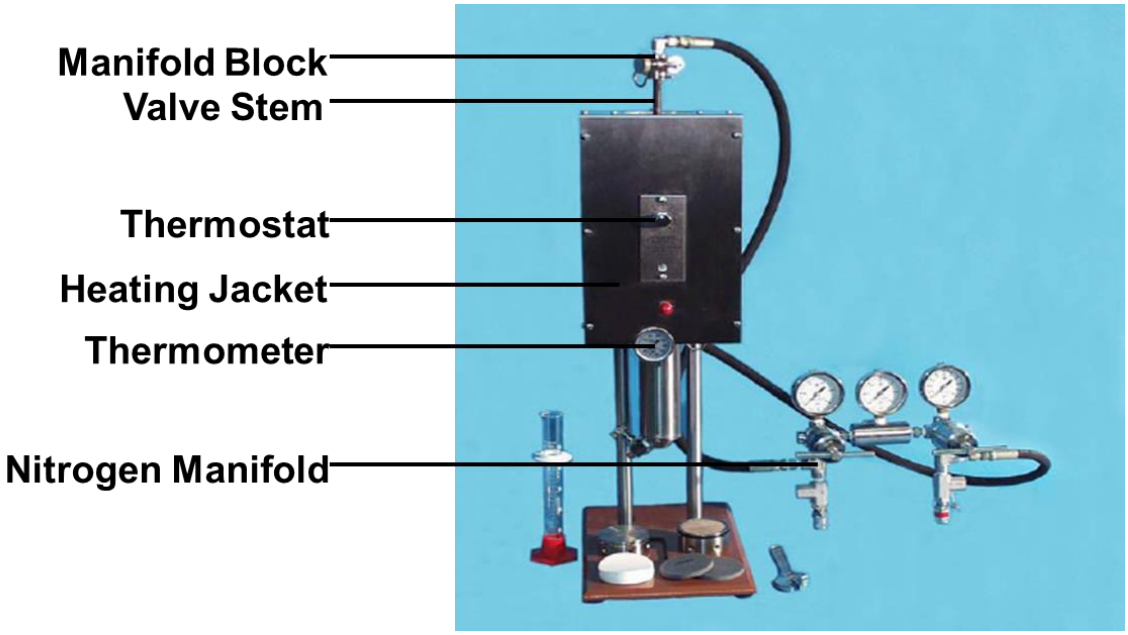

Fig. 15—Photo of HPHT filter press with parts labeled (Not the model used, displayed here for parts clarity). Source: OFITE website. Reprinted with permission.

After the cell is loaded, apply pressure (300 psi) with the manifold and set temperature, 200°F is chosen for filter cake buildup. After equilibration for 30 min (given by API recommendations), leakoff is performed for 30 min and data is collected to determine filter cake integrity. The cell is then cooled and the remaining drilling fluid decanted. The filter cake treatment solution is applied at 300 psi and the chosen temperature and after equilibration, left to react for the prescribed period of time. The cell is then cooled and the spent treatment solution collected and analyzed for enzyme concentration, residual starch concentration, carbohydrate distribution and total carbohydrate content. The formula for the drilling mud used in this study is taken directly from my predecessor's thesis (Dharwadkar 2011) is used without modification

and is given in **Table 7** and the formula for the making 200 g of 10 wt% enzyme solution is given in **Table 8**. More detailed treatment of the procedures involved in performing a filtration press may be found in Beall et al. (1996) and Samuel et al. (2010).

Table 7—Recipe for making drilling mud. Components are to be added in the order presented in the table, mixing time for each addition is 10 min

Material	Amount	Unit	Function
Distilled Water	308	g	Base
Defoamer	0.33	mL	Defoamer
Xanthan Polymer	1.5	g	Viscosifier
Pre-gelatinized Starch	6	g	Fluid Loss Control
Potassium Chloride	97.6	g	Density and Shale Inhibition
Potassium Hydroxide	0.3	g	pH Control
Calcium Carbonate	13	g	Weighting and Bridging Material
Sodium Sulfide	0.25	g	Oxygen Scavenger

Table 8—Recipe for making 200 g enzyme solution.

Material	Amount	Unit	Function
Enzyme	20	g	Enzyme
KCl	4	g	Salinity
Water	176	g	Base

2.5.2 Dynamic and Pre-Wash Conditions

For enzyme degradation experiments done under dynamic conditions, the filter cake is still formed under static conditions, but the degradation is done dynamically.

For the case of pre-wash, the pre-wash is done following filter cake buildup and before enzyme degradation. The pre-wash is applied for 2 hours before being decanted. Pre-washes are done under static conditions. The recipe for the pre-wash solution is adapted from Al-Otaibi and Nasr-El-Din (2005) and is presented in **Table 9**. The experiment performed in the paper is also replicated in this study to serve as a basis case (see results section).

Table 9—Recipe for pre-wash solution adapted from Al-Otaibi and Nasr-El-Din (2005).

Materials	Quantity	Units
4 wt% KCl in diH_2O	290	mL
HCl (15 wt%)	0.074405	mL
2-Butoxyethanol (Mutual Solvent)	10	mL
Sodium sulfite	0.624096	g
pH	3-4	

3. RESULTS AND DISCUSSION

The results of the experiments performed and discussions on their significance are given in this section. Firstly, calibration curves establishing the validity of the analytical methods chosen are presented. Secondly, the basis case, replication of experiment of Al-Otaibi and Nasr-El-Din (2005) is presented. Thirdly, results of the enzyme characterization experiments follow. Finally, the results various filter press experiments are given and compared to the basis case.

3.1 Calibration Curves

Below are calibration curves for the analytical methods used in this study. The calibration is valid only for interpolation within the curve given. Samples whose concentrations are higher than the calibration range must be diluted before testing.

3.1.1 Bradford Method

The calibration of the Bradford method is done upon vol% solutions of the company supplied enzyme. The recipe for making the Bradford reagent was taken directly from the relevant papers and may be found in the methods section. Initially, the Bradford method was calibrated for enzyme concentrations of up to 10 vol%. This

calibration revealed that the Braford response to enzyme concentration is non-linear within this range (**Fig. 16**).

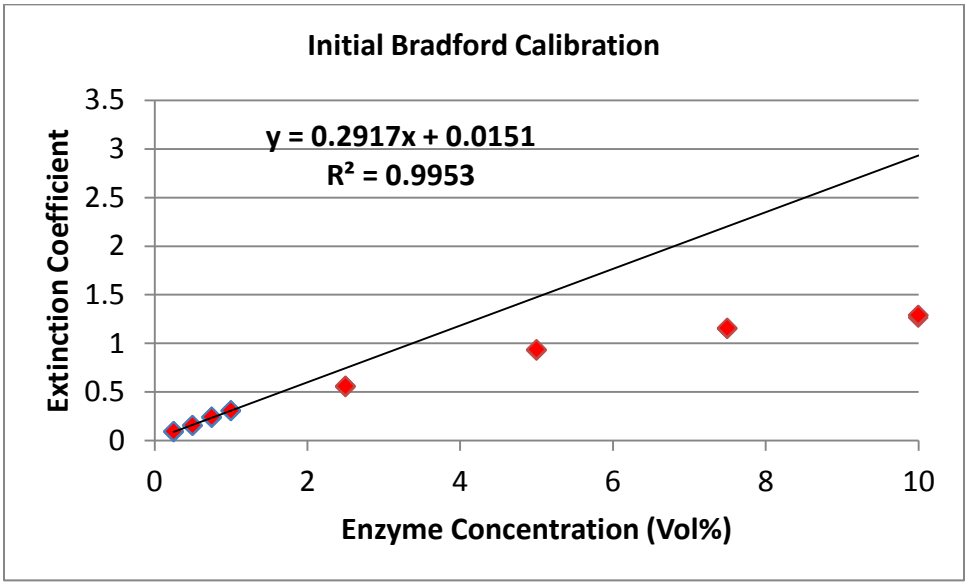

Fig. 16—Bradford calibration, 0.25 to 10 vol% enzyme.

From Fig. 15, the linear range of the Bradford method is found to be between 0.25 to 1 vol% enzyme for the range of enzyme concentration values tested by inspection. This part of the raw data is calibrated by itself in **Fig. 17**.

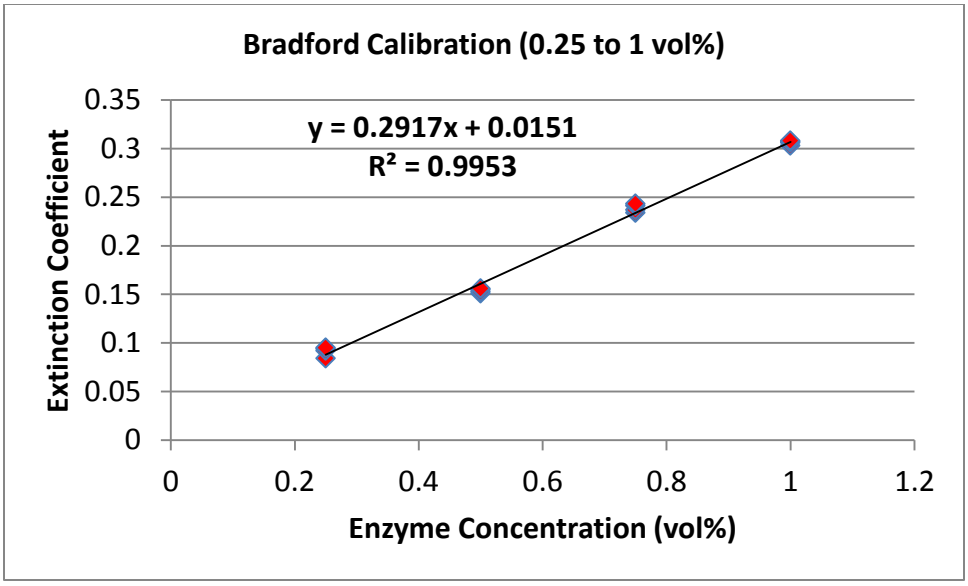

Fig. 17—Bradford calibration curve for determining enzyme concentration.

The Bradford calibration for the range of 0.25 and 1 vol% gives a linear relationship having a coefficient of correlation that is above 0.99 (R=0.99765) and is determined to be appropriate as a proper calibration for use in determining enzyme concentration in subsequent experiments. After this determination, the dependence is swapped and a more directly usable form of the correlation is found (**Fig. 18**). The trendline of the data gives a direct correlation between the optical density found by the spectrometer and solution enzyme concentration with the optical density as the independent variable. The trendline equation is valid only for resultant enzyme values of 0.25 to 1 vol% and any values which fall beyond this range requires dilution of an aliquot of the sample solution to this range in order to obtain a valid reading.

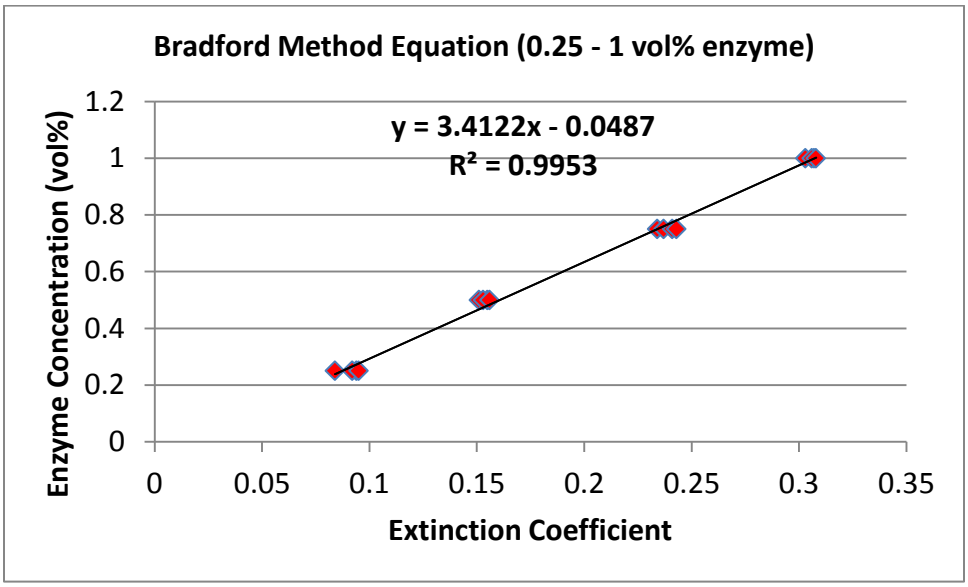

Fig. 18—Bradford method equation. Valid for resultant enzyme concentrations of between 0.25 and 1 vol%.

3.1.2 Starch-Iodine Assay

The calibration of the Starch-Iodine assay is performed on a weight by volume basis (w/v%) of the company provided starch. The starch was thermally activated before calibrating. The calibration conditions were tested according to **Table 10**. The results of these tests are presented in **Fig. 19, 20, 21 and 22** in numerical order by test number.

Table 10—Starch-Iodine calibration conditions confirmation tests

	Iodine Reagent Concentrations	
Optical Density Wavelength	5 mM I_2 and 5 mM KI	10 mM I_2 and 10 mM KI
580 nm	Test 1	Test 2
700 nm	Test 3	Test 4

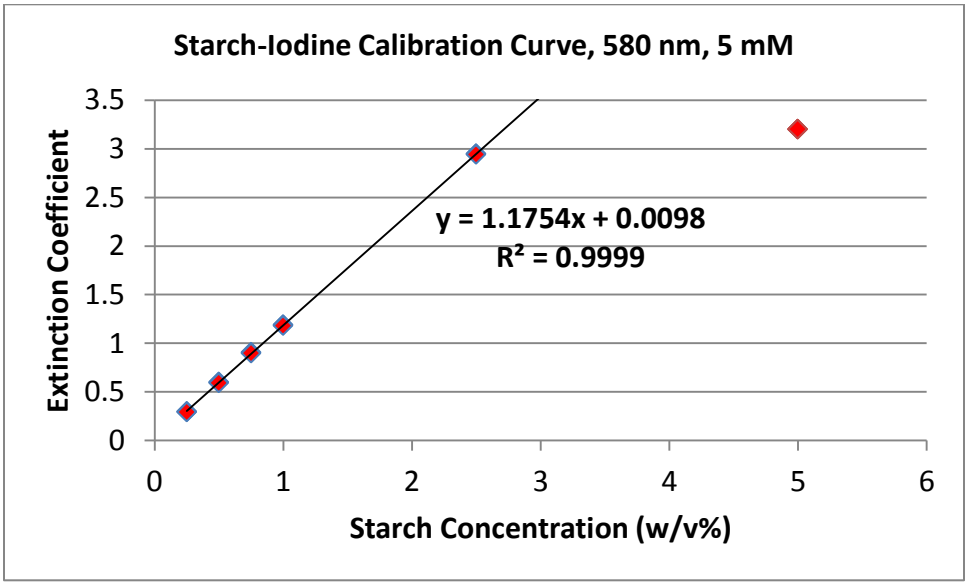

Fig. 19—Iodine calibration, test 1. Linear up to 2.5 w/v% starch solution.

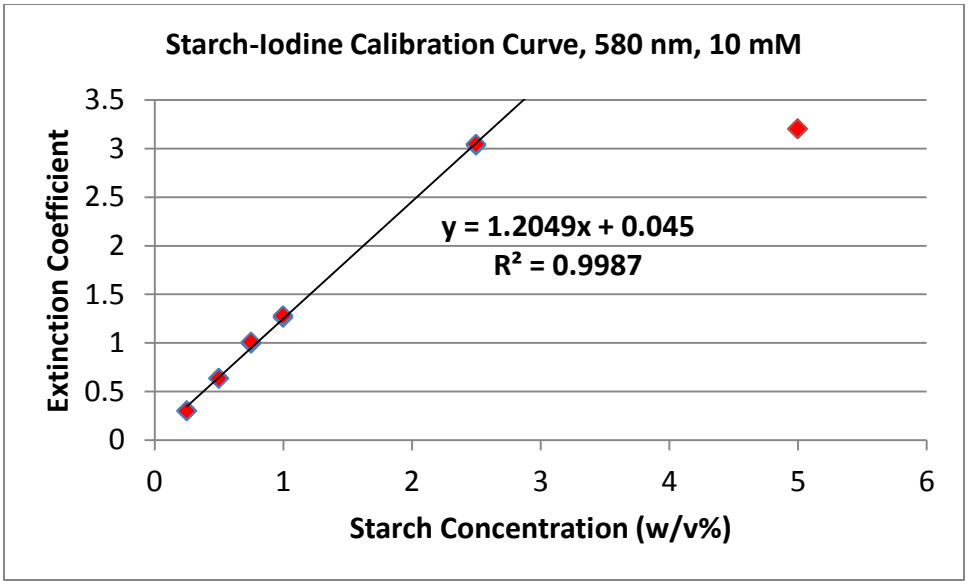

Fig. 20—Iodine calibration, test 2. Linear up to 2.5 w/v% starch solution.

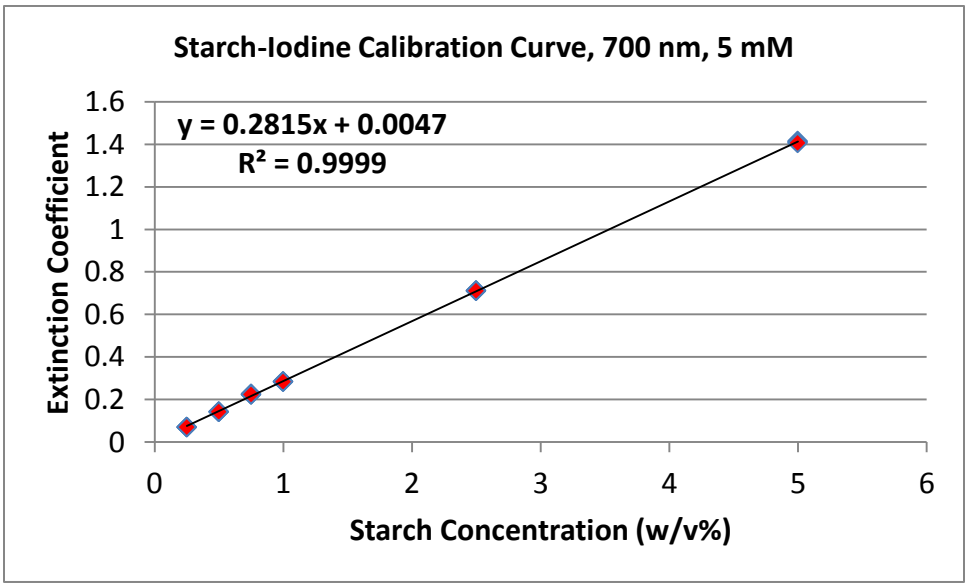

Fig. 21—Iodine calibration, test 3. Linear for entire tested range.

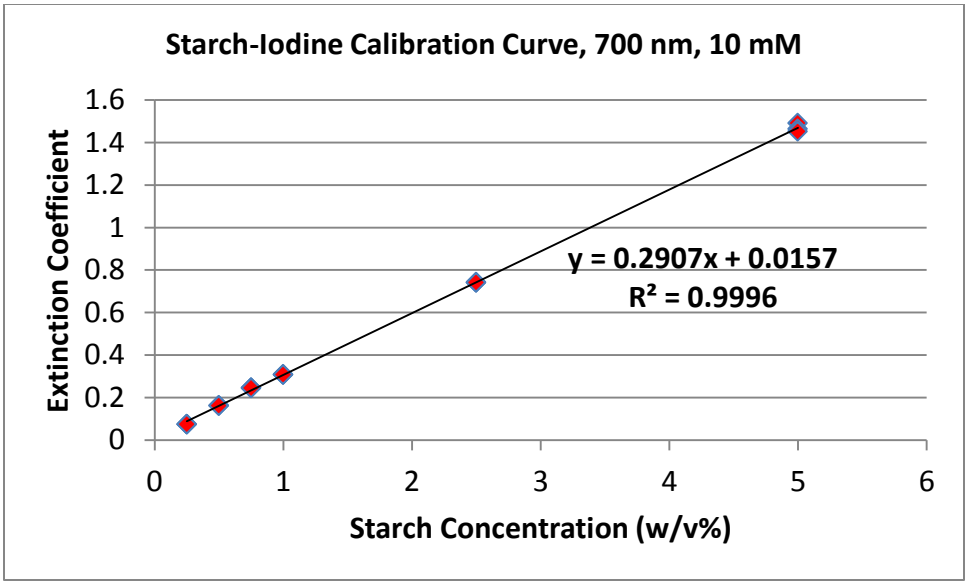

Fig. 22—Iodine calibration, test 4. Linear for entire tested range.

By direct comparison of the R^2-values, the conditions chosen for the Starch-Iodine assay calibration is chosen to be the conditions given in test 3, namely, 700 nm optical density wavelength and reagent concentrations of 5 mM for both components. This is given below in **Fig. 23**. The recipe for making the Iodine reagent may be found in the methods section.

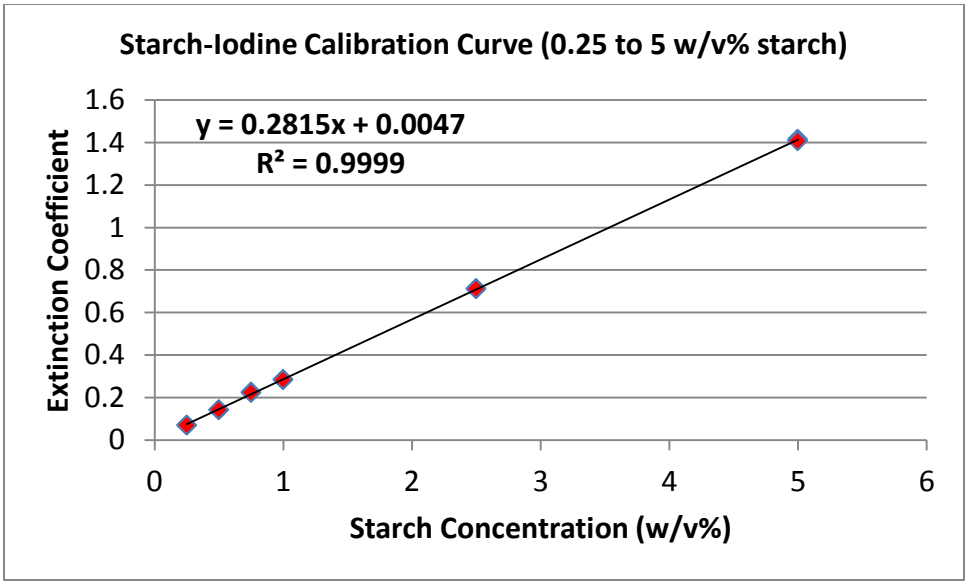

Fig. 23—Starch-Iodine calibration curve for determining starch concentration

The Starch-Iodine assay equation is generated in the same way as the Bradford method equation and is given in **Fig. 24**, the equation is valid for resultant starch concentration of 0.25 to 5 w/v% thermally activated, gelatinized starch.

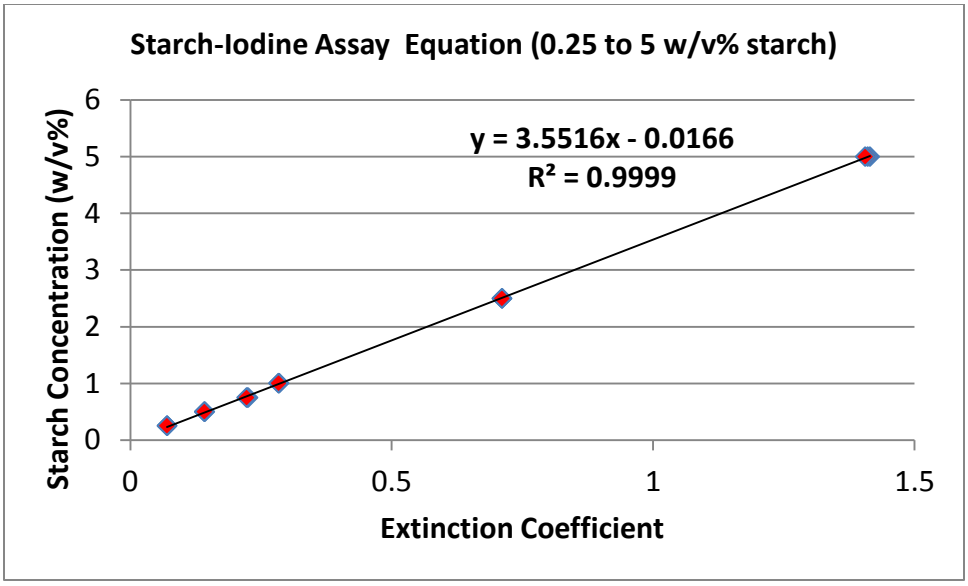

Fig. 24—Starch-Iodine assay equation. Valid for resultant starch concentrations of between 0.25 and 5 w/v%.

3.1.3 Anthrone Test

Anthrone method development which exactly matches the results of Al-Otaibi and Nasr-El-Din (2005) was not successful; therefore, this method is excluded from this study. The test conditions set forth in Morris (1948) did not give quantifiable measurements for the concentrations of carbohydrates found in Fig. 5 and as the exact test conditions for Al-Otaibi and Nasr-El-Din (2005) were not given consistency between literature results and results that could arise from this study cannot be established.

3.1.4 HPLC-RID

In general, prior to calibrations of the external standards (the analytes to be tested) the following items must be established beforehand:

1. Column Flow Conditions
2. Optimum Column and Detector Temperatures
3. Analysis Time
4. Analyte Focus
5. Internal Standard

At the time the experimental process began, the HPLC column was 3 years old (delivered in 2009) and confirmation of utility was necessary. Phenomenex's Column Performance Check Std. (P/N: ALO-3038) was ran to confirm utility (**Fig. 25**). The resultant chromatogram corresponded with the company provided chromatogram for proper column response and thus, the column was judged to be in good condition.

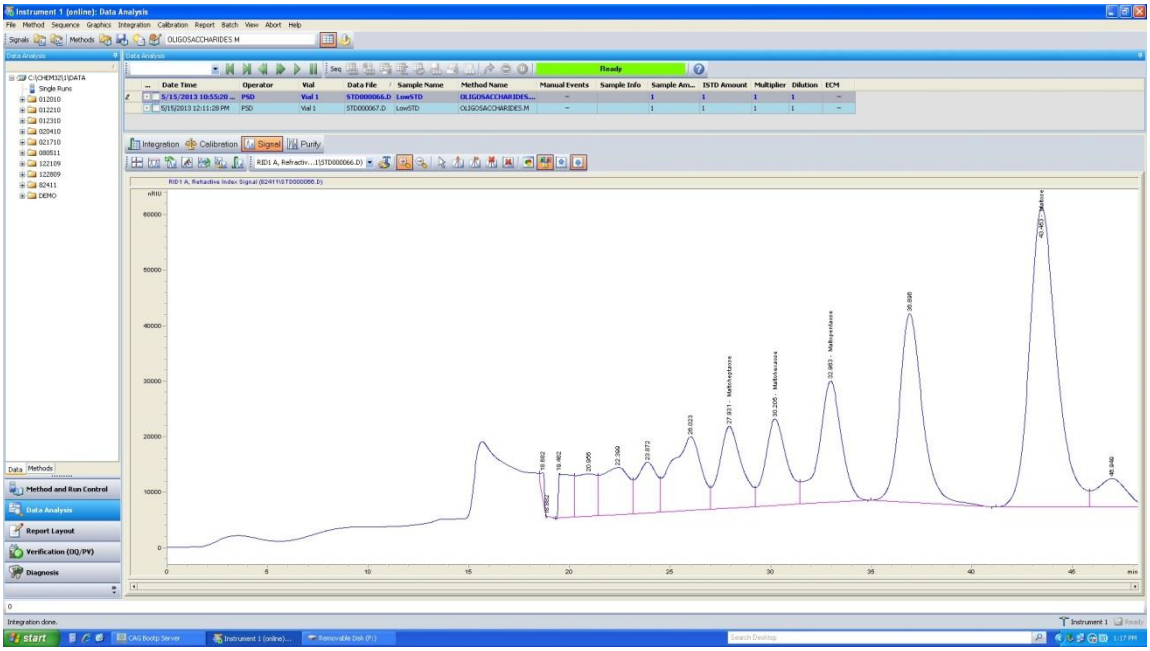

Fig. 25—HPLC column performance check run. Sample injector was fully flooded, meaning a sample of 20 µL of un-diluted performance check fluid was ran.

3.1.4.1 Column Flow Conditions

From column care guidelines (Phenomenex 2014a), the maximum flow rate is given as 0.3 mL/min and the maximum column backpressure is given as 300 psi (~20.7 bars) with the optimal backpressure given as 200 psi (~13.8 bars). Running the system at 0.3 mL/min gave a backpressure of 18-19 bars at room temperature. Therefore, to minimize sample axial mixing due to diffusion, 0.3 mL/min was chosen as the appropriate flowrate.

3.1.4.2 Optimum Column and Detector Temperatures

In general, the higher the temperature, the better the chromatogram resolution (Harris 2007). However, higher temperatures can degrade the intended analyte or cause sample reactions in the column, therefore an optimum must be found. Sucrose (a disaccharide made of glucose and fructose) was chosen as the test molecule for this test since it is very close chemically to the intended anticipated analytes (glucose and chains thereof) and it is the compound used by the RID as a reference compound. First, the maximum allowed column temperature of 80°C was tested (**Fig. 26**).

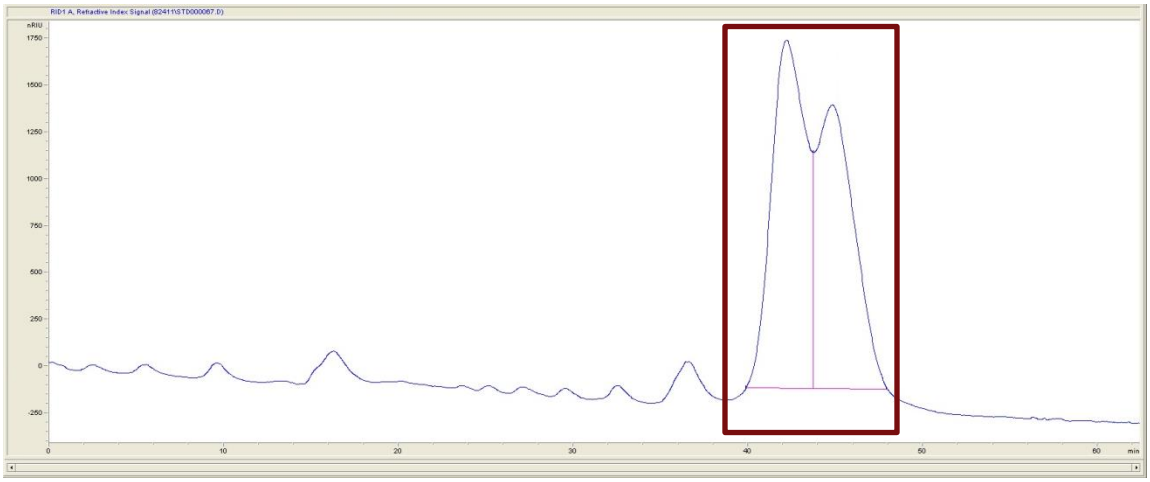

Fig. 26—Sucrose run for 1 mg/mL at 0.3 mL/min and 80°C. Pure compound gave two interlocked peaks, as displayed in the red box.

The chromatogram result shows two interlocked peaks for the pure compound. As the bond keeping the constituent glucose and fructose together is subject to heat

breaking, necessitates the need for testing at lower temperature to confirm HPLC-RID ability to resolve pure compound as single peak. Therefore, sucrose run at 30°C was conducted (**Fig. 27**)

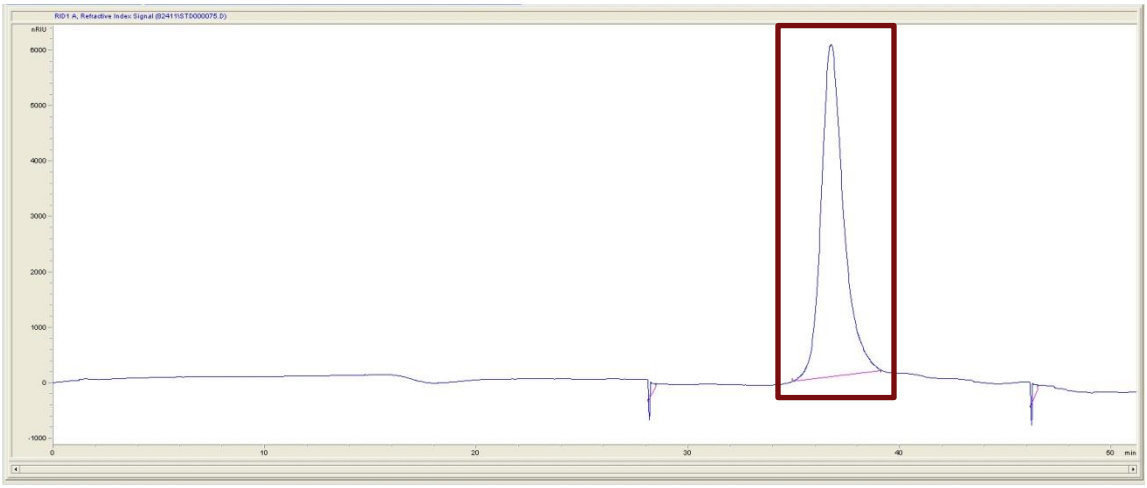

Fig. 27—Sucrose run for 1 mg/mL at 0.3 mL/min and 30°C. Single, strong peak is found at 36.8 min.

The characteristic elution time for sucrose is established at 36.8 min. The optimum temperature for the column is between 30 and 80°C. The next temperature to be tested was 60°C (**Fig. 28**).

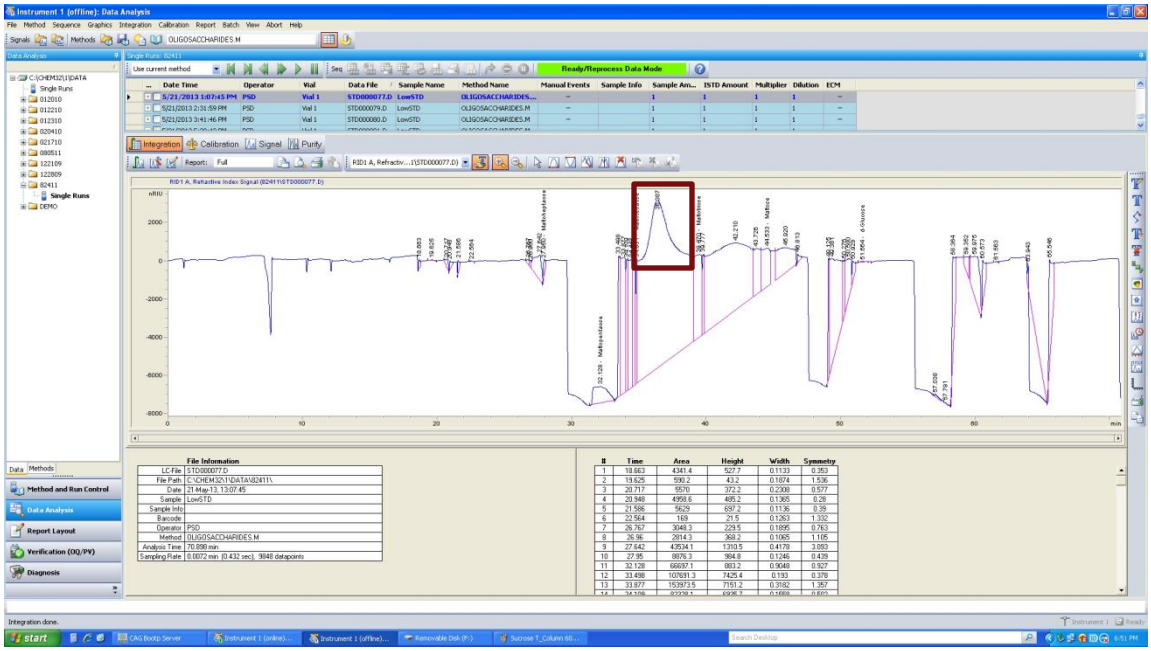

Fig. 28—Sucrose run for 1 mg/mL at 0.3 mL/min and 60°C. Single, strong peak is found at 36.8 min. A run with strong pressure pulsations (the periodic sharps shifts of the response down) is shown to illustrate what pressure pulsations look like. In the experience of the author, the HPLC is usually stable for 4 hours a day for runs.

Fig. 8 shows favorable results for 60°C in terms of peak resolution. This temperature was chosen as the proper column temperature to be used. This is due to the constraint from RID temperature selection. From private conversation with vendor company representative, it was suggested that the RID temperature be set no higher than 40°C. From literature sources (Chávez-Servín et al. 2004; Dee and Bell 2011; Paredes et al. 2008) the detector is set at either the same temperature or temperatures lower than the column. Therefore the RID temperature for operating conditions is set at 40°C.

3.1.4.3 Analysis Time

The run conditions were determined to be 60°C for the columns, 40°C for the RID, and 0.3 mL/min for the system flowrate. After this, products of an acid-starch reaction were ran in order to ensure that the column can resolve starch and starch degradation products properly (**Fig. 29, 30 and 31)**. The reaction involved 0.25 g of starch reacting with 100 mL of 4% HCl at 80°C. Running the product solution also allowed the determination of the range of elution times which are relevant for the starch-starch degradation product system.

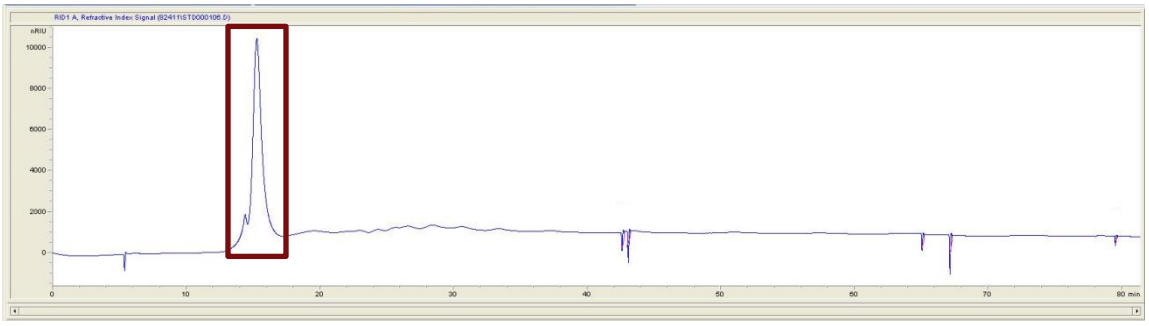

Fig. 29—4 hour starch-acid reaction.

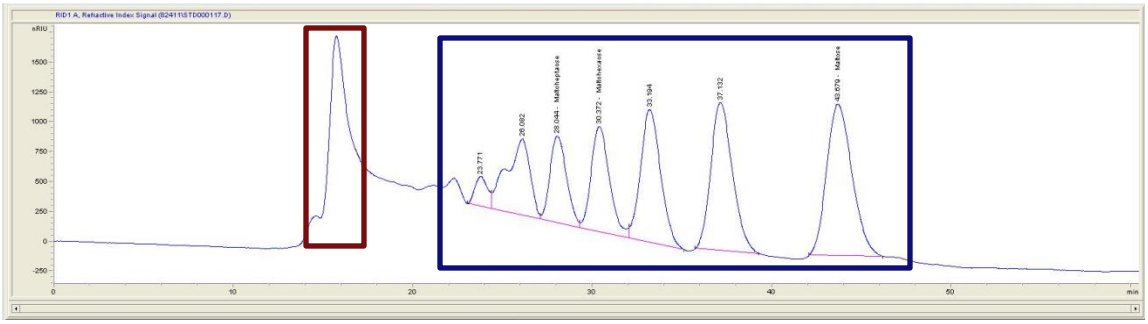

Fig. 30—16 hour starch-acid reaction.

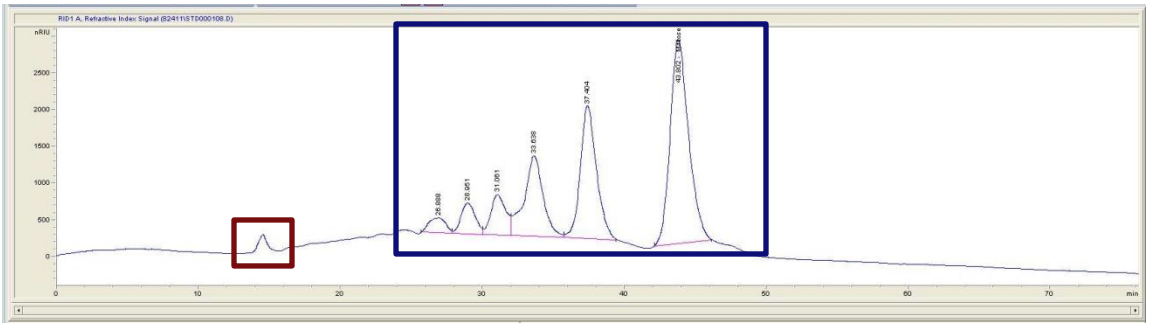

Fig. 31—24 hour starch-acid reaction.

The preceding figures establish that the acid reacts to degrade the starch and that the HPLC is able to capture the product distribution of the reaction. The figures strongly hint that the unreacted starch is eluting at around 15 min and the reaction products are eluted thereafter by decreasing degree of polymerization. Also, analysis time was determined to be 0 to 60 min for the starch reaction system.

3.1.4.4 Analyte Focus

Only maltose and glucose (Sigma-Aldrich P/N M5885-100G and Avantor P/N 4912-12 respectively) were available in HPLC grade (in the case of maltose, the compound was actually maltose monohydrate). Therefore, only the peaks of these two compounds are relevant analytically. These were ran separately (**Fig. 32 and 33**) and together (**Fig. 34**).

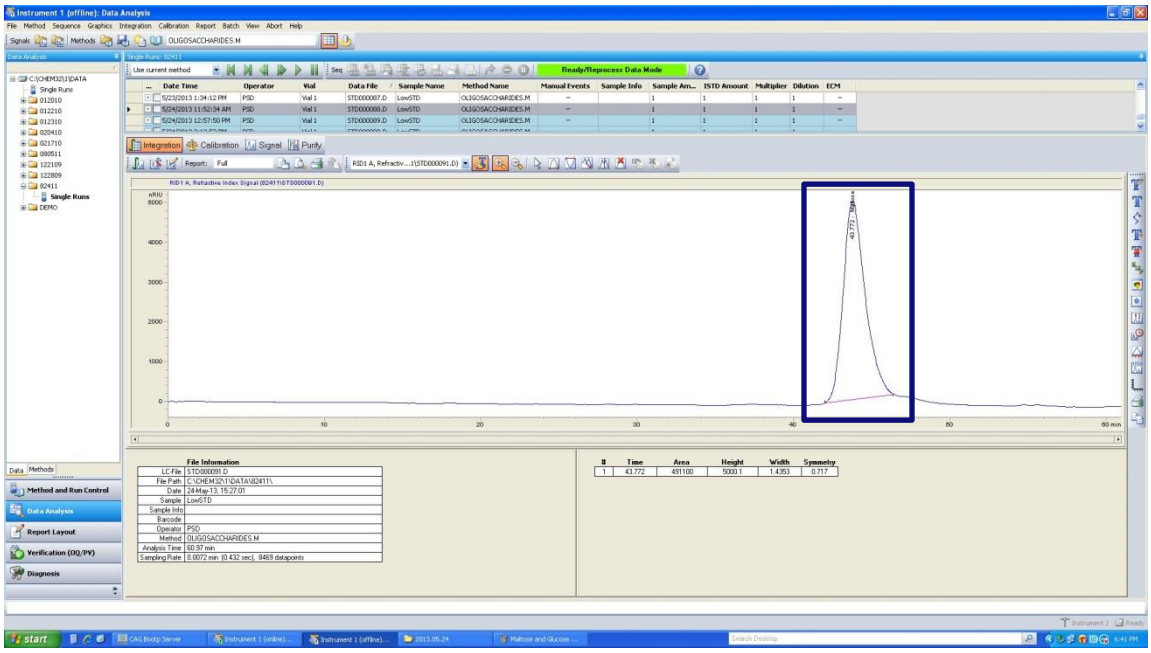

Fig. 32—Chromatogram of glucose by itself. For the operating conditions of this study, the glucose elutes at 43 min.

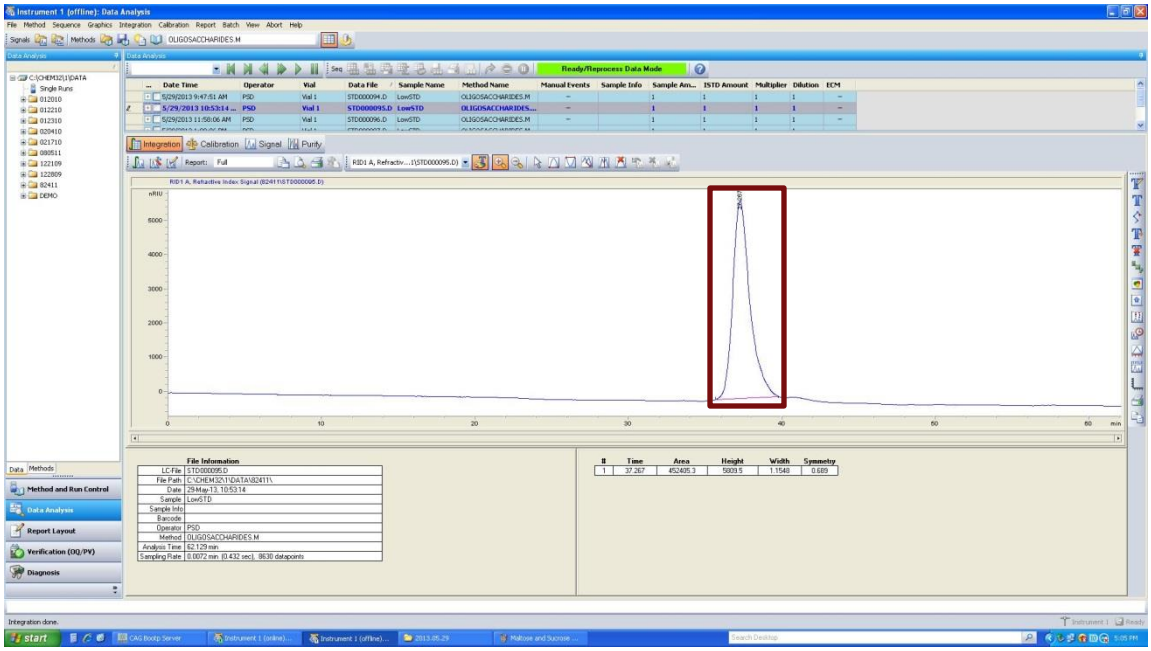

Fig. 33—Chromatogram of glucose by itself. For the operating conditions of this study, the glucose elutes at 37 min.

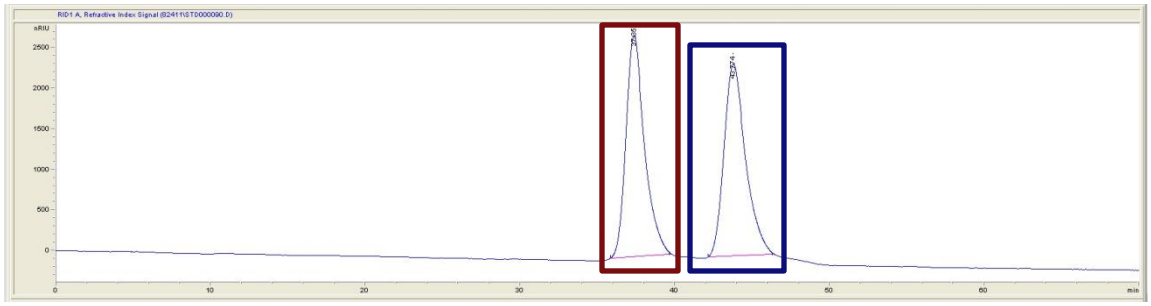

Fig. 34—Chromatogram of maltose and glucose. Shows strong separation between the two peaks.

3.1.4.5 Internal Standard

Due to the high sensitivity of the HPLC baseline to pressure fluctuations, real-time solution calibration of the machine is needed. Internal standards (IS) are spiked in known quantities within both the calibration solutions and the sample solutions. The ratio between the analyte response areas and IS response area is used to calculate sample quantity. Three possible candidates were tested,

a) Ethanol (**Fig. 35**)

b) β-Cyclodextrin (**Fig. 36**)

c) 1,6-Hexanediol (**Fig. 37**)

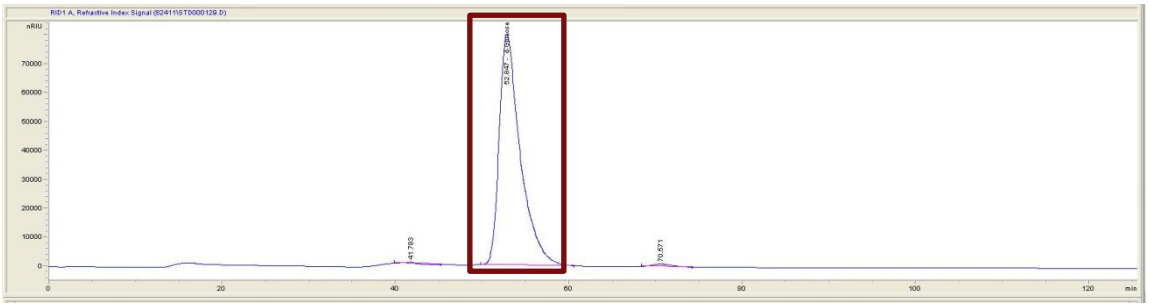

Fig. 35—Chromatogram of ethanol. Elution time 52 min. Within analysis time range. Inappropriate as IS.

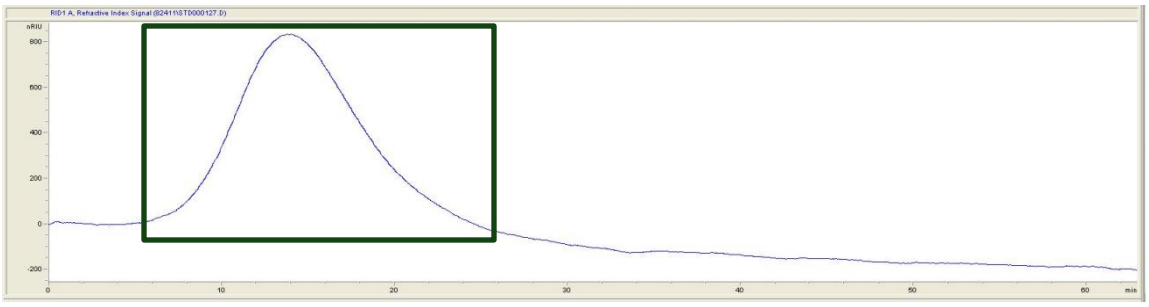

Fig. 36—Chromatogram of β-cyclodextrin. Elution time 8-25 min. Within analysis time range. Inappropriate as IS.

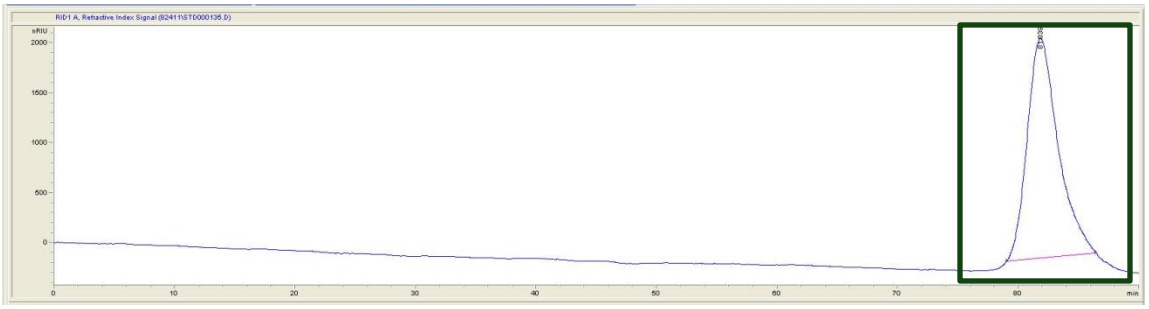

Fig. 37—Chromatogram of 1,6-hexanediol. Elution time 82 min. Beyond analytical range. Appropriate as IS.

3.1.4.6 HPLC Calibration Curves and Tables

With the internal standard established, calibration runs were performed, an example is given in **Fig. 38**. The resultant response areas were collected into calibration curves.

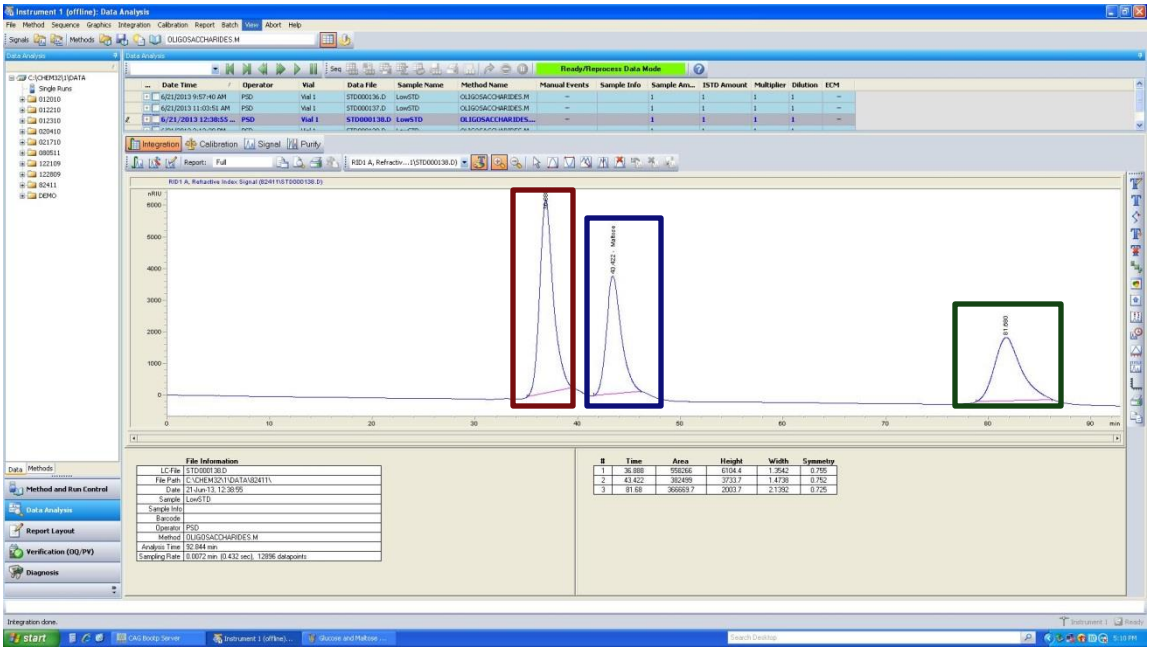

Fig. 38—Chromatogram of maltose, glucose, and 1,6-hexanediol.

The chromatograms of the purified compounds show that the HPLC gives appropriately high resolution peaks for the compounds of analytical significance to this study. It also shows that the internal standard is properly chosen barring any compounds in the enzyme-starch reaction which elutes at the same time.

The HPLC calibration curves for glucose and maltose are ratio calibrations. The analyte response area (maltose or glucose) is divided by the response area of the internal standard (1,6-hexanediol), this is the response ratio. For the calibration curve, the response ratio is related to the analyte concentration. For experimental tests, the response ratio is multiplied by the IS concentration before being multiplied by the scaling factor

in order to obtain the analyte concentration. The ratio calibration for maltose and glucose may be found in **Fig. 39** and **Fig. 40**, respectively.

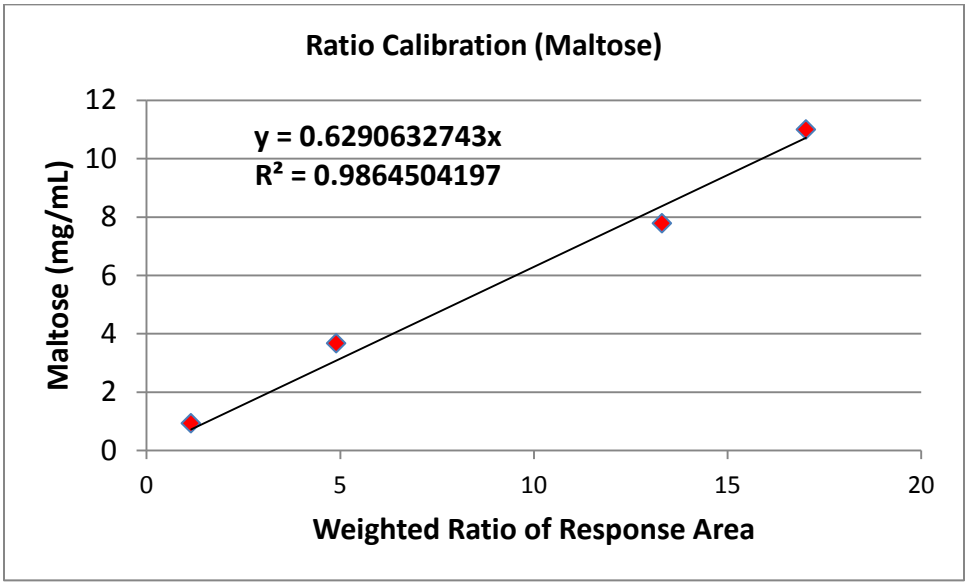

Fig. 39—HPLC ratio calibration for maltose.

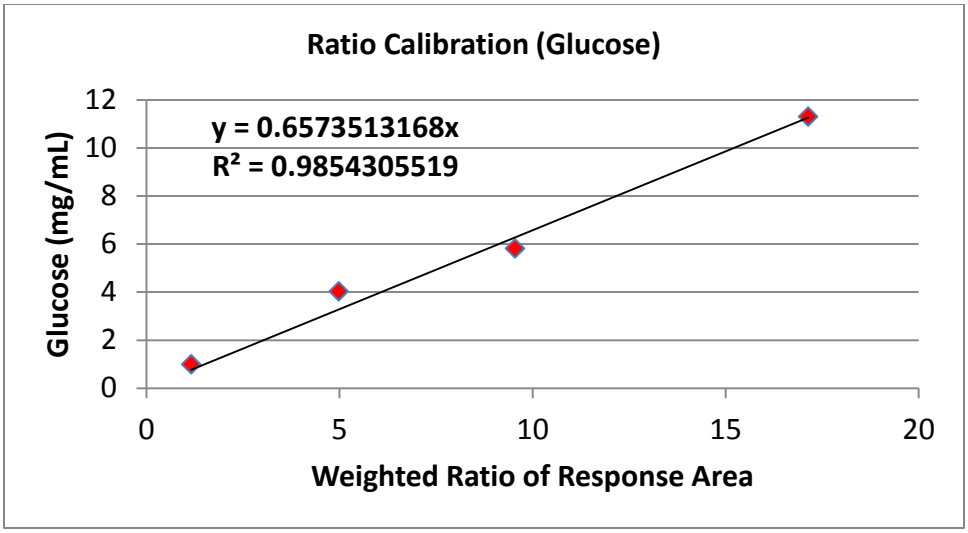

Fig. 40—HPLC ratio calibration for glucose.

3.2 Basis Case

The laboratory experiment performed in Al-Otaibi and Nasr-El-Din (2005) is used as the basis case for this study. The general steps are given below sequentially:

1. Filter cake buildup (200°F, 300 psi, 30 min)
2. Permeability test (200°F, 60 psi)
3. Pre-wash solution treatment (250°F, 300 psi, 2 hours)
4. Permeability test (200°F, 60 psi)
5. Enzyme Treatment (10 vol% solution, 250°F, 300 psi, 41 hours)
6. Permeability test (200°F, 60 psi)

3.2.1 Filter Cake Buildup

Water-based drilling mud was made according to the formulation given in the methods section. Filtration press was performed using the methods outlined in the same section. The result of the filter cake build up is outlined below in **Fig. 41**.

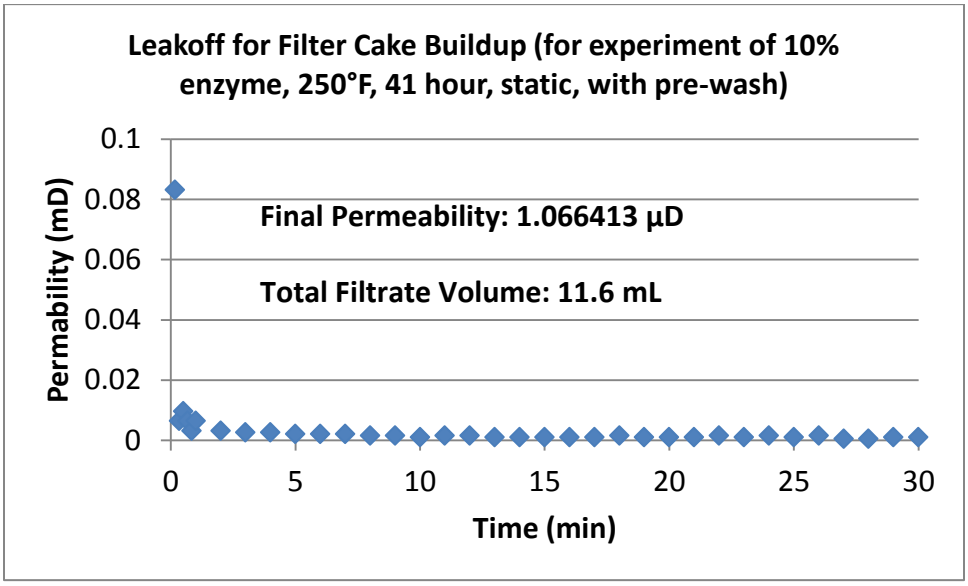

Fig. 41—Filter cake build up for basis case.

3.2.2 Retained Permeability

The permeability changes resulting from the application of the pre-wash solution (2 hours) and the enzyme solution (41 hours) is summarized below in **Table 11**.

Table 11—Retained permeability results for basis case.

Property	Value	Unit
Initial Core Permeability	92.06	mD
Filter Cake Permeability	1.07	μD
Post Pre-Wash Perm Test (a)	0.47	mD
Retained Permeability (a)	0.51%	1
Post Enzyme Treatment (b)	5.83	mD
Retained Permeability (b)	6.33%	1

With final retained permeability of merely 6.33%, this system for degrading filter cake is not as successful in contrast to the results reported in Al-Otaibi and Nasr-El-Din (2005), this could be due to the following factors:

1) The enzyme used in this study is made by DuPont specifically for use in the food industry
2) The exact surfactant used in the Al-Otaibi and Nasr-El-Din (2005) was not available, so instead, increase levels of mutual solvent was used to compensate
3) The enzyme formulation used in this study was exclusively starch degrading α-amylase. The formulation used in the paper reports enzymes for degradation both starch and xanthan gum.

The basis case was successful in the sense that it demonstrated degradation, albeit limited, of the formed filter cake with the enzyme solution system. This is sufficient for the purpose of this study, which limits itself to the elucidation of the enzyme-starch reaction system.

3.2.3 Analytical Chemistry

The results of the enzyme degradation as characterized by the Bradford and Starch-Iodine methods are given in **Fig. 42** and as characterized by HPLC-RID are given in **Fig. 43**.

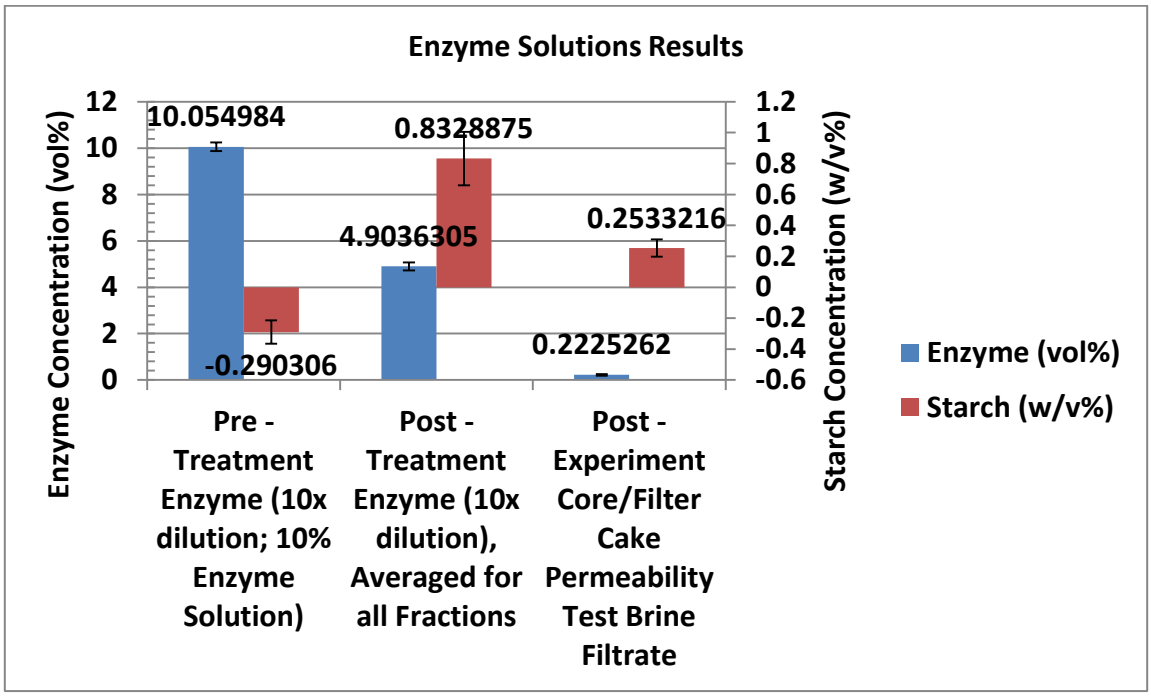

Fig. 42—Basis case Bradford and Starch-Iodine methods results. Figure shows a 51% collapse in the amount of enzyme in the treatment solution after the 41 hour treatment. It also shows a significant increase in the amount of gelatinized starch in the system.

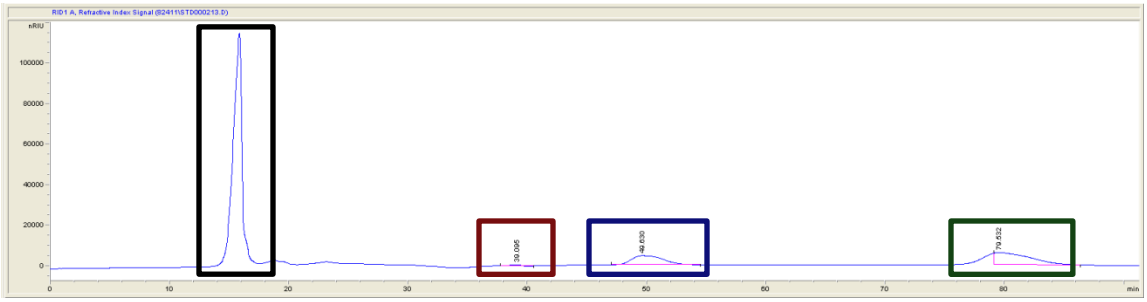

Fig. 43—Basis case HPLC results. Strong macromolecule peak, follow by maltose peak of concentration 0.045 mg/mL (0.00037 ppg), mystery peak at 51 min followed by IS peak.

3.2.4 Basis Case: Discussion

- Retained permeability is low, however, the purpose of this study is reagent fate focused, therefore, proceed to further experimentation without modification of the filter cake or reagents formulations
- Bradford results show enzyme is lost, this could be due to either degradation or deposition onto filter cake, characterization of enzyme is needed
- Starch-iodine results show increase in unreacted gelatinized starch in solution after 41 hours, this means that the reaction conditions can be further improved
- HPLC negligible maltose concentration confirms need to improve reaction conditions
- Identity of mystery peak must be known

3.3 Enzyme Characterization

3.3.1 Enzyme Thermal Stability

The experiments described in section 2.4.1 and laid out in Table 5 in the methods section were performed and the results given in **Fig. 44**.

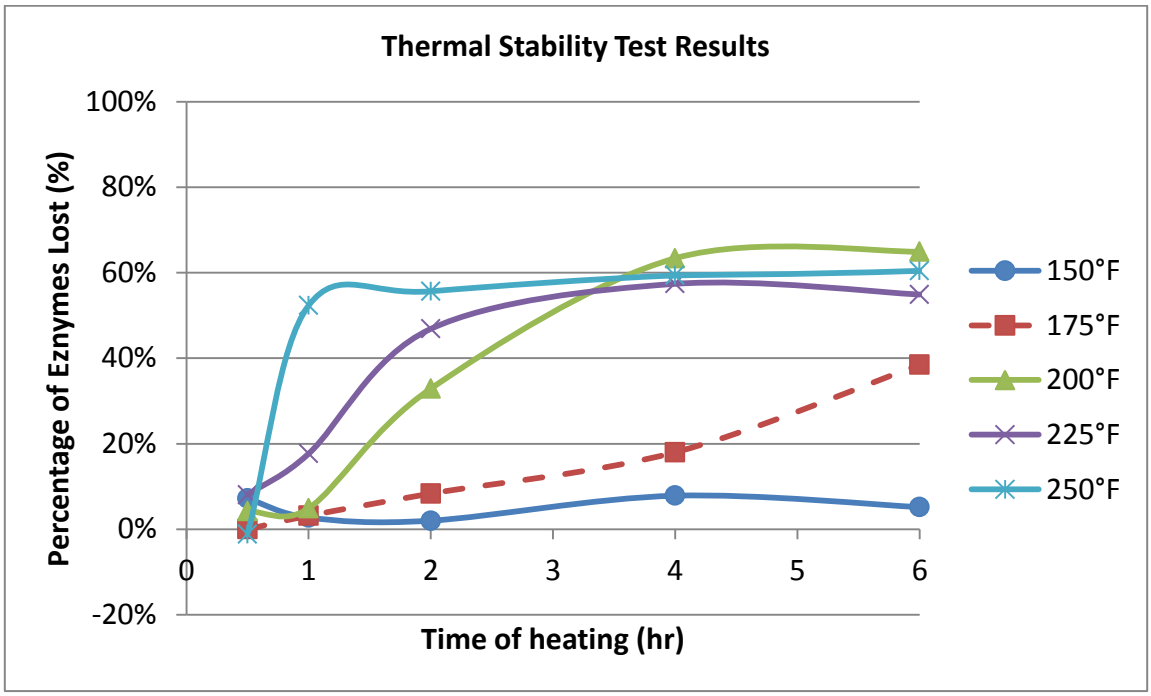

Fig. 44—Enzyme thermal stability results. Rate of enzyme denaturation increases as a function of temperature. For the time scales tested, temperatures around or above 200°F converges to 60% of original enzymes lost according to Bradford detection. For 175°F the enzyme solution shows significantly slower degradation. For the case of 150°F the solution shows no significant denaturation.

3.3.2 Enzyme Activity

The experiments described in section 2.4.2 and laid out Table 6 were carried out and the results are given in **Fig. 45**.

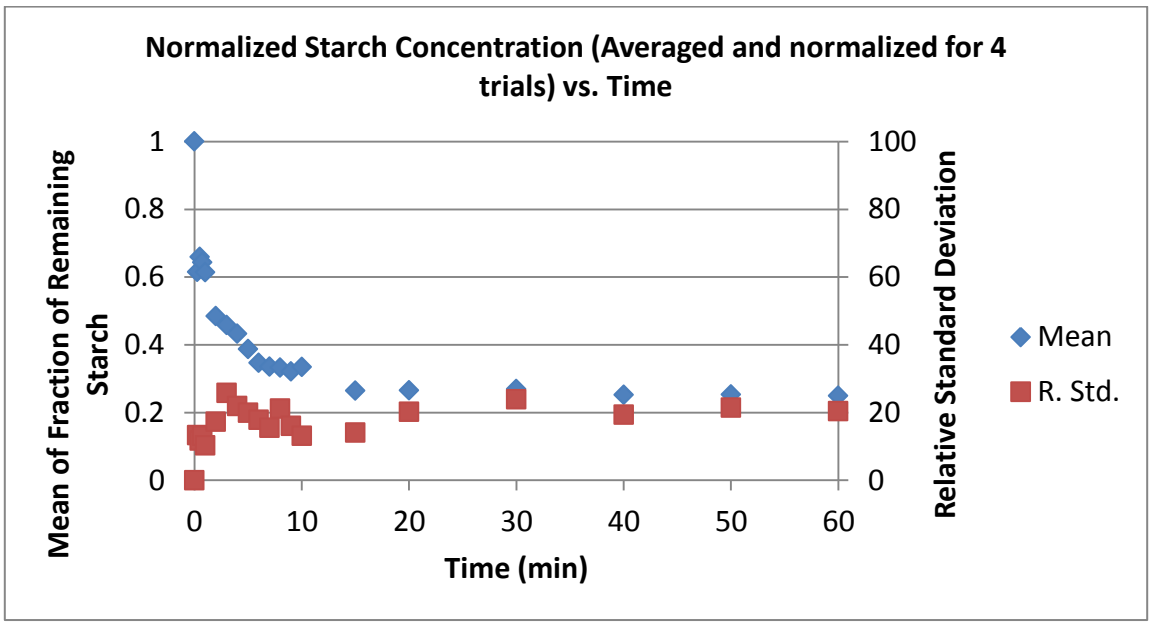

Fig. 45—Enzyme activity results. Reaction Condition: 5% starch degraded by 1% enzyme at 95°C at 1 atm. For well mixed solution, 66% of starch present degraded in 10 min of reaction.

3.3.3 Enzyme Solution: HPLC Run

A 5 vol% enzyme solution was filtered and ran on the HPLC in order to establish HPLC response to the enzyme solution itself (**Fig. 46**).

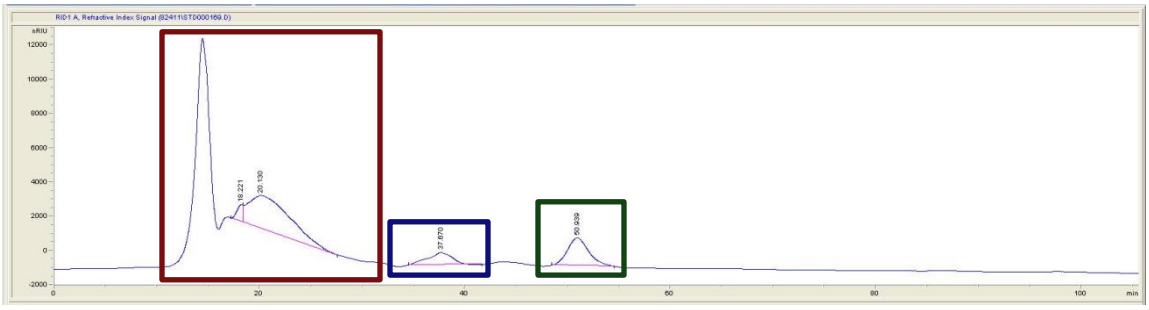

Fig. 46—Chromatogram of 5 vol% enzyme solution. Marcomolecule peak/region at the beginning, followed by maltose contaminant (0.235 mg/mL in 5 vol% enzyme solution), lastly mystery peak is found again at 51 min.

3.3.4 Enzyme Characterization: Discussion

- Enzyme thermal stability tests show that enzymatic degradation is a strong influence on Bradford response (strong evidence to suggest that enzyme fate in the basis case is retention in solution in a degraded state)
- Speed of enzyme reaction shown in the enzyme activity test coupled with enzyme solution stability at 150°F suggests that this is the best temperature for enzyme deployment
- Mystery peak identified as part of the enzyme solution itself. It does not interfere with the analytical peaks (glucose and maltose) and is therefore no longer relevant to this study.

3.4 Optimization of Enzyme Performance

The following conditions, given in **Table 12**, were tested. The results are given in the following sections. No pre-washes were applied in subsequent experiments because the focus of this study is enzyme focused. The initial tests with 150°F used a lower enzyme concentration of 5 wt% due to a desire on the part of the author to test assertions made by Hanssen et al. (1999) that a 5 wt% solution at 48 hours is sufficient to remove the filter cake completely.

Table 12—Enzyme performance optimization test conditions.

Test	Temperature	Pressure	Mixing	Pre-wash	Enzyme	Soaking Time
1	150°	300 psi	Static	No Pre-Wash	5 wt%	48 hours
2	150°	300 psi	Dynamic (150 rpm)	No Pre-Wash	5 wt%	48 hours
3	175°	300 psi	Static	No Pre-Wash	10 wt%	64 hours
4	250°	300 psi	Static	No Pre-Wash	10 wt%	41 hours

3.4.1 Test 1: 150°F, Static

Retained permeability result, Bradford and Starch-Iodine results and HPLC results are given below in **Table 13**, **Fig. 47** and **Fig. 48** respectively.

Table 13—Retained permeability result for 150°F, static.

150°F Static Test	Value	Unit
Initial Core Permeability	102	mD
Filter Cake Permeability	1.07	µD
Post Treatment Perm	2.24	µD
Retained Permeability	0.00224%	1

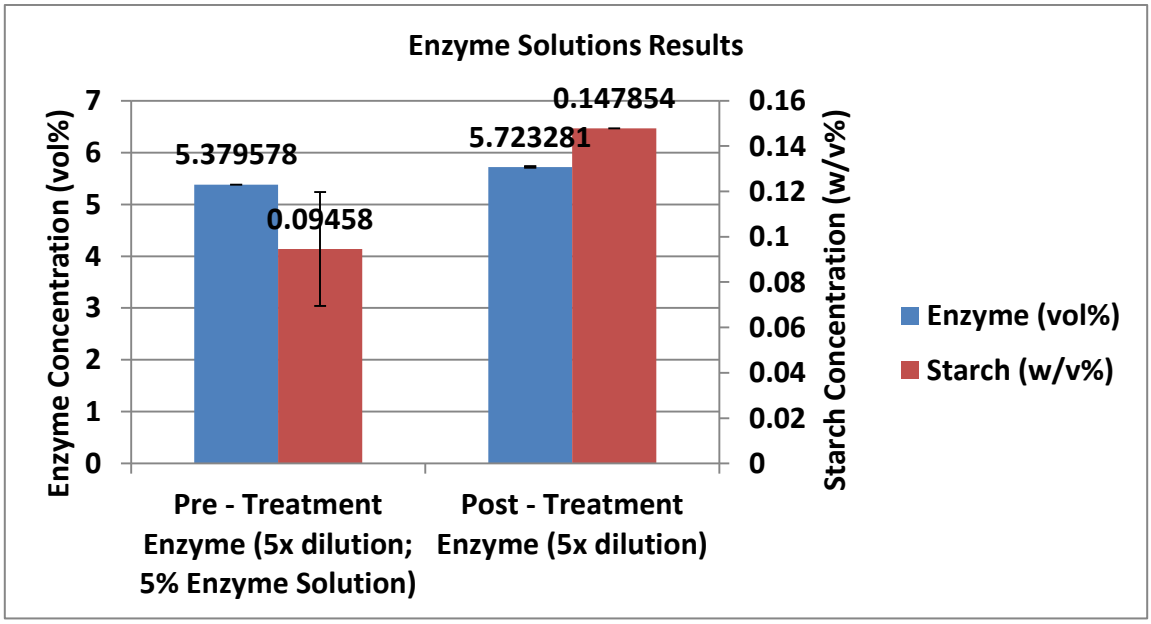

Fig. 47—Bradford and Starch-Iodine results for 150°F, static.

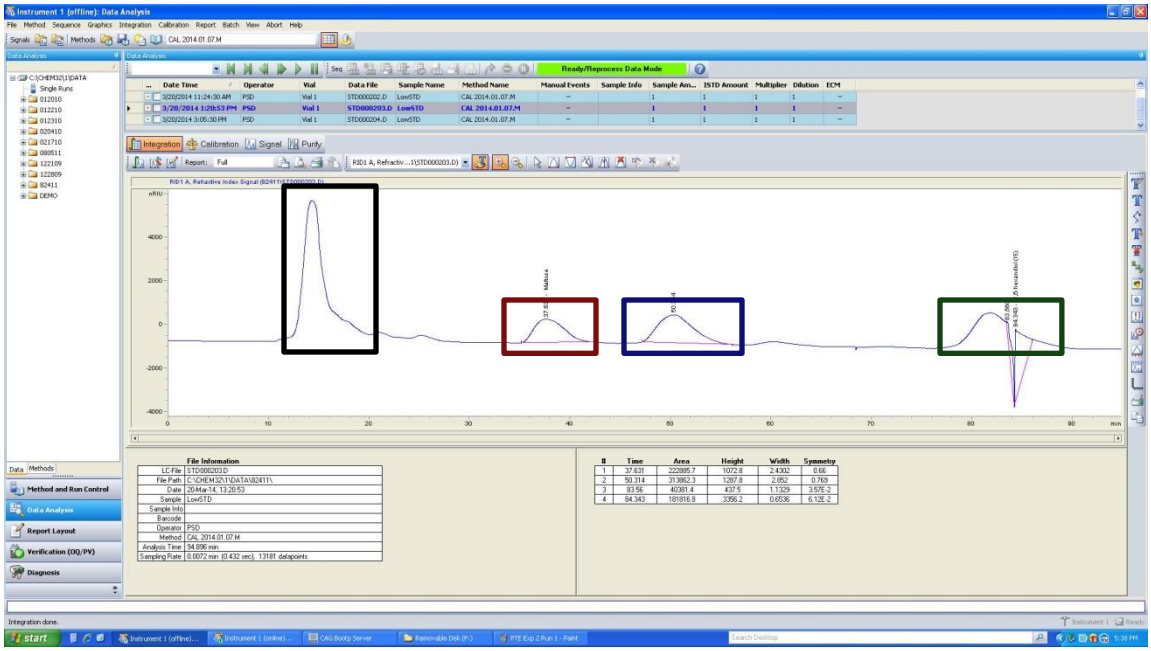

Fig. 48—HPLC results for 150°F, static. A fairly large macromolecule peak in front is followed by a maltose peak (at 0.63101 mg/mL). No glucose detected in the sample. The enzyme mystery peak is present at 51 min is followed predictably by the internal standard at 82 min.

3.4.1.1 Summary of 150°C, Static Results

- Filter cake showed no significant degradation from permeability test
- 100% of enzyme was retained within post-treatment solution
- HPLC results shows more extensive enzyme degradation of starch at 150°F (0.63 mg/mL) than at 250°F (0.045 mg/mL) or, a 14 fold increase in degradation
- Lack of filter cake removal and increase in residual starch concentration strongly suggest other forces at work

3.4.2 Test 2: 150°F, Dynamic

After the results of the 150°F, static test, due to the unfavorable retained permeability, the same test under dynamic conditions (150 rpm) was conducted. The retained permeability result as compared to the static result is given in **Table 14**. The HPLC result is summarized in **Table 15**.

Table 14—Comparison of 150°F static and dynamic filter press results. Dynamic result is 40 fold greater than static result. However, the small values involved suggest that more experimentation is necessary to confirm. Mass transfer effects is outside the scope of this study, therefore, further confirmation is not attempted in this study.

Perm Results	150°F Static Test	150°F Dynamic Test	Unit
Initial Core Permeability	102	152	mD
Filter Cake Permeability	1.07	0.533	µD
Post Treatment Perm	2.24	133	µD
Retained Permeability	0.00220%	0.0875%	1

Table 15—Comparison of 150°F static and dynamic HPLC results. Maltose concentration is roughly the same for both however; glucose appears in the dynamic case but not the static case. While this result is puzzling, due to the inability of the enzyme at this temperature to degrade filter cake, no further investigation into this matter was conducted.

HPLC	150°F Static Test	150°F Dynamic Test	Unit
Maltose	0.63101	0.605408	mg/mL
Glucose	0	0.196307	mg/mL

3.4.3 Test 3: 175°F, Static

The next degradation experiment increase temperature to 175°F, enzyme concentration to 10 vol% and soak time to 64 hours. The retained permeability results are given in **Table 16**.

Table 16—Retained permeability result for 175°F, static.

175°F, Static	Value	Unit
Initial Core Permeability	136.29	mD
Filter Cake Permeability	0.8	µD
Post Treatment Permeability	5.29	µD
Retained Permeability	0.0039%	1

3.4.4 Test 4: 250°F, Static

With the previous tests very unsuccessful when compared to the base case, the base case was repeated except without the pre-wash. The retained permeability result is shown in **Table 17**, the Bradford and Starch-Iodine results is shown in **Fig. 49** and the HPLC result is shown in **Fig. 50**.

Table 17—Retained permeability result for 250°F, static, no pre-wash. The retained permeability for this case is much more favorable compared to the previous optimization attempts

250°F, Static, No Pre-Wash	Value	Unit
Initial Core Permeability	92.06	mD
Filter Cake Permeability	0.53	µD
Post Treatment Permeability	0.85	mD
Retained Permeability	0.93%	%

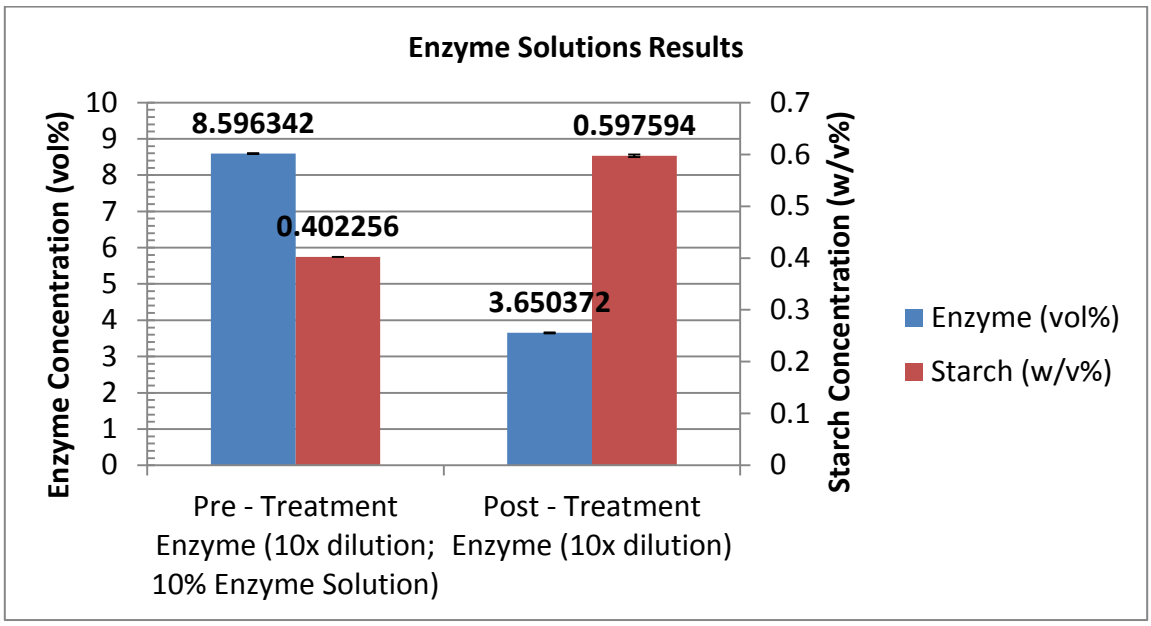

Fig. 49—Bradford and Starch-Iodine results for 250°F, static, no pre-wash. The enzyme concentration collapsed as expected and the starch concentration increased as expected, the same as the basis case.

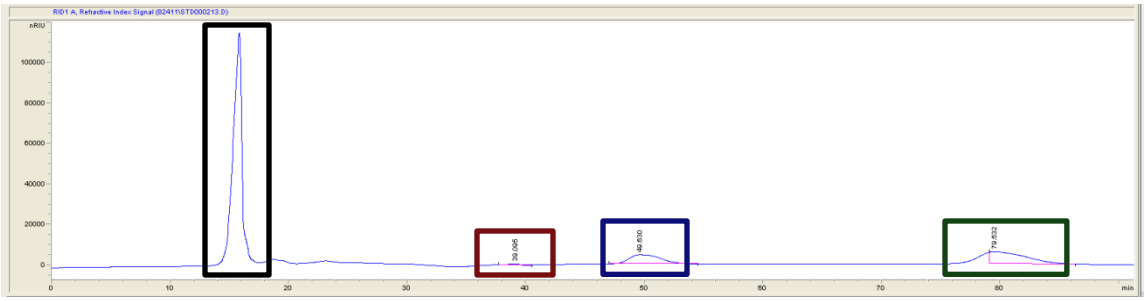

Fig. 50—HPLC result for 250°F, static, no pre-wash. The 0.055 mg/mL maltose in this sample is comparable to the 0.045 mg/mL sample for the basis case, thus suggesting same enzyme performance.

3.4.5 Optimization of Enzyme Performance: Discussion

- Greater enzyme performance achieved at lower temperature and lower concentration (14 fold increase) did not aid in greater filter cake degradation
- In all cases, residual starch concentration in solution actually increased.
- This is due to starch thermal activation (gelatinization). In Juansang et al. (2012) it was shown that efficient starch digestion by enzyme requires starch granule gelatinization. The speed of the gelatinization is highly dependent on temperature.
- The enzyme performance did increase, however, it was a small part in filter cake removal because the enzyme (in the case of this study) does not react with granular starch under static conditions, hence why reaction result increase by 40 fold under dynamic conditions

- The increase in starch concentration seen in solution is the starch granules being gelatinized, the maltose response provides the enzyme's effectiveness.

3.5 Degradation with Salt Solution

In order to test the hypothesis of starch thermal instability, the salt solution base of the enzyme solution (with no enzyme) was used to "degrade" the filter cake. The experimental setup is given in **Table 18**. The retained permeability result is given in **Table 19**.

Table 18—Degradation with salt solution experimental setup.

Test	Temperature	Pressure	Mixing	Pre-wash	Enzyme	Soaking Time
5	250°	300 psi	Static	No Pre-Wash	0 wt% (2% KCl)	41 hours

Table 19—Retained permeability result for 250°F, static, no enzyme. Retained permeability of 10% enzyme solution under same conditions was 0.93%, comparable to the 0.70% obtained here.

250°F, static, no enzyme	Value	Unit
Initial Core Permeability	94.79228	mD
Filter Cake Permeability	1.066413	µD
Post Treatment Perm	0.665187	mD
Retained Permeability	0.70%	1

4. CONCLUSIONS AND RECOMMENDATIONS

In accordance with the results of this study, the following conclusions may be drawn:

1) The combination of chemical methods presented in this work effectively characterizes the starch-α-amylase reaction system.
2) Without the exact core (ceramic disk, in the case of the literature source), drilling fluid formulation, reagents formulation and test conditions (equipment and pressures were different). The literature basis case was unable to be completely replicated; however, the basis case developed was still useful in further experimentation.
3) In all cases tested, there is strong evidence to suggest that the main mechanism for starch degradation is starch thermal instability and not enzymatic action.
4) In the case of the specific company enzyme provided:
    a. Fate of enzyme used to degrade starch in water-based filter cake is retention in reaction fluid for the basis case and for all other cases in which enzyme was added.
    b. Product distribution of enzyme-filter cake reaction is a limited oligosaccharide yield, primarily maltose

In accordance with the results the following recommendations may be given for future studies:

1) Fully explore the role that thermal activation of starch has in the enzyme-filter cake reaction
2) Add xanthan degrading enzyme to the enzyme solution system
3) Obtain other α-amylases from multiple companies and run the following tests with them:
    a. 250°F, static, with and without pre-wash
    b. 200°F, static, with and without pre-wash
    c. 150°F, static, with and without pre-wash

REFERENCES

Agilent. 2014a. Agilent 1100 Series Hplc Value System. http://www.chem.agilent.com/Library/usermanuals/Public/G1380-90000.pdf.

Agilent. 2014b. Agilent 1200 Series Refractive Index Detector. http://www.chem.agilent.com/Library/usermanuals/Public/G1362-90010_RID_User_ebook.pdf.

Al-Otaibi, M.B. and Nasr-El-Din, H.A. 2005. Chemical Treatments for Removal of Drill-in-Fluid Damage in Horizontal-Multilateral Gas Wells: Lab Studies and Case Histories. Presented at the SPE Europec/EAGE Annual Conference. SPE-94043-MS.

Beall, B.B., Brannon, H.D., Tjon-Joe-Pin, R.M. et al. 1996. Evaluation of a New Technique for Removing Horizontal Wellbore Damage Attributable to Drill-in Filter Cake. Presented at the SPE Annual Technical Conference and Exhibition. SPE-36429-MS.

Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* **72** (1–2): 248-254.

Brannon, H.D., Tjon-Joe-Pin, R.M., Carman, P.S. et al. 2003. Enzyme Breaker Technologies: A Decade of Improved Well Stimulation. Presented at the SPE Annual Technical Conference and Exhibition. SPE-84213-MS.

Chávez-Servín, J.L., Castellote, A.I., and López-Sabater, M.C. 2004. Analysis of Mono- and Disaccharides in Milk-Based Formulae by High-Performance Liquid Chromatography with Refractive Index Detection. *Journal of Chromatography A* **1043** (2): 211-215.

Compton, S.J. and Jones, C.G. 1985. Mechanism of Dye Response and Interference in the Bradford Protein Assay. *Analytical Biochemistry* **151** (2): 369-374.

Cuadros-Rodríguez, L., Bagur-González, M.G., Sánchez-Viñas, M. et al. 2007. Principles of Analytical Calibration/Quantification for the Separation Sciences. *Journal of Chromatography A* **1158** (1–2): 33-46.

Dee, S. and Bell, A.T. 2011. Effects of Reaction Conditions on the Acid-Catalyzed Hydrolysis of Miscanthus Dissolved in an Ionic Liquid. *Green Chemistry* **13** (6): 1467-1475.

Dharwadkar, P.S. 2011. Analyzing the Limits and Extent of Alpha-Amylase Catalyzed Removal of Starch-Based Filter Cake. Master's thesis, Texas A&M University.

Ding, Y., Longeron, D., Renard, G. et al. 2004. Modeling of Both near-Wellbore Damage and Natural Cleanup of Horizontal Wells Drilled with Water-Based Drilling Fluids. *SPE Journal* **9** (3): 252-264. SPE-82274-PA.

Dreywood, R. 1946. Qualitative Test for Carbohydrate Material. *Industrial & Engineering Chemistry Analytical Edition* **18** (8): 499-499.

Folin, O. and Ciocalteu, V. 1927. On Tyrosine and Tryptophane Determinations in Proteins. *Journal of Biological Chemistry* **73** (2): 627-650.

Gornall, A.G., Bardawill, C.J., and David, M.M. 1949. Determination of Serum Proteins by Means of the Biuret Reaction. *Journal of Biological Chemistry* **177** (2): 751-766.

Hanssen, J.E., Jiang, P., Pedersen, H.H. et al. 1999. New Enzyme Process for Downhole Cleanup of Reservoir Drilling Fluid Filtercake. Presented at the SPE International Symposium on Oilfield Chemistry. SPE-50709-MS.

Harris, D.C. 2007. *Quantitative Chemical Analysis*. 7th. W.H. Freeman and Company.

Juansang, J., Puttanlek, C., Rungsardthong, V. et al. 2012. Effect of Gelatinisation on Slowly Digestible Starch and Resistant Starch of Heat-Moisture Treated and Chemically Modified Canna Starches. *Food Chemistry* **131** (2): 500-507.

Kabir, M.A. and Gamwo, I.K. 2011. Filter Cake Formation on the Vertical Well at High Temperature and High Pressure: Computational Fluid Dynamics Modeling and Simulations. *Journal of Petroleum and Gas Engineering* **2** (7): 146-164.

Kresge, N., Simoni, R.D., and Hill, R.L. 2005. The Most Highly Cited Paper in Publishing History: Protein Determination by Oliver H. Lowry. *Journal of Biological Chemistry* **280** (28): e25.

Levitan, M.M., Clay, P.L., and Gilchrist, J.M. 2004. Do Your Horizontal Wells Deliver Their Expected Rates? *SPE Drilling & Completion* **19** (1): 40-45. 00087402.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. et al. 1951. Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193** (1): 265-275.

Morris, D.L. 1948. Quantitative Determination of Carbohydrates with Dreywood's Anthrone Reagent. *Science* **107** (2775): 254-255.

Nasr-El-Din, H.A., Al-Otaibi, M.B., Al-Qahtani, A.A. et al. 2007. An Effective Fluid Formulation to Remove Drilling-Fluid Mudcake in Horizontal and Multilateral Wells. *SPE Drilling & Completion* **22** (1): pp. 26-32. SPE-87960-PA.

Nasr-El-Din, H.A., Alhabib, N.S., Al-Mumen, A.A. et al. 2006. A New Effective Stimulation Treatment for Long Horizontal Wells Drilled in Carbonate Reservoirs. *SPE Production & Operations* **21** (3): pp. 330-338. SPE-86516-PA.

OFITE. 2014. Dynamic Hthp Filter Press. http://www.ofite.com/products/170-50.asp.

OFTIE. 2014. Pressurized Aging Cell, 500 Ml. http://www.ofite.com/products/175-30.asp.

Orbeco. 2014. http://www.orbeco.com/water/products/spectrophotometer-sp600.

Paredes, E., Maestre, S.E., Prats, M.S. et al. 2008. Single-Injection Calibration Approach for High-Performance Liquid Chromatography. *Journal of Chromatography A* **1185** (2): 178-184.

Phenomenex. 2014a. Hplc Column Care Guide. http://www.phenomenex.com/ViewDocument?id=hplc+column+care+guide.

Phenomenex. 2014b. Rezex Specifications and Operating Recommendations. http://www.phenomenex.com/ViewDocument?id=rezex+specifications+and+operating+recommendations.

Samuel, M., Mohsen, A.H.A., Ejan, A.B. et al. 2010. A Novel A-Amylase Enzyme Stabilizer for Applications at High Temperatures. *SPE Production & Operations* **25** (3): pp. 398-408. SPE-125024-PA.

Siddiqui, M.A.A. and Nasr-El-Din, H.A. 2005. Evaluation of Special Enzymes as a Means to Remove Formation Damage Induced by Drill-in Fluids in Horizontal Gas Wells in Tight Reservoirs. *SPE Production & Operations* **20** (3): 177-184. SPE-81455-PA.

Simonides, H., Schuringa, G., and Ghalambor, A. 2002. Role of Starch in Designing Nondamaging Completion and Drilling Fluids. Presented at the International Symposium and Exhibition on Formation Damage Control. SPE-73768-MS.

Smith, P.K., Krohn, R.I., Hermanson, G.T. et al. 1985. Measurement of Protein Using Bicinchoninic Acid. *Analytical Biochemistry* **150** (1): 76-85.

van der Maarel, M.J.E.C., van der Veen, B., Uitdehaag, J.C.M. et al. 2002. Properties and Applications of Starch-Converting Enzymes of the A-Amylase Family. *Journal of Biotechnology* **94** (2): 137-155.

Xiao, Z., Storms, R., and Tsang, A. 2006. A Quantitative Starch–Iodine Method for Measuring Alpha-Amylase and Glucoamylase Activities. *Analytical Biochemistry* **351** (1): 146-148.

Yang, B.H. and Flippen, M.C. 1997. Improved Flowback Analysis to Assess Polymer Damage. Presented at the SPE Production Operations Symposium. SPE-37444-MS.

Yoo, Y.J., Hong, J., and Hatch, R.T. 1987. Comparison of A-Amylase Activities from Different Assay Methods. *Biotechnology and Bioengineering* **30** (1): 147-151.