ANALYZING THE LIMITS AND EXTENT OF ALPHA-AMYLASE CATALYZED REMOVAL OF STARCH-BASED FILTER CAKE

A Thesis

by

PAVAN S. DHARWADKAR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Petroleum Engineering

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ABSTRACT

Analyzing the Limits and Extent of Alpha-Amylase Catalyzed Removal of Starch-Based Filter Cake. (December 2011)

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The ability of starch to impart functions including fluid-loss control, cuttings transport, and rheological characteristics to water-based drilling fluids has led to its widespread use in the oil industry. The filter cake deposited by these drilling fluids often employs sized solid particles and starch to inhibit fluid loss into the formation. This inherently causes damage to the formation by impairing the permeability and must be removed before production. An α-amylase enzyme treatment was found to provide an effective approach to degrading starch in filter cake.

In this work, an α-amylase enzyme treatment was analyzed by determining the extent of degradation of starch in filter cake using the iodine test, identifying degradants using high performance liquid chromatography, spectrophotometrically monitoring the concentration of enzyme, and measuring the cleanup efficiency of the enzyme treatment using a static filter press apparatus. The α-amylase enzyme used in this study was found to have a molecular weight under 30,000.

The activity of the α-amylase enzyme was found to be sensitive to pH and temperature. The α-amylase enzyme was found to denature at temperatures above 165°F

and reversibly deactivate at pH below 4. Optimal conditions for α-amylase activity were found to be 150°F and pH 6.5.

The enzyme treatment works by hydrolyzing the interior glycosidic bonds of amylose and amylopectin residues of starch, creating soluble poly- and oligosaccharides and glucose. The enzyme treatment did not dissolve the calcium carbonate sized solids and a 5 wt. % hydrochloric acid postflush was necessary. The cleanup efficiency of the enzyme at pH 6.5 and room temperature treatment in conjunction with the postflush in a static test was 73% at 10% v/v concentration. Degradants resulting from α-amylase were identified chromatographically. Enzyme concentration remained steady prior to and after treatment.

DEDICATION

This thesis is dedicated to my parents, who always believed in my abilities and me even against better judgment. Their encouragement and love were indispensable throughout this process.

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I would like to thank Dr. Hisham A. Nasr-El-Din for his unwavering support and indefatigable belief in me. The wisdom he has imparted to me will never be forgotten.

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INTRODUCTION

1.1 Drilling Fluids and Filter Cake

As openhole, high-angle, horizontal, and multilateral drilling techniques have increasingly been employed, drilling fluids have evolved to meet the needs of the industry with adjustments in their composition and properties. In order to maximize well productivity and return on investment, drilling fluids must be able to be cleaned up efficiently, simply, and without damaging the formation. The ultimate performance of a well is largely dependent on amount and significance of near-wellbore damage, most often caused during drilling operations (Battistel et al. 2011). This damaged zone is caused by reduced permeability around the wellbore due to contamination by mud particles and filtrate that result from several mechanisms including capillary phenomena, swelling and dispersion of indigenous clays by fluid filtrate, pore plugging by fluid particles, precipitation of soluble salts in filtrate and formation water, or aggregation of unconsolidated sands (Gray and Darley 1988).

The most reliable manner of preventing this sort of formation damage is to drill using an underbalanced fluid column to preclude solids or filtrate invasion.

However, underbalanced drilling is an inherently risky and difficult procedure that is not feasible in most wells. Therefore, in overbalanced drilling, drilling fluids must

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This thesis follows the style of *SPE Journal*.

be able to form a drilling low-permeability filter cake on the rock formation face that can minimize the invasion of fluid into the formation (Przepasniak and Clark 1998).

To form this filter cake, the drilling fluid must contain a sized distribution of bridging particles that serve to create a bridged zone in surface pores that prevents solids invasion into the formation, as well as fluid-loss control agents (Gray and Darley 1988). The first stage of the filter-cake formation is known as the *spurt,* wherein an internal cake is built after the rapid invasion of the rock matrix by solids present in the drilling fluid. This has also been identified as the first step of formation damage (Lomba et al. 2002). Then, an external cake consisting of fluid-loss control agents and other components of the drilling fluid is formed that is largely dependent on the hydrodynamic conditions of the fluid column (Guichard et al. 2007). Before a well is put into production, it is imperative that this filter-cake damage is removed to restore flow capacity around the wellbore and ensure unimpaired production.

During drilling operations, fluid rheology must be controlled over a range of temperatures and fluid loss must be minimized at all times (Simonides et al. 2002). Drilling fluids must also maintain good cuttings transport ability, cool the drill bit, maintain the stability of the borehole, reduce friction between the drillstring and the sides of the hole, and prevent the inflow of formation fluids (Gray and Darley 1988). In most circumstances, drilling fluids will be water-based or oil-based. Although water-based muds are preferred because of their cost and low environmental impact, oil-based muds have seen increased use in challenging environments including horizontal wells and reactive shales, especially at lower pressures (Al-Otaibi et al. 2007).

Despite their performance and potential, oil-based drilling fluids face significant drawbacks that are not shared by water-based fluids. Aside from being significantly more expensive than water-based drilling fluids, oil-based fluids are subject to stringent environmental regulations governing their use in many parts of the world and are difficult and costly to dispose of properly (Beihoffer et al. 1992). These difficulties have led the industry to look toward water-based fluids that can serve similar functions as oil-based fluid such as thermal stability and protection of the production zone.

Many of the features provided by oil-based drilling fluids can be reproduced by water-soluble polymers with the added bonus of cost-effectiveness and environmental sustainability. Polymers have been proven to exhibit excellent thermal stability in operations up to 400˚F, protect the production zone in combination with sized-salt systems, enhance economics and drillability as compared to oil-based fluids, and alleviate many environmental concerns (Strickland 1994).

New formulations of drill-in fluids have been designed and used to drill through productive zones while minimizing the damage caused by more traditional drilling fluids. Conventional drilling fluids are generally used to drill to the top of the pay zone, at which time they are switched with the cleaner drill-in fluids which allow for drilling through the pay with comparatively less damage (Beall et al. 1996). The requirements for these new fluids have evolved to necessitate the ability of drill-in fluid additives to serve multiple functions including fluid-loss control, cuttings transport, and viscosity modification while maintaining their stability in more extreme drilling environments (Simonides et al. 2002). Although the use of these cleaner drill-in fluids has been shown

to cause less damage than conventional fluids, they still cause filter-cake damage that must be remediated to realize the maximum productivity and return on investment of a well.

1.2 Polymer Additives in Water-Based Fluids

Natural, naturally modified, and synthetic polymer additives are used to provide properties such as filtration control, viscosity modification, flocculation, and shale stabilization for water-based drilling fluids (Carico and Bagshaw 1978). Along with these properties, they must also maintain their stability in accordance with the environment in which they are being employed. Various polymers perform different roles, although some can overlap, when added to water-based drilling fluids, with some used for filtration control and others used for viscosity modification. Polymers that largely serve as viscosifiers include xanthan gums, guar gums, cellulosic polymers, and acrylamides, while filtration control polymers include starch and acrylates (Carico and Bagshaw 1978).

Polymers have greatly variable properties that also affect their ultimate function in a water-based fluid application. Natural polymers have a variety of functions that depend on their molecular weight, source, and structure. Although starch is used largely as a fluid-loss control agent, other natural polymers such as xanthan gum, guar gum, and scleroglucan are instead used as viscosifiers and rheology control agents (Caenn and Chillingar 1996). Modified natural polymers such as carboxymethylcellulose are derived from natural polymers but have been processed to enhance properties such as viscosity

and solubility with the addition of carboxymethyl side chains onto a cellulose backbone. Such modifications allow polymers to be customized, thereby allowing them to have the flexibility to meet the requirements of the application (Carico and Bagshaw 1978). Synthetic polymers such as polyacrylates and polyacyrlamides can be made to the user's specifications wherein the molecular weight and degree of polymerization of the polymers affect the viscosity control properties of the drilling fluid.

As new drill-in fluids are formulated with the intent to create less formation damage, natural polymers such as starch have emerged to address the ever-increasing requirements sought by the industry. As a versatile and relatively inexpensive polymer, starch can provide for the requirements of rheology control and filter-cake deposition over a range of conditions. Starch has been used in the oil industry since the 1930s as a filtrate-control material and continues to provide that function today. The flexibility of this polymer is illustrated by the change in characteristics engendered by varying the degree of crosslinking of starch molecules by epichlorohydrin or by changes caused by carboxymethylation to improve heat and shear resistivity (Simonides et al. 2002). Higher molecular weight starch molecules provided by increased crosslinking exhibit better loss control and slower degradation rates at higher temperatures, as environmental conditions require (Ellis et al. 1998).

1.3 Starch

Starch is made up of amylose and amylopectin that are represented in a ratio of approximately 1:4, depending on the origin of the starch (Ellis et al. 1998). Amylose is

mainly linear in form along the α-(1,4)-glucosidic bond, whereas amylopectin has branches on between 1 in 20 to 25 units on the α-(1,6)-glucosidic bond (**Fig. 1**).

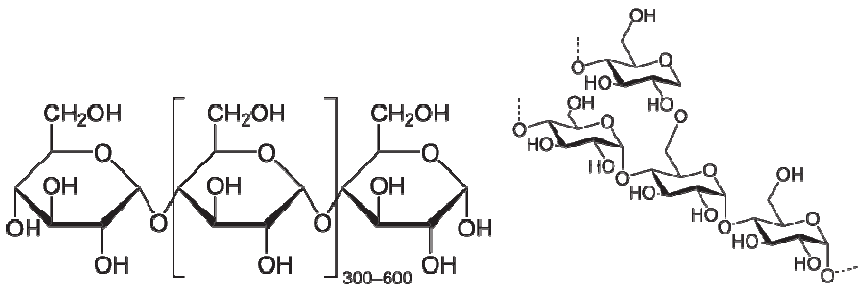

Fig. 1 — Amylose and Amylopectin Molecular Structures.

The molecular weights of amylose range between 10,000 and 100,000 while those of amylopectin fall between 100,000 and 1,000,000. The amylose-to-amylopectin ratio has an impact on properties such as the viscosity of starch solutions. Starch is fundamentally a polymeric condensation product of an anhydroglucose unit with degrees of polymerization ranging from 100 to 1,000 for amylose and 1,000 to 10,000 for amylopectin (Kearsley and Dziedzic 1995). Higher degrees of polymerization are indicative of longer starch chain length. The structural integrity of starch granules is due to intra- and intermolecular hydrogen bonding that is overcome by heating in water, which allows for the gelatinization of starch granules. Starch is sourced from a wide variety of crops including potatoes, corn, wheat, and taro. The properties of starch are heavily dependent on the species they are sourced from (Ellis et al. 1998). In the oil industry, potato starch is more often used, possibly because the relatively large grain size provides a preferred base compactibility (Al-Otaibi et al. 2004). Granule sizes for corn

starch range from 2 to 30 μm while potato starch granules exhibit sizes from 5 to 100 μm (van der Maarel et al. 2002).

Starch has been used extensively because of its fluid-loss control properties and strong economic justification (Gray and Darley 1988). Before starch can be incorporated into drilling fluids, it goes through a gelatinization process under heat and moisture that disrupts its original granular structure thereby affecting its rheological properties and making it cold-water soluble. This is accomplished by cooking the starch granules at temperatures between 140°F and 212°F to release the starch polymers, amylose, and amylopectin. The subsequent slurry is then dried and milled, at which point it can be used in drilling fluids (Ellis et al. 1998). Further modifications can be made chemically to change the properties of starch by substituting the hydroxyl functionalities of the glucose monomer with organic compounds or crosslinking with epichlorohydrin to increase its molecular weight and thereby change its physical properties (Ellis et al. 1998).

Simonides et al. (2002) showed that cross-linked carboxymethylated starch provided an increased viscosity and better temperature resistance. Unmodified pre-gelatinized starch exhibits thermal stability in the wellbore up to 200°F, at which time it breaks down and succumbs to degradation by heat and agitation. The degradation of the starch molecule is caused by an oxidation at the glycosidic linkage between two glucose units or a hydrolysis of an ether linkage at the glucose ring. This causes a depolymerization of the starch molecule that compromises the fluid-loss control function of the starch and also decreases its viscosity. Other mechanisms of starch degradation

include acid hydrolysis and enzyme catalyzed hydrolysis (Thomas 1982). Starch has been reported to co-precipitate with calcium when sodium hydroxide is added to drilling fluids with dissolved calcium salts (Gray and Darley 1988).

Compared to other polymers used as additives to water-based fluids, starch is the most effective at fluid-loss control. Audibert et al. (1999) ranked starch ahead of polyanionic cellulose and xanthan gum as fluid-loss control agents. Although polymers have been shown to invade the formation during the initial spurt phase, the concentration of polymers in filtrate levels off after the establishment of a filter cake and starch was seen to have relatively low polymer content within the filtrate (Loeber et al. 1996). Audibert et al. (1999) postulated that this is due to strong polymer/particle interactions when used with solid particles such as barite and carbonate particles. Adsorption tests on the particles confirmed that starch has the most interaction with solid particles, forming bridges within the pores of the cake as visualized by scanning electron microscopy (Plank and Gossen 1991). Starch that did invade the filtrate also exhibited lower molecular weights than polyanionic celluloses and xanthan gum thereby causing less damage to rock permeability (Simonides et al. 2002).

1.4 Starch-Based Filter Cake

The loss of fluid into the formation can cause a substantial reduction in oil and gas production rates, particularly in openhole completions. Organic polymers such as starch can be used as fluid-loss agents, which in combination with drilled solids can form a low-permeability filter cake on the rock formation face (Przepasniak and Clark

1998). Particulates such as calcium carbonate are often added to provide an additional plugging component to the fluid system. These can be specially sized to bridge the pore throats of the formation, but they use starch to stop the fluid flow into the matrix (Ross et al. 1999). Starch swells and gelatinizes rapidly and can reduce fluid loss by removing free water from the system and plugging the openings in the filter cake in conjunction with sized particles (Sah).

Filter-cake formation is subject to myriad factors and incompletely understood. Although a distribution of particles can be approximated when formulating a drilling fluid, the shear experience at the leakoff surface segregates smaller particles from the larger ones, effectively changing the distribution and causing smaller particles to reach the surface (Navarrete et al. 2000). Starch can then be used to fill the spaces left by the solid particles and, as mentioned previously, form bridges within the filter cake thereby decreasing its permeability.

However, starch and other polymers can themselves create formation damage. Large polymer fragments can block pores in the formation and reduce the permeability of formation rock. Polymeric additives in drilling fluids also induce strong effects in rock pores by plugging pores and elevating the residual water saturation because of the high molecular weight of the species (Audibert et al. 1999). Therefore, polymers that are used in drilling fluids must be easily removable from the wellbore. One of the advantages starch enjoys is the ease with which it can be removed.

1.5 Conventional Methods of Filter Cake Removal

Before production can begin, the filter cake deposited by the fluid needs to be removed by mechanical or chemical means. This is especially challenging in openhole completions along long, horizontal wells. Current methods of removing filter cake include strong acids, oxidizers, and enzymes. Aggressive treatments such as live acids and strong oxidizers will break down and remove filter cake at the first point of contact (Kyaw et al. 2010). This is a severe complication in horizontal completions where the treatment can leave large amounts of filter cake untreated and compromise production or even cause high-permeability streaks that can produce from gas or water zones. The fast reaction time and nonspecific nature of these treatments can cause production problems and corrosion damage and can shorten well life (Suhy and Harris 1998).

Field experiences have demonstrated that such treatments are not uniformly effective (Beall et al. 1996). In openhole horizontal intervals, acids and oxidative solutions have performed poorly. Extended openhole intervals complicate efforts to provide adequate contact between the filter cake and removal solution. A nonspecific reactive species such as an acid or oxidizer will preferentially leak off to the zone of highest permeability and can also produce wormholes that divert the solution away from the filter cake that is to be removed (Suhy and Harris 1998). Aside from the potential leakoff and poor contact with filter cake, acids and oxidizers may not effectively remove polymer from the wellbore, either. They will react at any active sites available on the polymer chain, not necessarily on the polymer backbone, and thereby leave significant unreacted polymer strands in place (O'Driscoll et al. 1998).

1.6 Starch-Degrading Enzymes

To provide a more specific, less damaging method to remove starch-based filter cake, enzymes have been employed as a replacement, precursor, and sporadic addition to acid treatments. Enzymes are complex proteins produced by living organisms that are made up of amino acid residues. The functionality of an enzyme is specified by its molecular weight, shape, and composition which determine the reaction it will catalyze, the substrate it is specific to, and the mechanism by which it catalyzes the reaction (van der Maarel et al. 2002). Enzymes catalyze chemical reactions by lowering their required activation energy, increasing the rate of reaction in some cases by as much as a billion times, but have the unique characteristic of not being consumed by the reaction they are catalyzing (Suhy and Harris 1998). Myriad types of enzymes exist that serve a wide variety of industrial purposes ranging from the paper industry to food processing. They have largely replaced chemical processes in several industries by offering greater yields, higher degrees of purity, and specific action under mild conditions (Kearsley and Dziedzic 1995).

Enzymatic methods of filter cake removal have several advantages over the more traditional methods of acids and oxidizers. Enzymatic processes present a more deliberate approach to the goal of removing filter cake. Although starch retains good degradability with acids and oxidizers, the specificity of the reaction an enzyme catalyzes allows for a more even and thorough removal, preventing issues of undisturbed filter cake and acid/oxidizer reaction with the formation or tubulars (Cobianco et al. 2007). The corrosion issues seen with the use of acids and oxidizers are also mitigated

by using the enzymes that exclusively catalyze the hydrolysis of specific linkages in polymers.

With the continued use of biopolymers such as starch to provide functions such as fluid-loss control and rheology modifications in drilling fluids, interest has grown in effective methods to remove the filter cakes they create. An entire industry has been established around the need to effectively process starch, with enzymatic processes largely supplanting acid hydrolysis methods (Ellis et al. 1998). These starch-converting enzymes are of four related, but distinct, groups: endoamylases, exoamylases, debranching enzymes, and transferases.

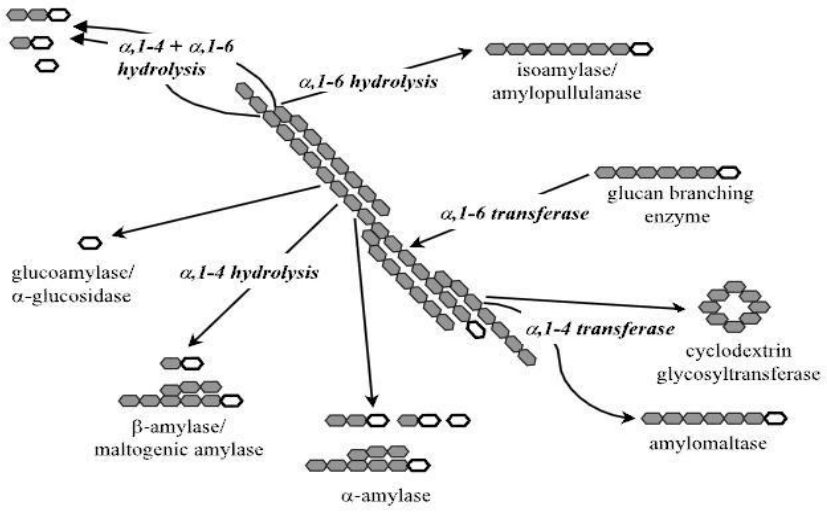

Fig. 2 — Starch-Degrading Enzymes. Amylases degrade starch in a variety of manners (van der Maarel et al. 2002).

As described in **Fig. 2**, these enzymes catalyze different actions on a starch substrate. Transferases such as amylomaltase have extremely limited hydrolytic activity, instead forming new α, 1-4 glycosidic bonds creating a linear product (van der Maarel et al. 2002). Debranching enzymes, such as isoamylase, hydrolyze α, 1-6 glycosidic bonds

in amylopectin, leaving linear molecules that can be further hydrolyzed by other, more commonly employed enzymes. Exoamylases such as β-amylase hydrolyze external glucose residues and are limited by the fact that they exclusively attack the nonreducing end of amylose chains (Kearsley and Dziedzic 1995). The enzymes that are most commonly applied to degrade starch are endoamylases, mainly α-amylase. Present in a wide variety of animal, plant, and microbial kingdoms, including human saliva, α-amylase is industrially produced from bacterial and fungal sources (Gupta et al. 2003). Endoamylases such as α-amylase randomly cleave α, 1-4 glycosidic bonds in the inner part of amylose and amylopectin chains, leaving linear and branched oligosaccharides of various chain lengths (Gupta et al. 2003; van der Maarel et al. 2002).

α-Amylase enzymes have molecular weights that range between 10,000 and 210,000, smaller than the macromolecules of starch they attack (Gupta et al. 2003). The structure of the α-amylase enzyme includes a $(\beta/\alpha)_8$ or triosephosphate isomerase (TIM) barrel that contains the catalytic site residues. This conserves the glycolytic activity that enables the α-amylase enzyme's catalysis of the hydrolytic degradation of starch (van der Maarel et al. 2002). The catalytic mechanism of the α-amylase enzyme is a double-displacement reaction that involves the formation of a covalent intermediate. Since the reaction catalyzed by the α-amylase does not consume it, the enzyme will still remain after the starch substrate has been degraded. This raises important questions about the potential of the enzymes themselves to cause damage in the formation once they have degraded starch. Even the products of the hydrolysis reactions have the potential to invade the formation and cause damage or alter solubility capacities of the smaller, more

soluble oligosaccharides. These degradation products must be explored and addressed to ensure the most effective cleanup.

The mechanistic effects and degradation products of enzymatic action are unclear in the context of the oil industry. The hydrolysis of insoluble starch substrates, which would be seen on starch-based filter cakes, consists of several steps including diffusion to the surface, adsorption, and catalysis (Leloup et al. 1991). The limiting factor of hydrolysis is the enzyme adsorption onto starch, although factors such as the potential for inhibition by metal ions or even hydrolysis products has been documented (Leloup et al. 1991). The hydrolysis of starch by α-amylase has limits that would leave a significant quantity of small oligosaccharides (Marchal 1999). Although α-amylase has proven to provide the best method of starch hydrolysis to remove filter cake, the factors that affect its performance and the post-treatment fate of the enzymes themselves and their degradation products are still ambiguous.

1.7 Current Knowledge

Enzyme use in oilfield applications began in earnest in the 1990s. They were often used to remove fracturing fluid residues (Samuel et al. 2010). Since then, their use has increased and enzymes have been seen as a viable alternative to traditional oxidative and chemical means of cleanup of fracturing fluids and drilling fluids. The factors that most influence enzyme activity have been identified as temperature, pH, salt concentration, the presence of calcium and chloride ions, solvents, and enzyme concentration (Samuel et al. 2010).

Studies conducted by Kameda et al. (2007) showed that temperature was the parameter with the greatest statistical significance for enzyme stability. They also saw variation in the stability of enzyme based on salt concentration, with higher NaCl concentrations moderating inactivation rate. Samuel et al. (2010) noted that the effect of chloride ions is more significant in animal α-amylases and not those more commonly used industrially that are derived from bacteria and fungi. They also found that at salt concentrations near zero, the side chains of amino acids on the enzyme will be attracted to each other and promote its denaturation, while high salt concentrations can cause enzyme precipitation by blocking normal interactions of the charged groups.

Gupta et al. (2003) showed that α-amylase is a metalloenzyme that contains at least one Ca^{2+} ion, which provides enzyme stability and high temperature tolerance. That Ca^{2+} ion can be removed by chelating agents such as EDTA, deactivating the enzyme and rendering it ineffective. However, reintroduction of Ca^{2+} ions or their replacement with other ions such as Sr^{2+}, Mg^{2+}, or Ba^{2+} can reactivate the enzyme. Depending on the source of the enzyme, the pH and temperature optima and stability will be different. At extremely high pH levels, Samuel et al. (2010) found that the enzymes can denature completely and irreversibly. Gupta et al. (2003) provided a comprehensive table that detailed the active temperature range for several α-amylases from various sources.

A mechanistic study on enzyme transport in filter cake by Hanssen et al. (1999) showed that enzyme accumulates in the filter cake until the concentration of enzyme builds up to the point that there are not enough substrate sites for the enzyme solution. At this point, the enzyme passes through into the filtrate. This action raises questions

about the potential for enzymes themselves to cause formation damage once degrading the starch in the filter cake. This work augmented the theory put forth by Leloup et al. (1991) that the adsorption of α-amylase onto a suitable substrate is the limiting factor for enzyme activity.

Various studies have detailed the hydrolysis products of α-amylase activity on starch. Bouchard et al. (1988) found that α-amylase-catalyzed starch hydrolysis yields a variety of hydrosylates largely dependent on reaction temperature, with higher concentrations of larger polysaccharides present at lower reaction temperatures and higher concentrations of monosaccharides and oligosaccharides at higher temperatures. Ivanova et al. (1991) showed that up to 80% of a starch substrate is hydrolyzed to oligosaccharides within one hour and illustrated the time-dependence of the degrees of polymerization of the reaction products with reaction time.

1.8 Research Objectives

The main objectives of this research were to evaluate the extent and limitations of α-amylase catalyzed degradation of filter cake formed by starch-based drilling fluids by employing methods that allowed me to:

1) Quantify the effectiveness of enzyme-catalyzed remediation of starch-based filter cake by measuring the cleanup efficiency of enzyme treatments.

2) Explore the effects of temperature and pH on α-amylase hydrolysis of starch and the subsequent effects on filter-cake remediation by measuring amylolytic activity.
3) Analyze the effectiveness of enzyme treatments on starch-based filter cake by using ultrafiltration centrifugation and high-performance liquid chromatography (HPLC) to obtain a distribution of degraded polymer fractions.
4) Monitor the fate of the enzyme after catalyzing the hydrolysis of starch in filter cake by spectrophotometrically measuring the concentration of enzyme in solution.

CHAPTER II

EXPERIMENTAL STUDIES

In order to develop a comprehensive picture of the α-amylase catalyzed degradation of starch in filter cake, several experimental methods were employed. Three main procedures were used extensively in this analysis. These included spectrophotometric measurement of protein concentration by near UV absorbance, analysis of degradation products by high performance liquid chromatography, and molecular weight cutoff analysis performed using ultrafiltration centrifuge tubes. Supporting experimentation included enzyme activity assays to determine the temperature and pH limitations and optima for subsequent analysis of degradation efficiency.

2.1 Experimental Design

The preliminary step of this process was performed in order to better inform the subsequent experimental procedures. An analysis of enzyme activity over a range of pH and temperatures was conducted using the 3,5-dinitrosalicylic acid method as described by in *Methods of Enzymology*, Colowick et al. (2009).

An investigation of the efficiency of filter-cake remediation was then conducted using a Fann static filter press apparatus. Enzyme treatment solutions were prepared based on the enzymatic activity assay in order to quantify the efficiency of filter-cake remediation over a range of conditions. The concentration of these enzyme treatment solutions was recorded spectrophotometrically for comparison with enzyme concentration in treated solutions. A qualitative measurement of the removal of starch from filter cake was performed using the iodine test. The method for the quantification of cleanup efficiency was adapted from Beall et al. (1996) It should be noted that treatment with enzyme fluid alone was ineffective and a mild acid wash was needed to remove calcium carbonate from the filter cake. This will be discussed further in the discussion chapter.

A starch-based drilling fluid was used to produce the filter cake and was prepared according to the formulation described in **Table 1**:

Table 1 – Formulation of Drilling Fluid		
Material	Amount	Function
Distilled Water	308 g	Base
Potassium Chloride	97.6 g	Shale Inhibition
Calcium Carbonate	13.0 g	Weighting Material
Pre-gelatinized Starch	6.0 g	Fluid Loss Control
Xanthan Polymer	1.5 g	Viscosifier
Potassium Hydroxide	0.3 g	pH Control
Sodium Sulfide	0.25 g	Oxygen Scavenger
Defoamer	0.33 mL	Defoamer

The drilling fluid itself and filtrate collected from this drilling fluid on the static filter press was analyzed to provide a baseline against which the enzyme solutions and enzyme treated samples can be compared.

Samples were analyzed using ultrafiltration centrifugation and high performance liquid chromatography. The control samples helped ascertain the initial polymer composition that was expected prior to treatment. The ultrafiltration centrifugation provided molecular weight cutoff filters to gauge the initial degree of hydrolysis of starch. The samples were then run through a high performance liquid chromatography unit equipped with an oligosaccharide column to identify degradation products of the hydrolysis of starch by α-amylase.

2.2 Methods

2.2.1 Colorimetric Enzyme Activity Assay

The 3,5-dinitrosalicylic acid method will be used to determine the activity of α-amylase under conditions an enzyme treatment may be subjected to in the oilfield. Enzymatic activity is dependent on several factors, the most important of which are temperature and concentration. This analysis will determine the optimum temperature and pH for enzyme activity. The effect of enzyme concentration will also be investigated. As a colorimetric procedure, this analysis required the use of a spectrophotometer. A ThermoScientific Genesys 10UV Spectrophotometer was used and is shown in **Fig. 3**:

Fig. 3 — ThermoScientific UV-Vis Spectrophotometer.

Enzymatic activity was measured at the optimum pH of α-amylase activity at 6.5 (Somogyi 1960) at a range of temperatures to determine the temperature at which α-amylase has the greatest activity. Subsequent analyses varied the pH of the enzyme treatment solution to determine the optimal pH.

This analysis works on the principle that reducing groups (maltose) released by α-amylase hydrolysis of starch are measured by the reduction of 3,5-dinitrosalicylic acid. One unit of enzyme activity is defined by the liberation of 1.0 mg of maltose from starch in 3 minutes. This method was adapted from Colowick et al. (2009) *Methods in Enzymology.*

- Prepare a standard curve using maltose standard solution at 0.2% w/v with color reagent solution of sodium potassium tartrate and 96 mM 3,5-dinitrosalicylic acid.
- Add 1% w/v starch solution into a sample test tube and 1 mL of 20 mM sodium phosphate buffer into a blank test tube, respectively.
- Mix by swirling and equilibrate to test temperatures.
- Add to sample test tube 1 mL of enzyme solution, mix by swirling, and incubate for exactly three minutes at test temperature.
- Add 1 mL of color reagent solution to sample test tube and blank test tube and 1 mL of enzyme solution to blank test tube.
- Cap both tubes and place in a boiling water bath for 15 minutes, cool on ice to room temperature and add 9 mL of deionized water to the sample

and blank test tubes.

- Record the A_{540} for both the sample and blank test tubes using the ThermoScientific spectrophotometer.
- Determine the enzyme activity by calculating the amount of maltose released using the standard curve.

The data garnered from the colorimetric enzyme activity assay was then used to select candidates for further investigation using other analytic techniques.

2.2.2 Molecular Weight Cutoff Analysis Using Ultrafiltration Centrifugation

Starch, as discussed in the introduction, is made up of amylose and amylopectin, high molecular weight polymers of glucose. The molecular weight of these components range between 10,000 and 100,000 MW for amylose and between 100,000 and 1,000,000 MW for amylopectin. α-amylase enzymes have been shown to have molecular weighs between 10,000 and 40,000. Molecular weight cutoff analysis was used to obtain molecular weight distribution information about larger components of the treatment solutions and the treated products while also providing filtration to inhibit damage to the column subsequently used in high-performance liquid chromatography. A modification of the method described in Brannon and Pin (1995) was used to perform this analysis. A ThermoScientific Sorwall Legend Mach 1.6 centrifuge was used in conjunction with GE VivaSpin2 centrifuge tubes with ultrafiltration membranes as seen in **Fig. 4**.

Fig. 4 — Centrifuge and Tube. ThermoScientific Legend Mach 1.6 centrifuge and VivaSpin2 molecular weight cutoff centrifuge tube.

VivaSpin2 centrifuge tubes provided molecular weight cutoffs of 1,000,000, 100,000, and 30,000. The procedure used for the molecular weight cutoff analysis is described here:

- Molecular Weight Cutoff Centrifuge Tube Priming Procedure
    - Place 2 mL deionized water into each centrifuge tube
    - Place centrifuge tubes into centrifuge along with additional tubes required for balance
    - Spin at 4000 rpm for 10 minutes (0.166 on centrifuge)
    - Empty water from centrifuge tube and refill with deionized water
    - Spin again at 4000 rpm for 10 minutes (0.166 on centrifuge)
    - Empty water from centrifuge tube and refill with deionized water
    - Spin again at 4000 rpm for 10 minutes (0.166 on centrifuge)

- Molecular Weight Cutoff Determination Procedure
    - Weight centrifuge collection section
    - Add 2 mL of sample into each sample centrifuge tube
    - Spin at 4000 rpm for 30 minutes (0.5 on centrifuge)
    - Weigh centrifuge collection section

Molecular weight cutoff analysis of controls and samples served a dual purpose in serving as a filtration mechanism to protect the oligosaccharide column in the HPLC as well as generating data that was used to analyze the degradation of starch macromolecules using the enzyme treatment solutions.

2.2.3 Determination of Protein Concentration by Near UV Absorbance

To better understand the consequences of the action of enzyme breakers on starch-based filter cake, a quantification of enzyme concentration before treatment, in any filtrate from the treatment, and after treatment was conducted. By determining the initial and final enzyme concentrations, information about enzyme transport and inactivation while degrading starch-based filter cake was garnered. Enzyme molecular weight data determined from ultrafiltration tests were used in conjunction with enzyme concentration measurements to provide a more complete picture of enzyme molecular weight distribution. The enzyme concentration was measured in the following manner, adapted from Aitken and Learmonth (2002):

- Warm up lamp of the ThermoScientific Genesys 10UV spectrophotometer for 15 minutes

- Adjust wavelength to 280 nm
- Calibrate to zero absorbance with deionized water
- Generate standard curve of enzyme concentration vs. absorbance
- Collect samples of post-treatment enzyme solutions as well as any filtrate that result from the enzyme treatment of filter cake
- Measure absorbance samples at A_{280}
- Determine concentration of enzyme in the solutions using the standard curve previously generated

An α-amylase treatment fluid, GBW-16C, was acquired from BJ Services and found to have an initial concentration of 100 mg/mL. A standard curve was used to determine enzyme concentration and based on this initial condition and is presented in **Fig. 5**.

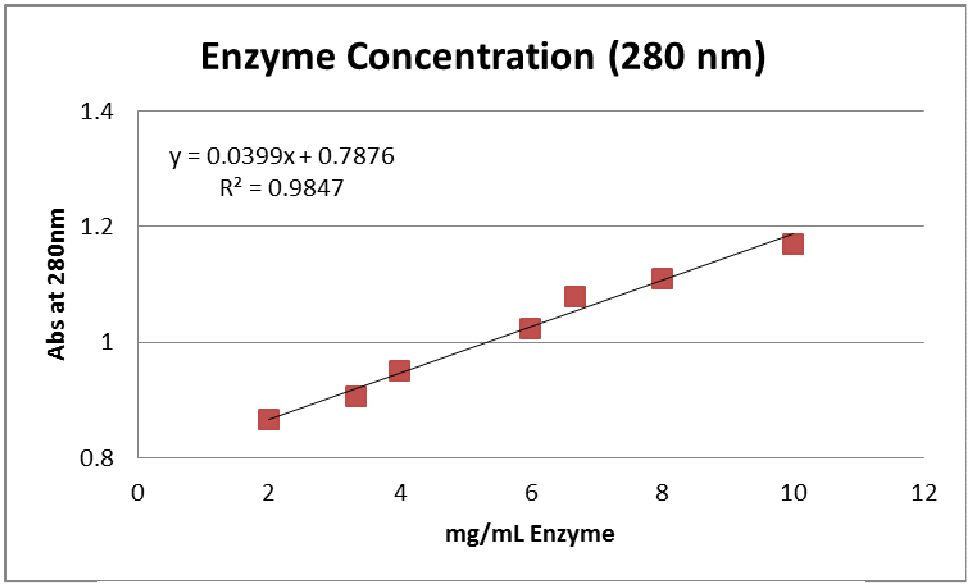

Fig. 5 — α-amylase Concentration Standard Curve.

2.2.4 Starch Degradant Distribution Determination Using HPLC

An overlooked consideration in the use of α-amylase enzyme treatment fluids to remove starch-based filter cake is the degradation products that result from the polymer hydrolysis catalyzed by the enzyme. This was explored using a high performance liquid chromatograph to determine the distribution of starch degradants hydrolyzed by the α-amylase enzyme. Samples collected previously from the comparison of cleanup efficiency and analyzed through ultrafiltration and near UV absorbance were subsequently analyzed using the HPLC to determine the presence of glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose against standards.

This analysis was conducted using an Agilent 1200 HPLC system with a refractive index detector, degasser, quaternary pump, and ChemStation software. The column used was a Phenomenex Rezex RSO-Oligosaccharide column capable of resolution of oligosaccharides of up to 18 degrees of polymerization. The system is shown in **Fig. 6**.

Fig. 6 — HPLC Apparatus. From top to bottom: degasser, quaternary pump, column thermostat, refractive index detector.

Per the instructions of the distributor, the analysis was conducted using degassed deionized water as the mobile phase at a flow rate of 0.25 mL/min. The Rezex RSO-Oligosaccharide column was kept at 75°C and the optical unit in the refractive index detector at 30°C. Samples were filtered through the ultrafiltration centrifugation method as described above and then run with the following procedure:

- Prepare oligosaccharide standard solutions using glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose standards for low and high concentrations
- Inject 20 µL using 1 mL syringe of standard solution and use ChemStation software to create standardization curves and verify oligosaccharide retention and elution
- Inject samples of only enzyme solution and untreated filtrate and starch solutions to provide control

- Samples from ultrafiltration at 30,000 MW are drawn into 1 mL syringe without dilution
- 20 µL of sample is injected into manual injection port and analysis is started on ChemStation software
- The presence of oligosaccharides will be determined from a standard curve

The sensitivity of the HPLC instrument necessitated multiple runs in order to provide useful data. Several samples were derived from the filter-cake remediation efficiency procedure.

Standard solutions of oligosaccharides were prepared and are displayed in **Fig 7**. These were made up of glucose units with a degree of polymerization of up to 7. In an attempt to provide a method of quantification of oligosaccharides in samples multiple standard curves were generated.

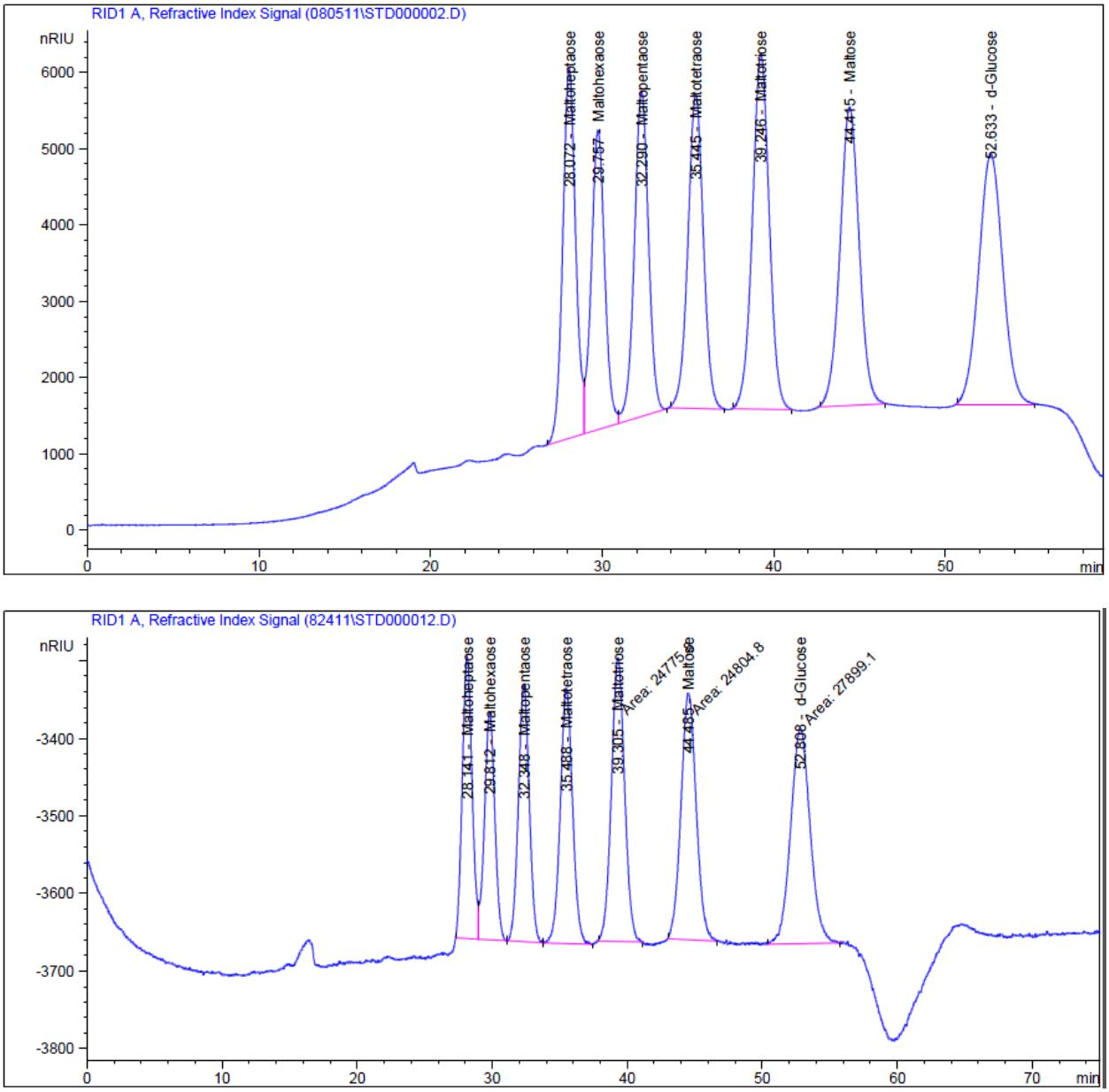

Fig. 7 — Oligosaccharide standard solutions. 0.1 wt. % and 0.03 wt. %, respectively.

However, samples of known concentration did not reflect the result expected from the standard curves and the quantitative measurement was found to be unreliable. Observations were made qualitatively using chromatographic data in order to maintain high confidence in results.

2.2.5 Filter Cake Removal Efficiency

A comparison of the filter-cake removal efficiency of starch-based filter cake was conducted using a method described by Beall et al. (1996) and modified to adapt to the Fann static filter press equipment in **Fig. 8** and Fann filter paper with particle size retention of 2-5 µm.

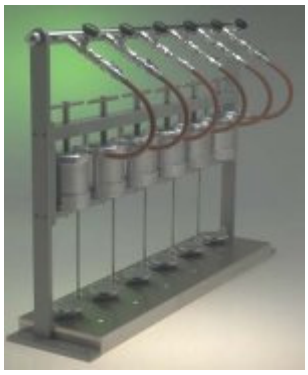

Fig. 8 — Fann API Filter Press Apparatus.

Filter-cake cleanup efficiency was determined qualitatively by checking for the presence of residual starch using the iodine test and quantitatively by determining cleanup efficiency based on fluid flow before the deposition of a filter cake and after cleanup treatment.

- Prepare filter-cake cleanup solutions based on data from enzyme activity assays
- Determine initial rate of fluid flow through filter paper by recording the time for 250 mL of 2% KCl to pass at 50 psi using nitrogen
- Bleed pressure, flip filter paper and fill cell with drilling fluid
- Pressurize apparatus to 150 psi and record fluid loss at 1 minute and at 30 minutes

- Bleed pressure, extract drilling fluid, and leave filter paper with filter cake.
- Add breaker, pressurize cell to 150 psi and shut-in cell for 8 hours
- Bleed pressure, extract excess treatment fluid for further analysis, remove filter paper, and test for residual starch using iodine test.
- If there is any filtrate, note the quantity and keep fluid for further analysis.
- Dissolve calcium carbonate with a flush of 5 wt% HCl
- Fill cell with 2% KCl water, pressurize to 50 psi, and record time for 250 mL to pass through filter paper
- Determine cleanup efficiency by comparing the initial time for 250 mL of 2% KCl to pass through filter with the final time after the treatment

Determining the cleanup efficiency provided a comparison of the filter-cake removal treatments while also providing samples for further analysis. Filtrate collected during the formation of the filter cake was further analyzed to determine its composition.

CHAPTER III

ENZYME ACTIVITY ASSAY

3.1 α-amylase Activity

The determination of the limits and optima of enzyme activity is a crucial step in this work. The function of α-amylase is the catalysis of the hydrolysis of the α,1-4 glycosidic bond, usually an exceptionally stable bond. The enzyme is able to increase the rate of this hydrolysis by up to 10^{15} fold (van der Maarel et al. 2002). The active site, shown in **Fig. 9** of α-amylase contains acidic groups of glutamate and aspartate that work together to hydrolyze the glycosidic bond (Matsuura et al. 1984).

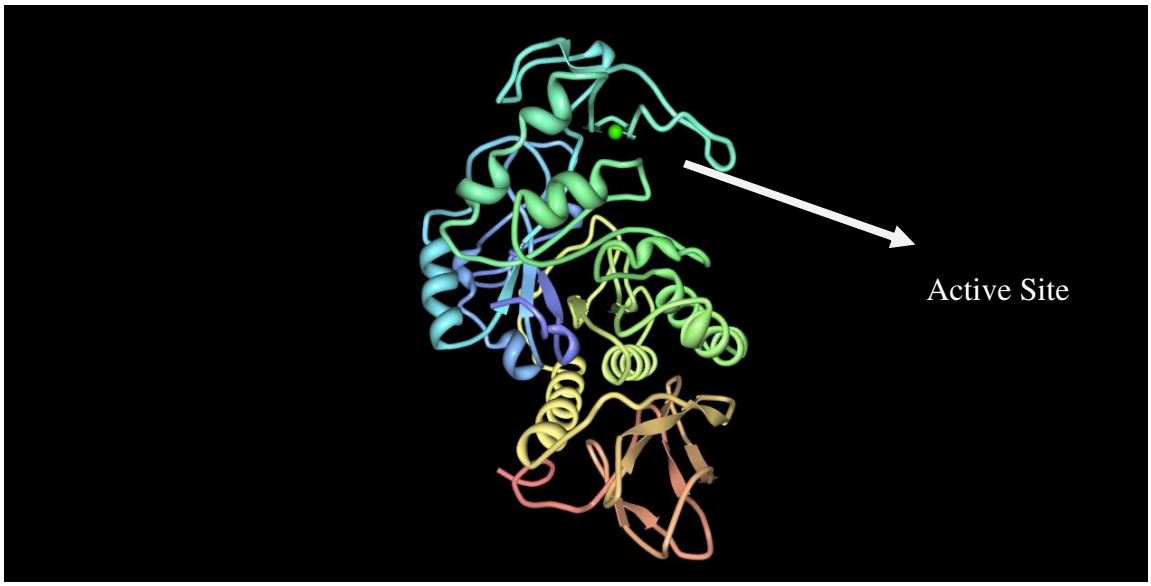

Fig. 9 — α-amylase visualization. Protein structure with active site defined, (Matsuura et al. 1984)

Several factors are known to have an impact on the activity of α-amylase. These include substrate concentration, temperature, pH, calcium ions, chloride ion, salt

concentration, and enzyme origin. It has been found that a salt concentration of approximately 0.9% wt. is the optimum concentration for enzyme activity (Samuel et al. 2010). Conditions downhole will not always meet the conditions favorable or even tolerable for enzyme activity. Therefore, it is important to know the limitations and optima of α-amylase activity in the context of expected application conditions. For the purposes of this work, the conditions that were considered most sensitive to variation in the oil industry were tested. These factors included temperature, pH, and enzyme concentration. Temperature and pH are commonly variable in the oil industry and are known to be incredibly important to enzyme stability. Enzyme concentration, however, is a factor that can play an important role in the overall cost of a treatment.

The 3,5-dinitrosalicylic acid method of determining enzyme activity used the liberation of the reducing groups of maltose to provide a colorimetric response. This response is measured as the absorbance at 540 nm. The enzyme activity unit is defined as a unit that liberates 1.0 mg maltose from starch in 3 minutes. A standardization curve, **Fig. 10** was developed using a maltose standard solution.

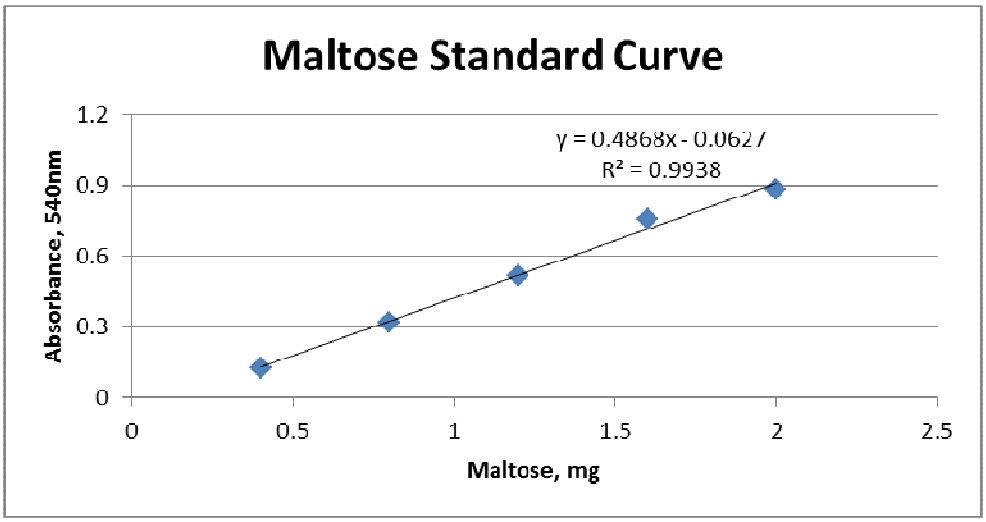

Fig. 10 — Maltose standard solution Absorbance at A_{540}.

3.1.1 α-amylase Activity vs. Temperature

This standard curve allowed the determination of milligrams maltose released to define enzyme activity. As temperature has been noted by Kameda et al. (2007) as the parameter of the greatest statistical significance on enzyme activity, an analysis of enzyme activity over a range of temperatures was conducted in **Fig. 12**. An important observation to note was that the 10% v/v enzyme solution kept at pH 6.5 began to cloud up at around 150°F and started to denature at 165°F. At 180°F, the enzyme solution largely denatured as demonstrated in **Fig. 11**. The precipitated denatured protein was sticky and adhered to the glassware. It was not possible to resolubilize the precipitate, indicating the likelihood of α-amylase inducing damage if used at high temperatures.

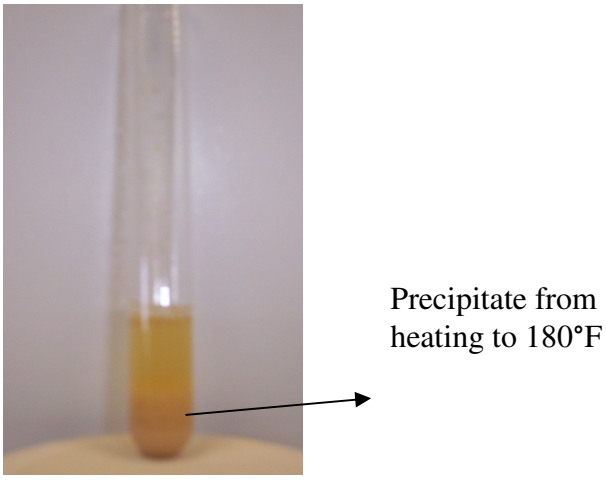

Fig. 11 — Denatured α-amylase. 10% v/v enzyme at pH 6.49
α-amylase denatured at high temperatures.

Although the some of the enzyme began to denature above 165°F, it did retain some of its function at high temperatures before it completely denatured.

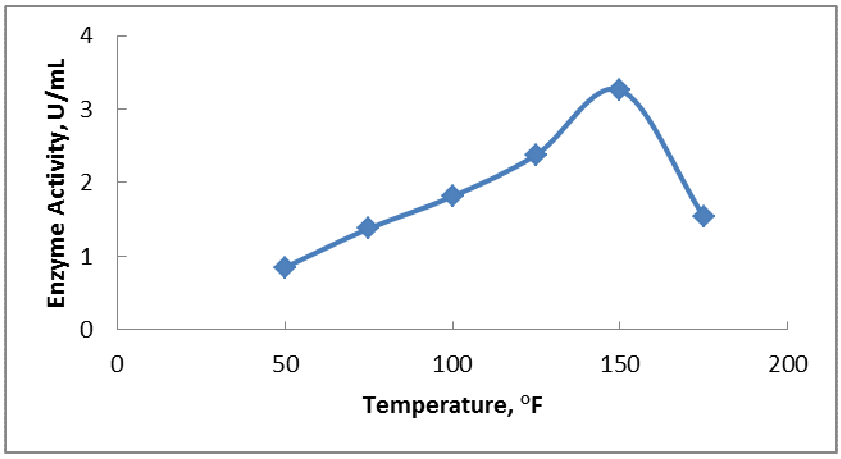

Fig. 12 — α-amylase activity vs. temperature. pH 6.5.

3.1.2 α-amylase Activity vs. pH

Enzyme activity also varied widely with pH. As with high temperatures, extreme pH values can cause significant loss of activity. Changes in pH can restrict activity by changing the shape of the enzyme's active site and can also cause it to denature.(Nielsen and Borchert 2000) α-amylase solutions with pH values under 4 resulted in a conspicuous and apparent precipitation of the protein. However, in **Fig. 13**, when the pH was moderated to above 4, the enzyme was able to resolubilize into solution and retained its activity. Enzyme activity was found to be highest between pH 5.75 and 8 as shown in **Fig. 14**.

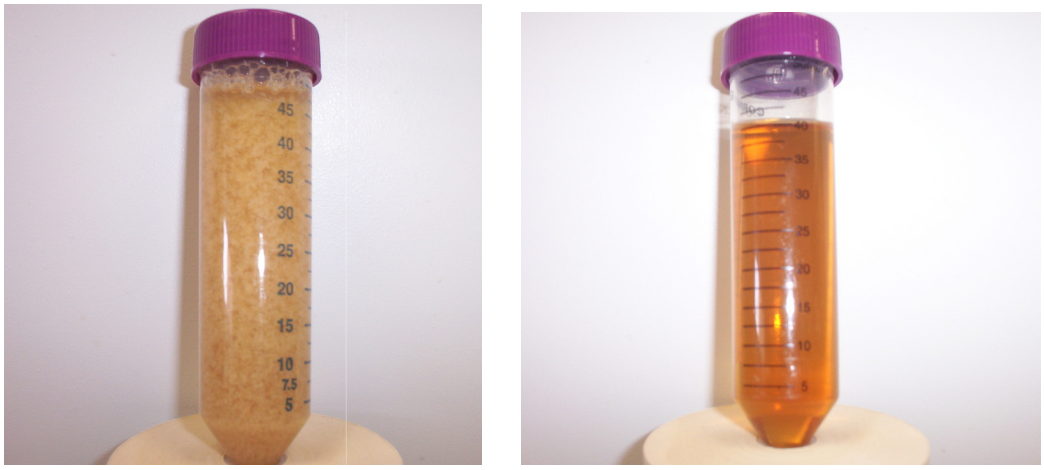

Fig. 13 — α-amylase low pH deactivation . 10% v/v at room temperature at pH 2.9 and solubilized at pH 6.5

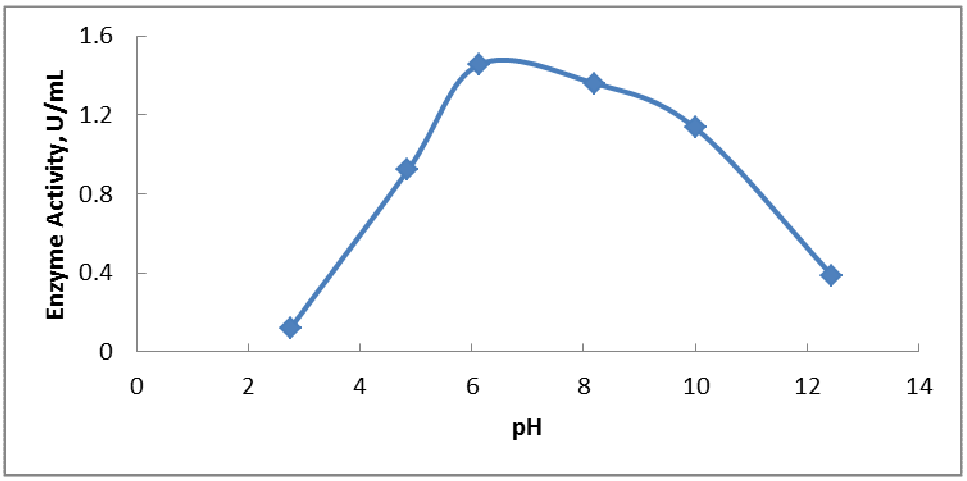

Fig. 14 — α-amylase activity vs. pH.10% v/v at room temperature.

3.1.3 α-amylase Activity vs. Enzyme Concentration

Although enzyme activity is known to be governed by substrate concentration in the context of classic Michaelis-Mentin enzyme kinetics, it was useful to explore the role of enzyme concentration from the angle of minimizing material cost. For the measurement of α-amylase activity, starch substrate was left constant at 1% w/v. α-amylase concentration was varied between 1% v/v to 10% v/v. With the constant

substate concentration, increasing enzyme concentration was not seen to correspondingly increase overall enzyme activity, as seen in **Fig. 15**.

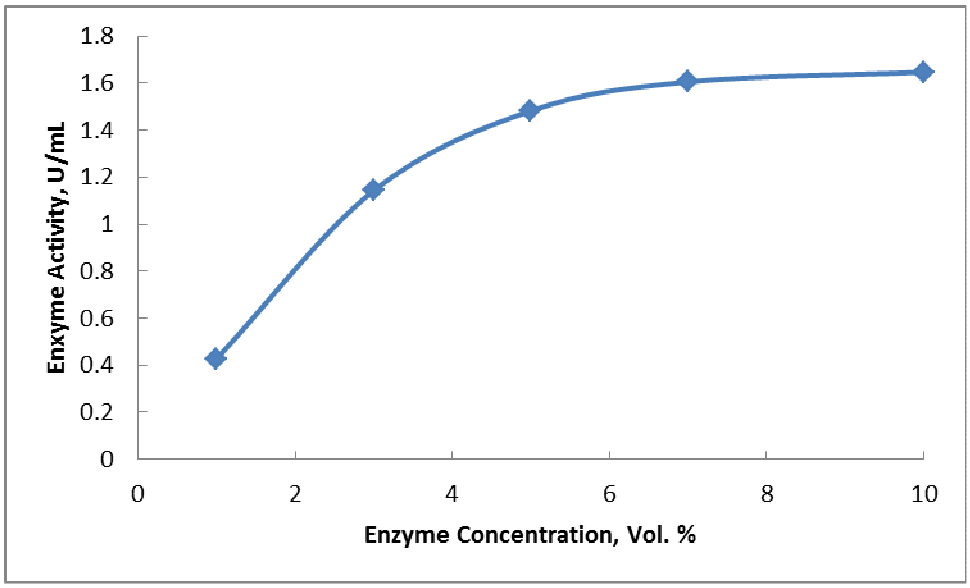

Fig. 15 — α-amylase activity vs. concentration. Enzyme activity at room temperature and pH 6.5 stagnated at higher enzyme concentrations in a test with 1% w/v starch substrate.

The efficacy of lower enzyme concentrations is further explored later in this work. However, in order to make a definitive determination on the efficacy of using lower enzyme concentrations to remove starch-based filter cake, field trials would be necessary.

3.2 α-amylase Activity Discussion

The exploration of α-amylase activity yielded results that provided important insight about enzyme activity. Temperature considerations must be addressed as high temperatures (above 165°F) not only deactivate the enzyme but also irreversibly denature it, opening the potential for causing, rather than alleviating, damage to the formation. Similarly, the application of enzyme in conjunction with acid or into a basic environment will not achieve the desired effects. However, unlike the irreversible denaturing and potential damage that high temperatures cause, the enzyme will not be irreversibly damaged by extremely high or low pH. This was seen in the solubilization of α-amylase when the pH of the solution was raised above 4.

By preliminarily subjecting the α-amylase samples to a variety of tests to gauge its activity at conditions relevant to its application, we were able to provide pre-selection screening for further testing. The results of the activity tests ruled out the intriguing possibility of a combined enzyme/acid treatment that could remove both starch and calcium carbonate in one stage. The activity results led to the development of tests that explored enzyme action under extreme as well as more moderate conditions. Subsequent testing of α-amylase treatment solutions was performed by varying the parameters of temperature and initial enzyme concentration. A comparative sample of a strong acid treatment was also included at room temperature.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Untreated Drilling Fluid, Filtrate, & 1% w/v Starch Solution

As mentioned in the introduction of this work, non-damaging drilling fluids are of great interest in the oil industry, especially with the proliferation of long horizontal intervals, high angle wells, and other drilling innovations. The drilling fluids must be able to provide good carrying capacity to remove drill cutting, adequately cool the drill bit, and protect the formation by minimizing fluid loss into the matrix (Przepasniak and Clark 1998). Starch has been identified as a useful additive to these fluids and was found to provide the requisite properties sought for these applications.

However, all drilling fluids inherently cause a degree of damage to the formation. The sources of this damage include solids invasion, viscous filtrates, and polymer deposition. This damage is not always bad and indeed some degree of damage is intended to create an external filter cake on the borehole wall to prevent further fluid invasion.

The extent of damage caused by starch-based drilling fluid was investigated by Audibert et al. (1999) who found that the amount of starch in drilling fluid filtrate, or the fluid that leaks off through the matrix face, was small. The starch-based, calcium carbonate weighted drilling fluid used in this work was analyzed to serve as a baseline. The suite of tests carried out on the drilling fluid and its filtrate served to establish the initial molecular weight of the fluid and filtrate and provide initial chromatographic

measurements.

Before preparation of the starch-based drilling fluid, a 1% w/v starch solution was subjected to the iodine test to ensure its effectiveness. Iodine forms an inclusion complex with amylose expressing a characteristic color that is dependent on chain length. Starch-iodine complexes form a deep blue color due to the conformational characteristics of the amylose molecule where one iodine molecule is held within a turn of the helix, or six glucose units (Kearsley and Dziedzic 1995). The result of the iodine test of the 1% w/v at room temperature was a strong, deep blue color, as seen in **Fig. 16**.

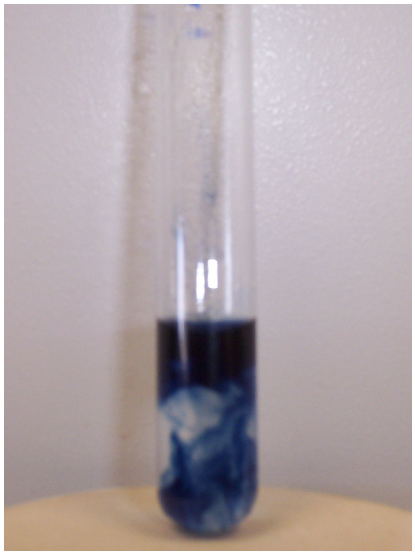

Fig. 16 — Starch-iodine complex. 1% w/v starch solution.

After confirmation of the effectiveness of the iodine test, the starch-based drilling fluid was prepared according to the formula in section 2.1. A filter cake was deposited on Fann filter paper using the method described in 2.2.5. The iodine test was also performed on a sample of this filter cake to gauge if the deposition of the starch-based filter cake was effective. The positive result of this test is shown in **Fig. 17**.

Fig. 17 — Iodine test on untreated filter cake. Filter cake deposited by starch-based drilling fluid on Fann paper filter.

Filter cake formed by the drilling fluid described in **Table 1** provided a colorimetric response to the iodine test, confirming the deposition of starch onto the filter cake formed by the drilling fluid on the Fann paper filter.

Filter cake removal efficiency was tested on with a control sample without treatment as well. Using the method described in 2.2.5, the flow of 250 mL of 2% KCl solution through the Fann paper filter was measured to be 42.12 seconds. After the establishment of filter cake and a wash with deionized water, the time for 250 mL of 2% KCl to flow through the filter cake was unmeasureable as the total outflow did not reach 250 mL. After 30 minutes and a total outflow of 3 mL, the relative impermeability of the filter cake was confirmed.

The drilling fluid, its filtrate, and the 1% w/v starch solution were analyzed to determine its molecular weight distribution according to the method described in section 2.2.2. A 2 mL sample of the fluids was centrifuged through a series of VivaSpin2 molecular weight cutoff filters.

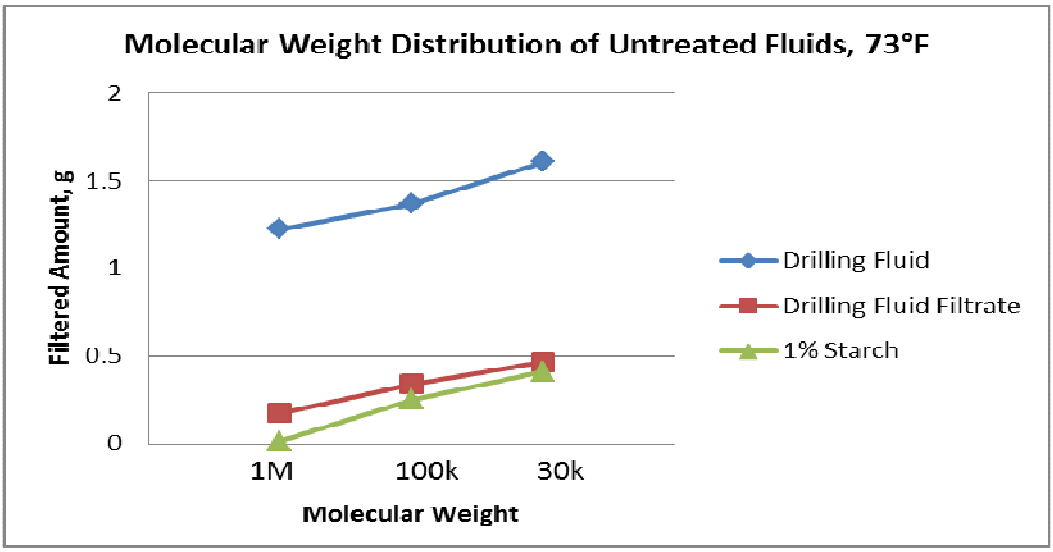

Fig. 18 — Initial molecular weight cutoff test. Cumulative molecular weight cutoff of untreated fluids based on ultrafiltration results.

As can be seen in **Fig. 18**, the untreated drilling fluid retains a significant amount of particles with molecular weight over 1,000,000. The discrepancy between this and the 1% starch and drilling fluid filtrate can be explained by the fact that the drilling fluid also contains xanthan polymer for use as a viscosifier. Literature has shown xanthan polymer to have a molecular weight between 0.4 to 15 × 10^6 (George 1978). More speculative reasoning includes the viscous nature of the drilling fluid as opposed to its filtrate or the starch solution enabling retention regardless of ultrafiltration level.

By using the centrifugal ultrafiltration to prepare the samples for chromatographic analysis the potential for losing additional information by filtering HPLC samples was mitigated. Samples were analyzed according to the procedure outlined in 2.2.4.

High performance liquid chromatography showed the initial, untreated samples to be a complex mix of poly- and oligosaccharides when filtered through the 30,000

molecular weight ultrafilter. The 1% w/v starch solutions exhibited a well-distributed mix of oligosaccharides as well as some unidentified molecules.

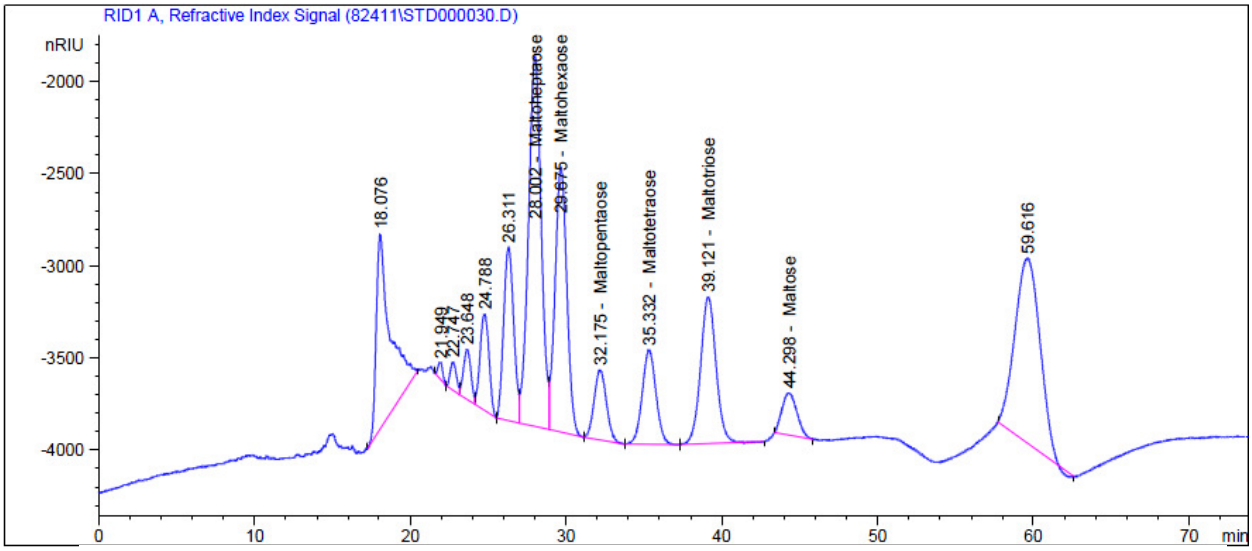

Fig. 19 — 1% w/v starch solution chromatograph. Conducted at room temperature.

A quantitative estimation of the oligosaccharides was made but needed to be disregarded due to inconsistencies and lack of confidence in the measurements. However, this data was able to provide a clear, qualitative picture of the molecular distribution of samples after ultrafiltration, as displayed in **Fig. 19**.

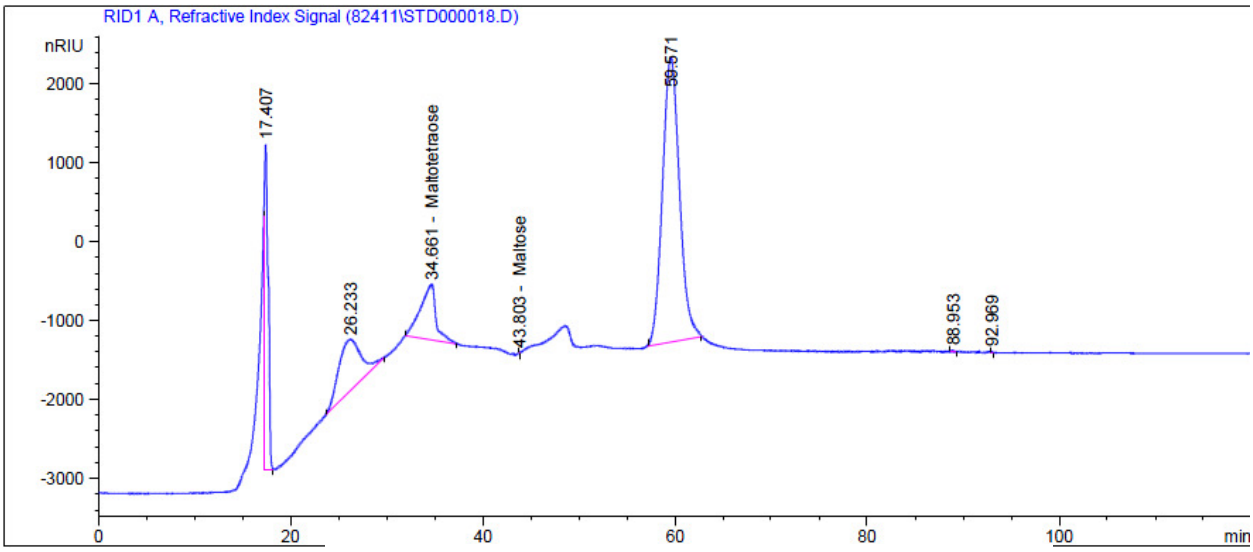

Fig. 20 — Untreated drilling fluid chromatograph.

The clarity seen in the 1% w/v starch solution was not reflected by the analysis of the drilling fluid. As can be seen in **Fig. 20**, the chromatograph of the untreated, ultrafiltered drilling fluid shows no discernible strong response at the retention times of any of the standards. The complexity of the drilling fluid also provided unidentified responses. Drilling fluid filtrate, as seen in **Fig. 21**, also exhibited a similar, unclear chromatographic response.

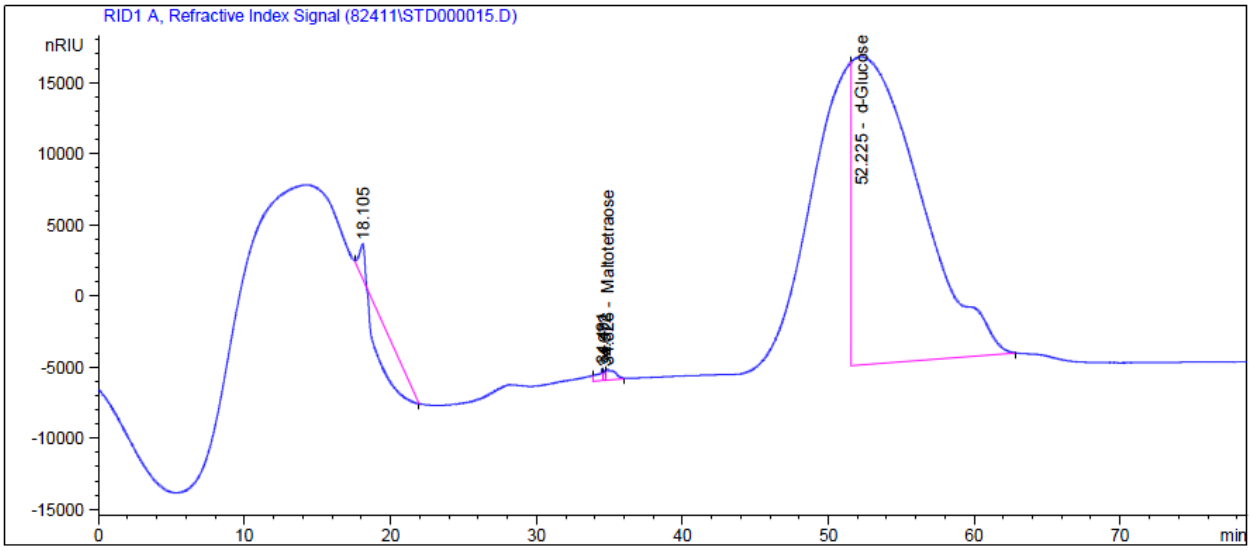

Fig. 21 — Drilling fluid filtrate chromatograph. Ultrafiltered drilling fluid filtrate, filtered through Fann paper filter.

Although the drilling fluid filtrate shows a strong response at the retention time of glucose, the reason for this is not clear and there is also significant unknown response at earlier retention times.

4.2 α-amylase Analysis

α-amylase treatment solution GBW-16C received from BJ Services was analyzed prior to its use in treating starch-based filter cake in order to investigate its molecular weight range and spectrophotometric and chromatographic response. Given values found in literature for α-amylase molecular weight ranges of 10,000 – 40,000, initial molecular weight cutoff analysis was done at the 30,000 MW centrifugal filtration level. However, concentrations of enzyme under 5% v/v did not exhibit any particle retention at the 30,000 MW level. Higher concentrations of 5% and 10% v/v were then investigated and showed some retention at the 30,000 MW filter.

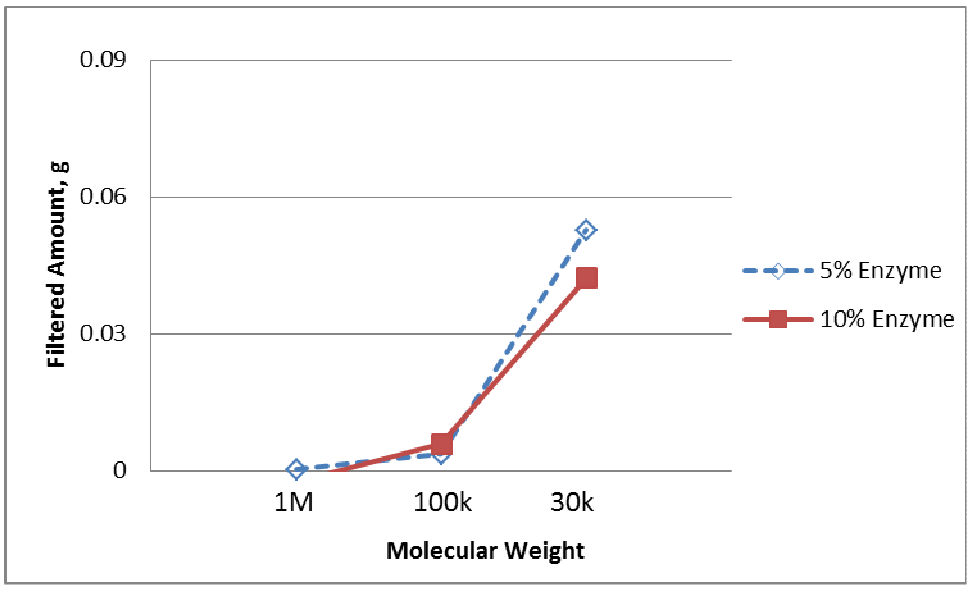

Fig. 22 — α-amylase 30,000 molecular weight cutoff. Limited enzyme particle retention exhibited by above 30,000 MW using molecular weight cutoff analysis at room temperature. Enzyme molecular weight can be concluded to be largely under 30,000.

Fig. 22 shows that a small amount of enzyme was retained in the 30,000 MW filter at enzyme concentrations of 5% v/v and 10% v/v. This information is important in the context of comparison with the molecular weight cutoff analysis of treated solutions. In order to gauge the effectiveness of the treatment, the initial properties of the enzyme itself were needed to compare against.

Spectrophotometric analysis using near UV absorbance confirmed the limited amount of enzyme over 30,000 MW. Concentration determinations of enzyme made prior to and after centrifugal ultrafiltration at 30,000 MW as shown in **Fig. 23** demonstrated some concentration decrease in accordance with the data from the molecular weight cutoff analysis.

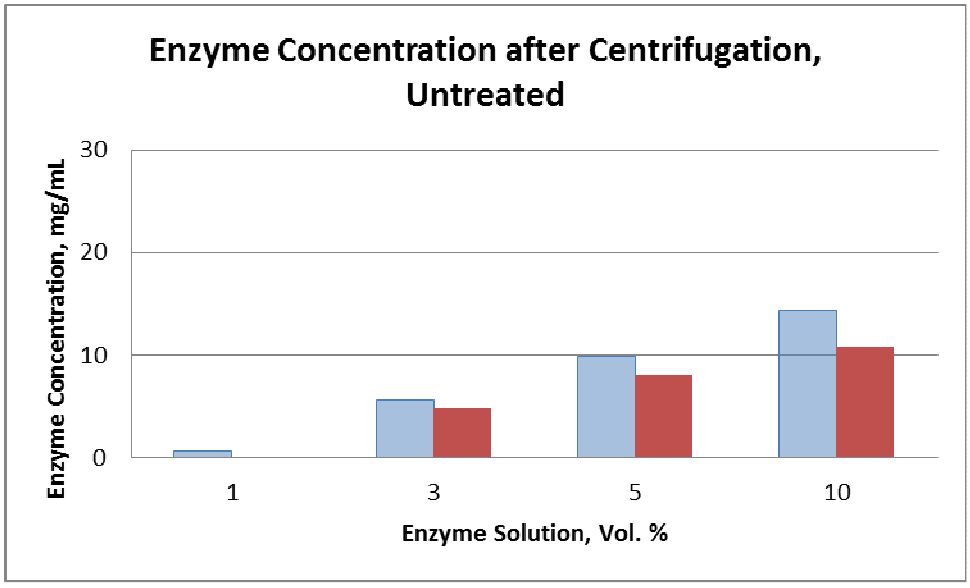

Fig. 23 — α-amylase concentration, 30,000 MW cutoff. Enzyme concentration changes after centrifugal ultrafiltration.

The chromatographic response of the enzyme solutions also needed to be taken into consideration when attempting to analyze the extent of α-amylase catalyzed hydrolysis of starch-based filter cake. **Fig. 24 and Fig. 25** show 5% v/v and 10% v/v α-

amylase treatment solutions that were run through the HPLC apparatus after centrifugal ultrafiltration at 30,000 MW.

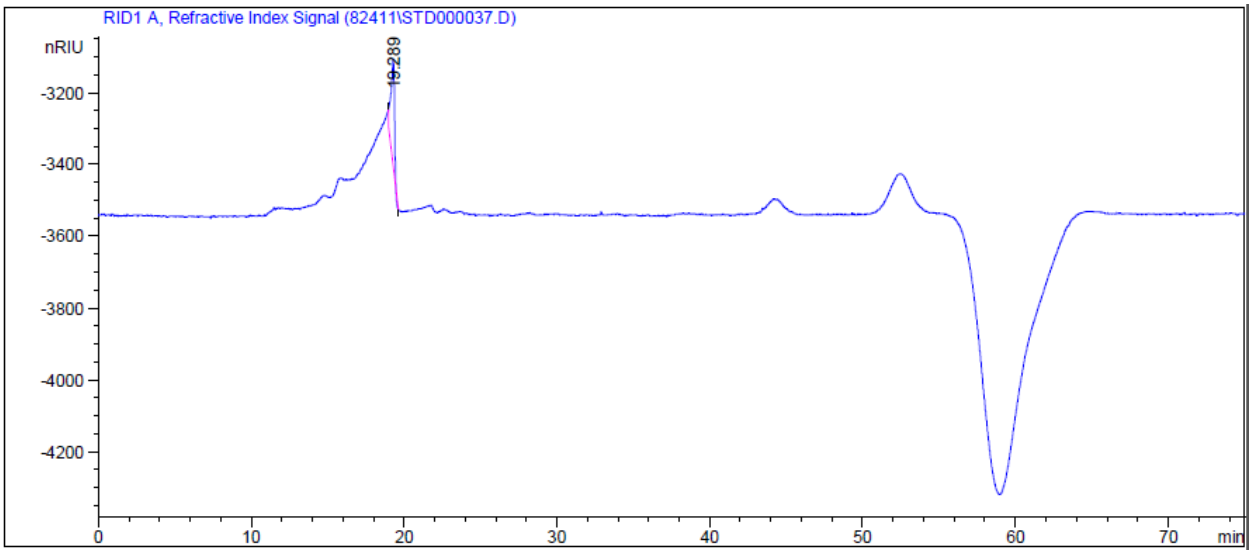

Fig. 24 — 5% v/v α-amylase chromatograph. After centrifugal ultrafiltration at 30,000 MW.

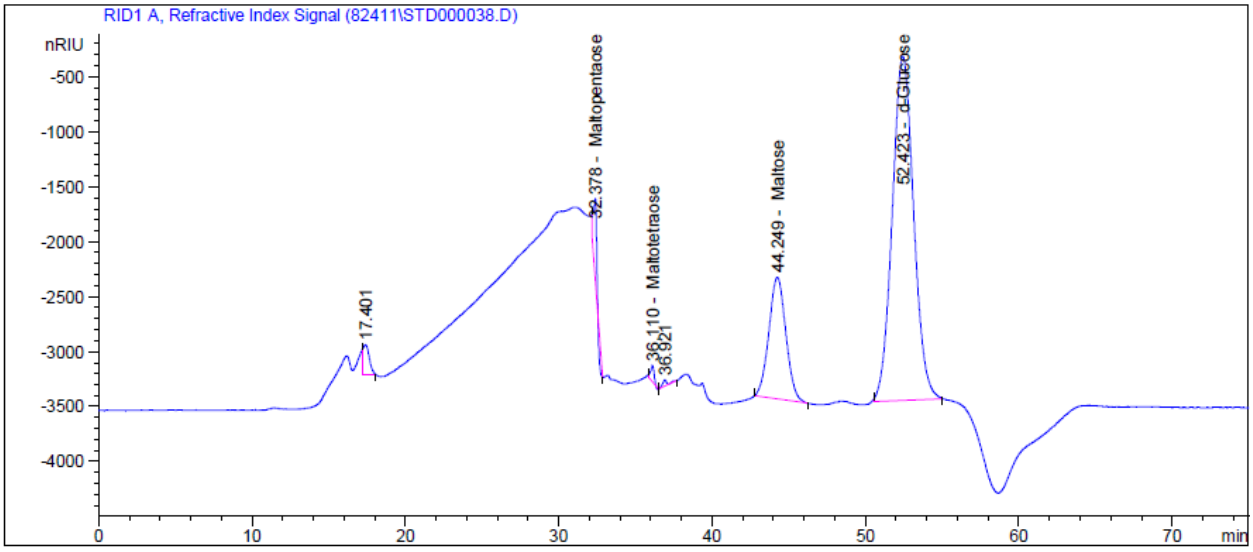

Fig. 25 — 10% v/v α-amylase chromatograph. After centrifugal ultrafiltration at 30,000 MW.

Both the 5% v/v and 10% v/v exhibited some evidence of glucose and oligosaccharide content to a degree. However, the reason for this was unknown.

Significant interference was apparent between retention times of 17-20 minutes in both the 5% and 10% treatment samples demonstrating a chromatographic response to α-amylase enzyme content with a molecular weight under 30,000.

4.3 HPLC Analysis of α-amylase Catalyzed Starch Degradation

A time-step picture of α-amylase hydrolysis of starch was deemed to be useful. This was accomplished using a 10% v/v α-amylase in deionized water treatment solution to degrade 1% w/v starch solution at room temperature. A sample of the 1% w/v starch tested positive for starch using the qualitative iodine test as seen in **Fig. 16**. An experimental vessel was prepared with 20 mL 1% w/v starch and 10 mL 10% v/v α-amylase treatment solution. Samples were taken at 30 minutes, 1 hour, 2 hours, and 4 hours to gauge the extent of starch degradation catalyzed by the enzyme treatment solution.

The original, untreated starch solution can be seen in **Fig. 19**. Thirty minutes of α-amylase catalyzed hydrolysis yielded a failed iodine test and the results seen in **Fig. 26**.

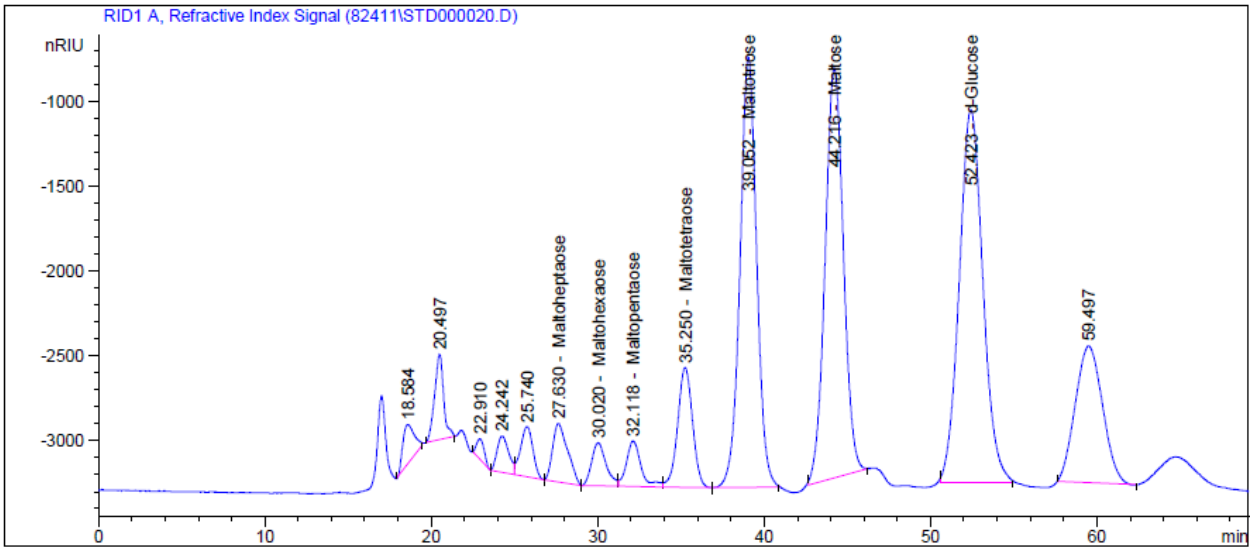

Fig. 26 — 30 minute α-amylase treated starch chromatograph. 10% v/v α-amylase treatment of 1% w/v starch at 73°F, pH 6.5.

A shift to lower order oligosaccharides and glucose as compared to the untreated solutions is readily apparent. Although quantification of the oligosaccharides was not attempted due to the problems discussed earlier in this work, a qualitative examination of this result shows the quick action of the enzyme in degrading starch and higher order oligosaccharides. This trend continues in subsequent samples as displayed in **Figs. 27, 28, and 29**.

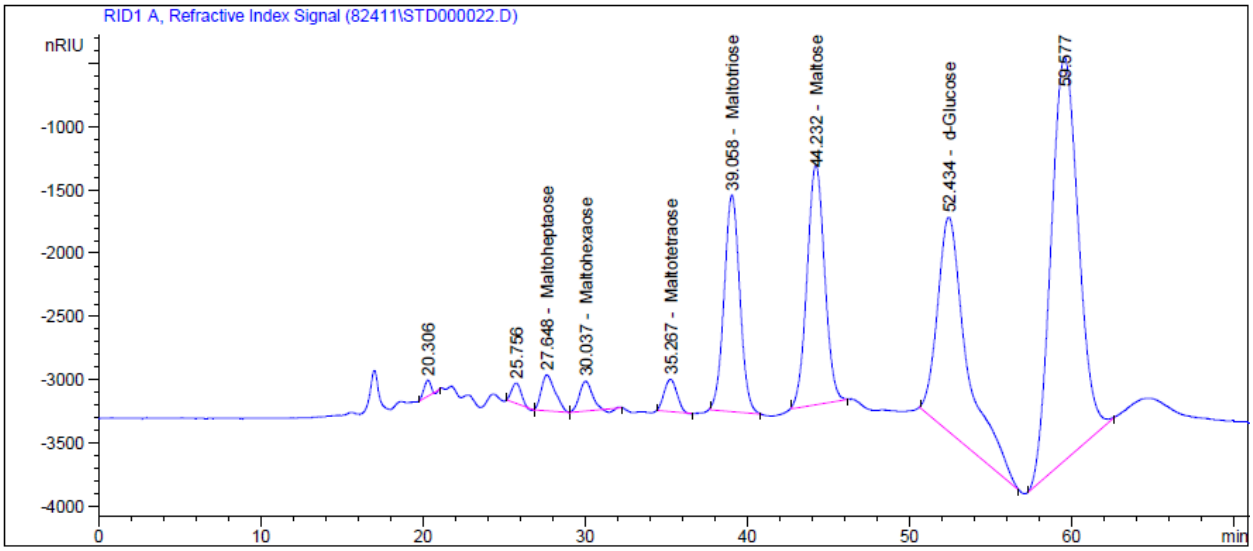

Fig. 27 — 1 Hour α-amylase treated starch chromatograph. 10% v/v α-amylase treatment of 1% w/v starch at 73°F, pH 6.5.

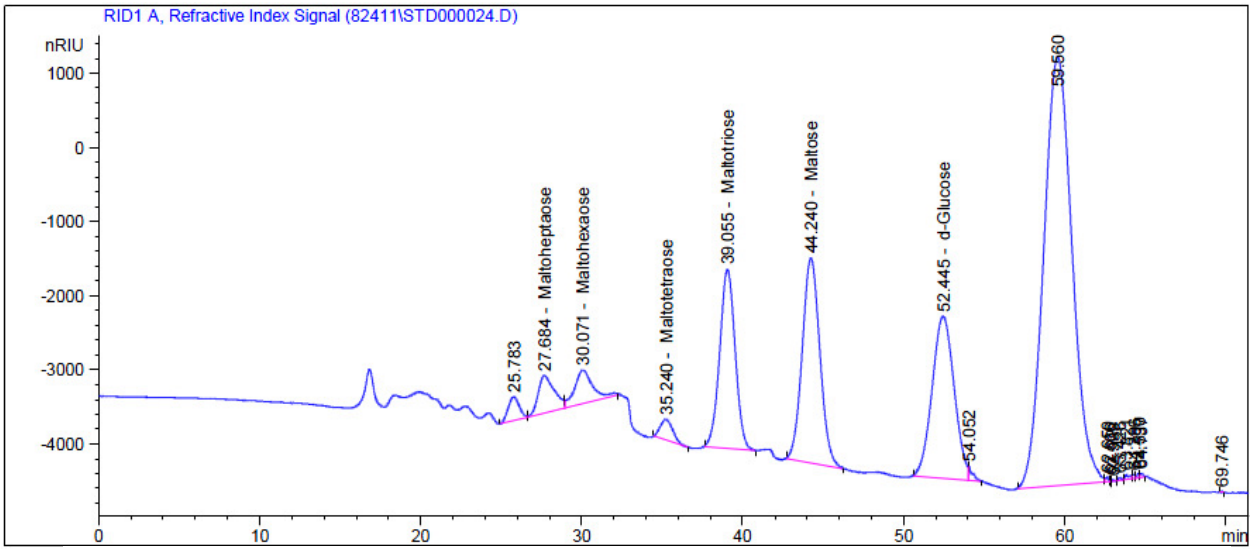

Fig. 28 — 2 Hour α-amylase treated starch chromatograph. 10% v/v α-amylase treatment of 1% w/v starch at 73°F, pH 6.5.

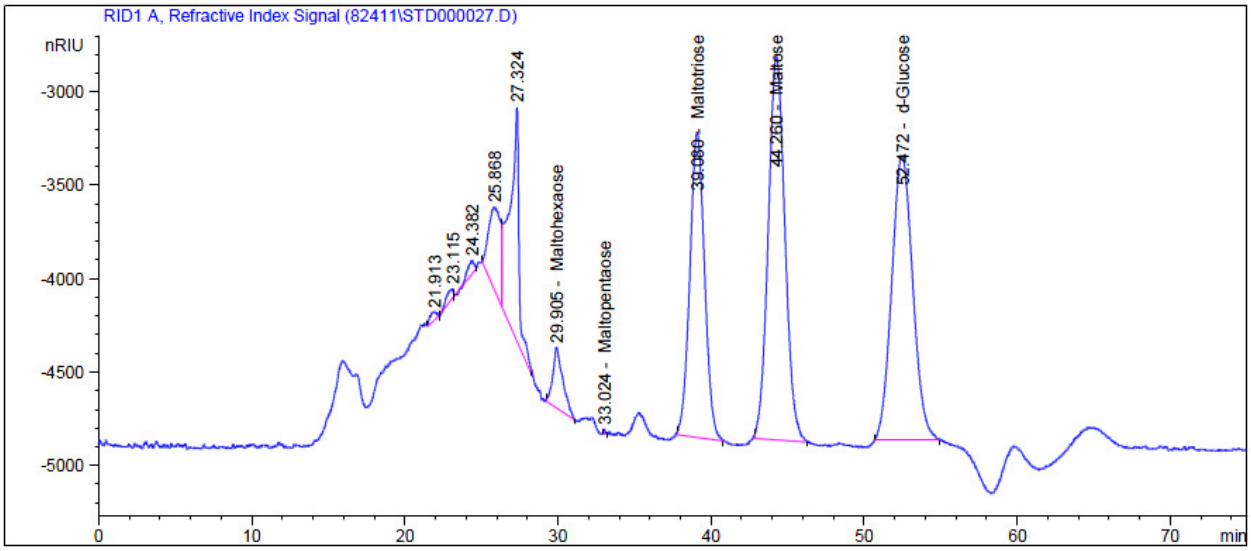

Fig. 29 — 4 Hour α-amylase treated starch chromatograph. 10% v/v α-amylase treatment of 1% w/v starch at 73°F, pH 6.5.

As the treatment extended over longer periods of time, the α-amylase treatment began to approach the limits of hydrolysis leaving glucose, maltose, maltotriose, and limit dextrins. Limit dextrins, or the branched chains that make up amylopectin, are branched by the α-1,6 glycosidic bond which is not hydrolyzed by α-amylase. None of the samples of 1% w/v starch treated with the enzyme treatment exhibited a positive response to the iodine test.

The hydrolysis of starch catalyzed by α-amylase demonstrated the swift degradation of starch to low order oligosaccharides and limit dextrins. These degradation products are highly soluble and enzyme-catalyzed hydrolysis products do not have a tendency to recrystallize (Kearsley and Dziedzic 1995). These results showed the effectiveness of the α-amylase treatment on starch.

4.4 Filter Cake Remediation Using α-amylase Treatment

After exploration of the limits and optima of α-amylase enzymes, their spectrophotometric and chromatographic response, and effectiveness in degrading a starch solution, an investigation of the results and efficiency of an α-amylase was conducted. The effectiveness of the enzyme treatment over a range of initial enzyme concentrations at optimal pH of 6.5 and room temperature was explored and compared to the effectiveness of a 5 weight percent HCl with corrosion inhibitor treatment.

Filter cake was deposited on to Fann filter paper by the drilling fluid described in **Table 1** and according to the procedure in 2.2.5. The enzyme treatment solution was shut-in for 8 hours and then collected for analysis. Samples were assessed using centrifugal ultrafiltration to determine the presence of material with molecular weights above 30,000 in **Fig. 30**.

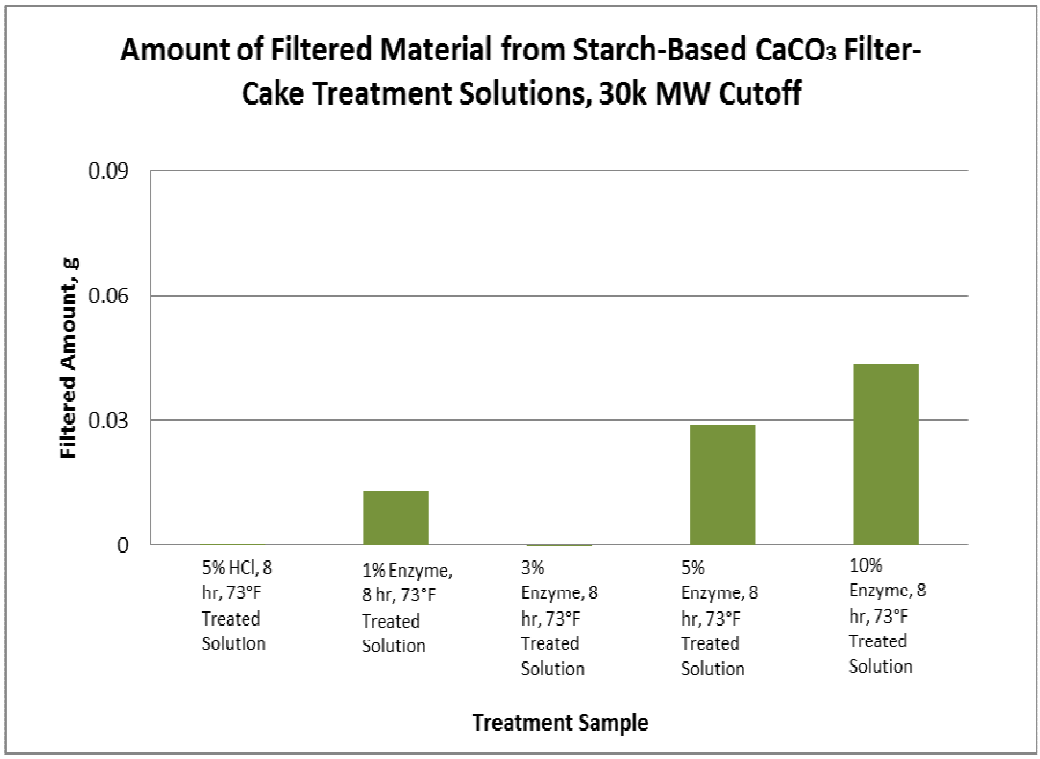

Fig. 30 — Treatment solution molecular weight cutoff. Centrifugal ultrafiltration of α-amylase solution after treatment of filter cake.

These results show a similar profile for the 5% and 10% v/v treated solutions as the untreated solutions shown in **Fig. 22**. Large polymer fragments are not apparent in the recollected treatment solution. This is confirmed spectrophotometrically in **Fig. 31**.

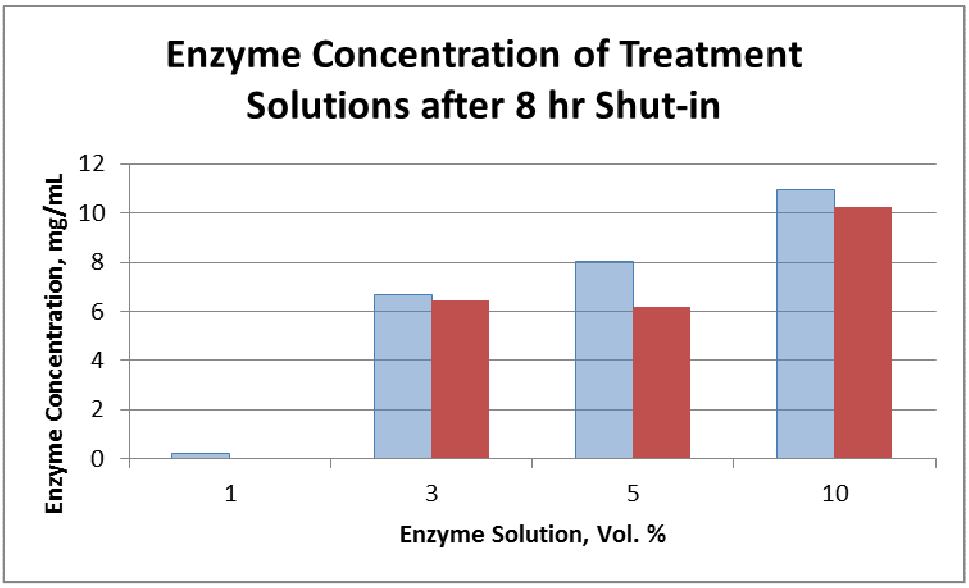

Fig. 31 — Treatment solution concentration determination. α-amylase concentration determined spectrophotometrically.

The results of the treatment were also made apparent with the use of the iodine test. Filter cake treated with α-amylase solutions did not exhibit a response to the iodine test. However, the 5% HCl treated sample was ineffective as a treatment alternative as the iodine test was positive on the filter paper, as seen in **Fig. 32**.

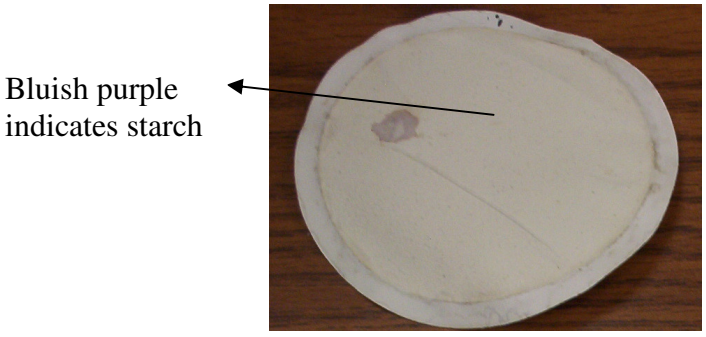

Fig. 32 — 5 wt. % HCl treated filter cake iodine test. Positive for starch on filter cake treated with 5% HCl.

Samples treated with enzyme solutions did not exhibit residual starch as determined by the iodine test. The ineffectiveness of the HCl treatment was also apparent in the chromatographic analysis, as seen in **Fig. 33**.

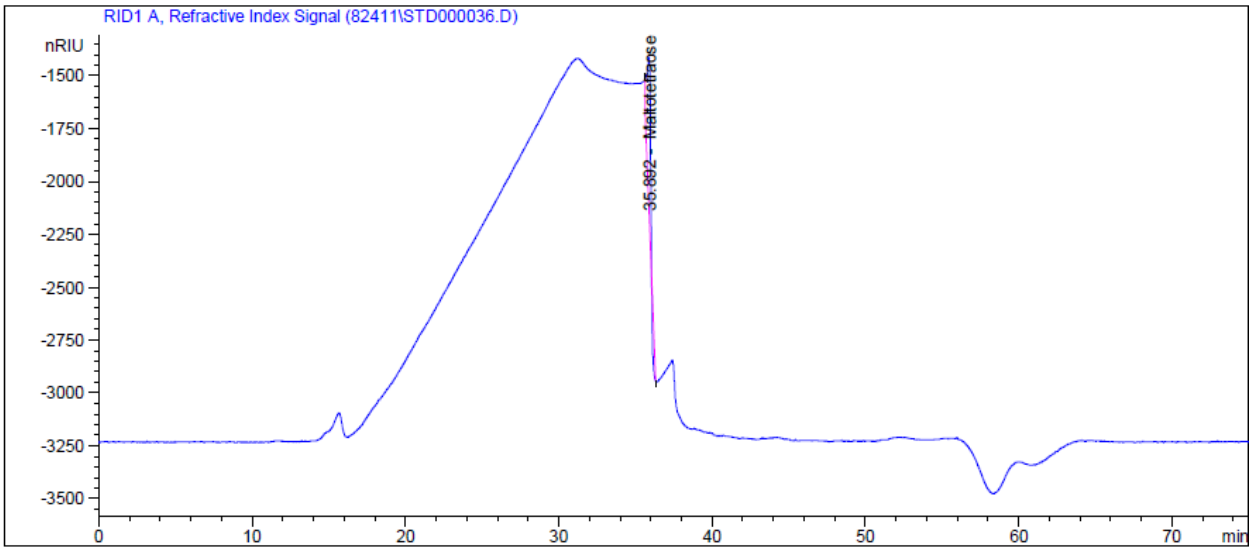

Fig. 33 — 5 wt. % HCl treated solution chromatograph. Starch-based filter cake treated with 5wt. % HCl and shut-in for 8 hours.

No noticeable small oligosaccharides are apparent and the response includes significant noise. However, even a low enzyme concentration treatment exhibited degradation of starch to glucose and small oligosaccharides.

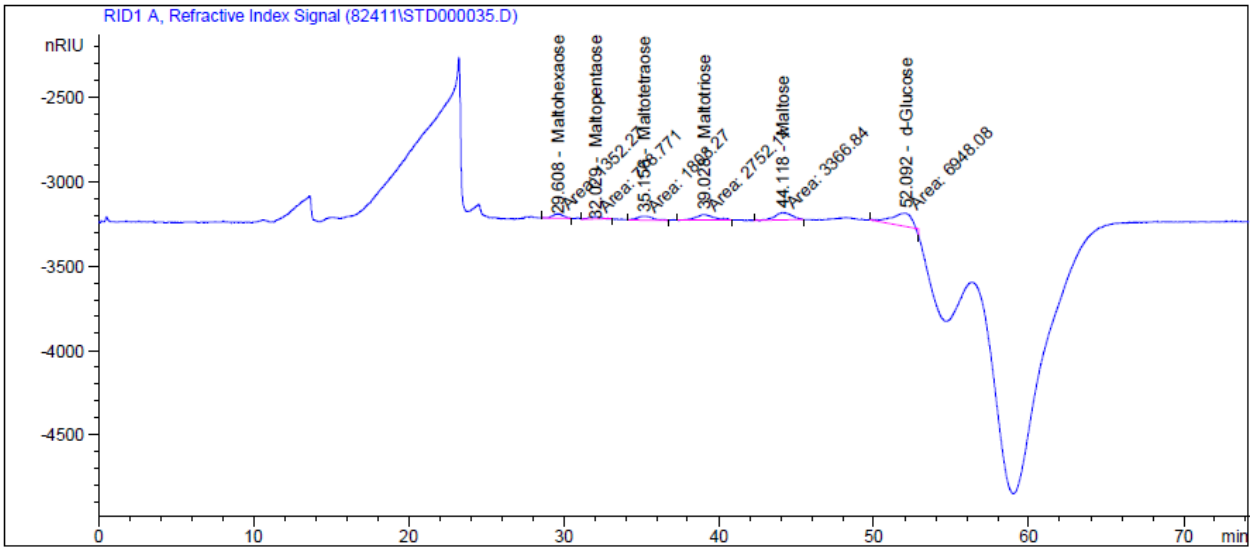

Fig. 34 — 1% v/v α-amylase treated solution chromatograph. Starch-based filter cake treated with 1% v/v α-amylase treatment solution exhibits limited degradation to small oligosaccharides.

Although the 1% v/v α-amylase treatment shown in **Fig. 34** did not exhibit the presence of undegraded starch in the iodine test, the limited degradation was apparent.

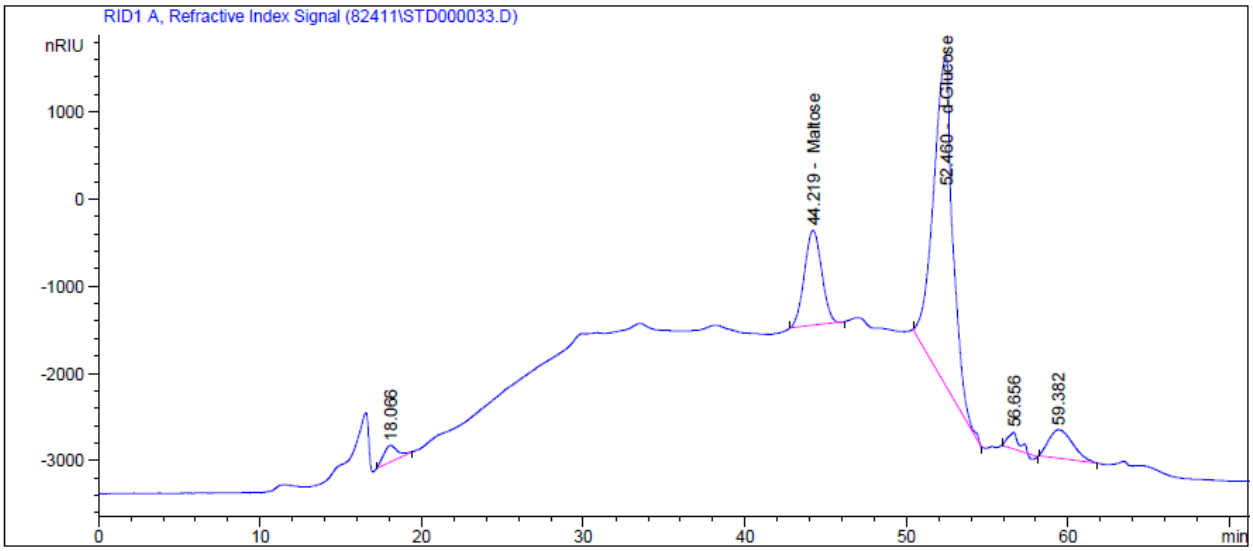

Fig. 35 — 3% v/v α-amylase treated solution chromatograph. Starch-based filter cake treated with 3% v/v α-amylase treatment solution exhibits greater degradation.

As seen in **Fig. 35**, increasing the concentration of α-amylase to 3% v/v demonstrated a significant augmentation of hydrolyzing capacity. Successively concentrations of α-amylase in treatment solutions exhibited further degradation of the small oligosaccharides into mostly maltose and glucose, 2 and 1 degrees of polymerization respectively. Although the evidence for improved starch degradation with higher concentrations of enzyme treatment is clear, it must be noted that this treatment was done under static conditions and did not provide for any flow of the clean-up fluid which could increase its contact with the starch substrate, potentially allowing lower concentration treatments to be more effective.

The effectiveness of the α-amylase treatment is best illustrated by the 10% v/v sample which showed a dominant peak for the glucose monomer, as seen in **Fig. 36**.

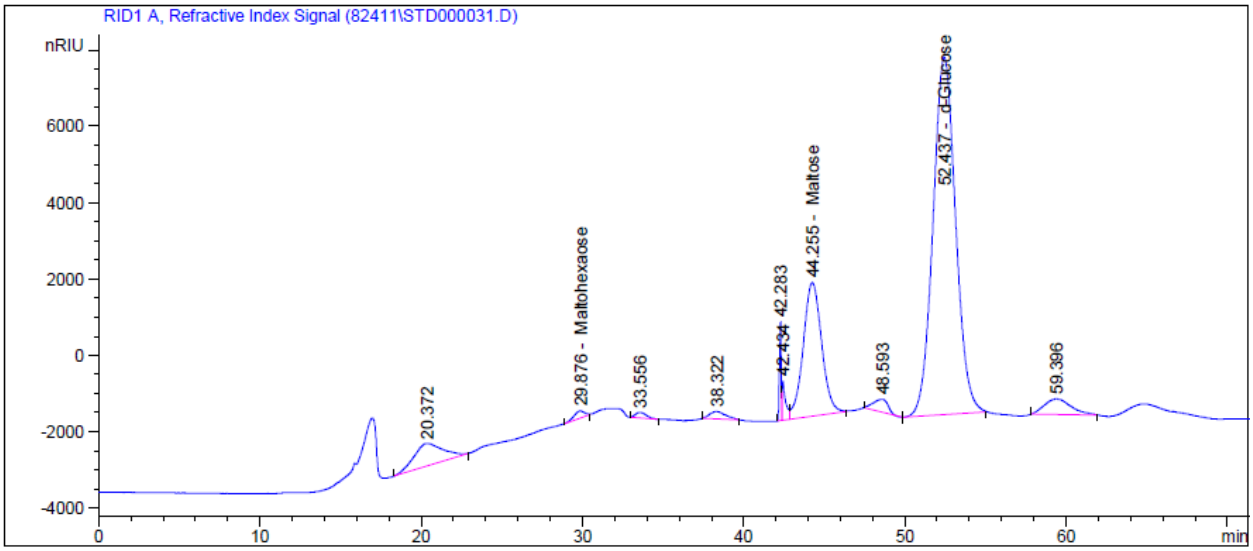

Fig. 36 — 10% v/v α-amylase treated solution chromatograph. Starch-based filter cake treated with 10% v/v α-amylase treatment solution.

removal efficiency of the α-amylase and deionized water solutions was negligible. This was due to the fact that after the degradation and effective removal of the starch

polymer, particles such as sized calcium carbonate remained on the filter. This problem was compounded by the fact that at high enough concentrations, significant enzyme residue remained on the filter cake the solution was designed to treat. **Fig. 37** shows that enzyme treatment solutions of 3%, 5%, and 10% are heavily stained by the enzyme solution after treatment.

Fig. 37 — Residual α-amylase on filter cake. Enzyme solutions leaving residue on the remaining filter cake after treatment of starch.

Accompanying the issue of enzyme residue remaining on filter cake is the necessity of removing the calcium carbonate from the paper filter to allow for flow. This was accomplished using the 5% HCl treatment solution that failed in the degradation of starch to remove the calcium carbonate.

Using the method described in 2.2.5, the cleanup efficiency was defined as the final time for 250 mL of 2% KCl to flow through the filter compared to the initial time before the deposition of filter cake and is presented in **Fig. 38**.

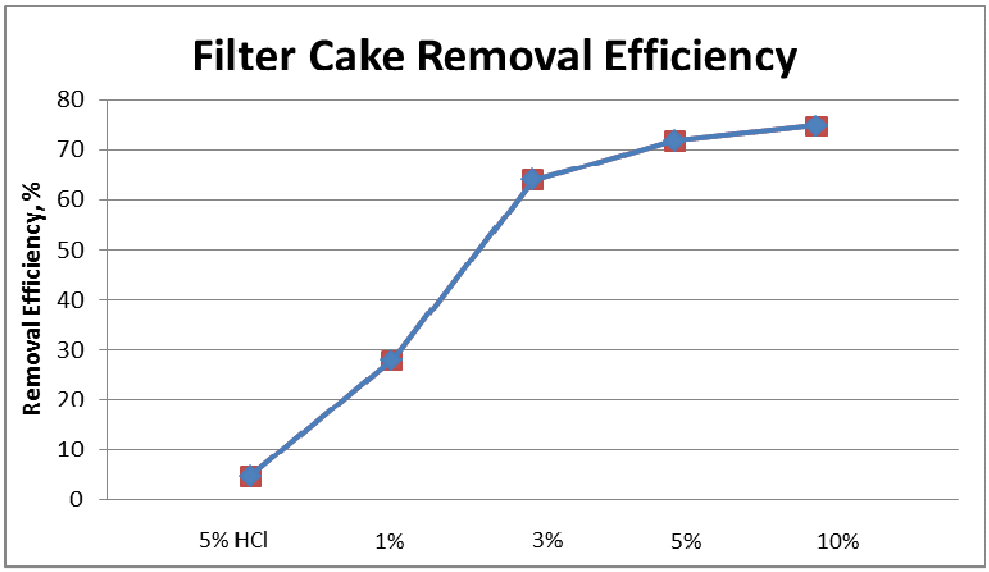

Fig. 38 — Filter cake removal efficiency. Filter cake removal efficiencies of treatment solutions tested.

Although the enzyme treatments proved to be very useful in the degradation of starch, the overall filter cake removal efficiency was not as robust as expected. The hydrochloric acid treatment was surprisingly poor and the higher concentration enzyme treatments were inhibited from reaching maximum return flow rates.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The diverse array of experiments utilized in this study served to guide me to the following conclusions.

1. The α-amylase enzyme irreversibly deactivates and begins to denature at temperatures above 165°F and reversibly deactivates at pH under 4.
2. α-amylase exhibits optimum activity at a pH near 6.5 and temperatures up to 150°F.
3. The α-amylase enzyme had a molecular weight mostly under 30,000.
4. Starch was effectively degraded by the α-amylase enzyme into limit dextrins and small oligosaccharides.
5. A chromatographic description of α-amylase hydrolysis of starch over time and starch in filter cake was furnished.
6. Spectrophotometric analysis of the enzyme treatment solution prior to and after treating filter cake did not show a discernible change in the α-amylase concentration of the solution.
7. A 5 weight percent hydrochloric acid solution is not effective and removing starch-based filter cake.
8. Enzyme treatment solutions were effective at degrading the starch component of filter cake but the process is incomplete until dissolution of calcium carbonate.

9. Higher concentrations of the enzyme treatment solution were observed to leave a residue on the filter cake they were used to treat that needs to be removed before the use of a strong acid to remove calcium carbonate.

Although much of this discussion has pointed out drawbacks and limitations of this treatment, the fact that it was able to effectively and completely degrade the starch component of filter cake is of paramount importance. Despite the fact that such a treatment would need to be done in more than one stage, incomplete degradation of starch would lead to an erratic and inconsistent treatment that would harm performance over the production interval.

The use of enzyme treatments that with greater temperature and pH tolerance is continues to be investigated. Genetic engineering of fungus and bacteria that produce these enzymes can allow for the production of thermo-stable α-amylase that can be of use in the deeper, high temperature/high pressure wells commonly drilled today. Despite the drawbacks associated with this treatment, it holds a great deal of promise in the future, and its applicability will expand as enhancements such as new stabilizers and genetic manipulation are implemented.

5.2 Recommendations for Future Work

Several aspects of this work could be further investigated.

An investigation of the return permeability generated by an enzyme treatment on a damaged coreface using a coreflood apparatus would provide important information

regarding the utility of the treatment. Similarly, a quantification of the damage potential for α-amylase used at high temperatures or low pH could be generated using this process.

Optical and electron microscopy of filter cake being treated by α-amylase could provide a better understanding of the interaction of enzyme and starch in filter cake.

The use of a dynamic filter press apparatus would allow for an investigation of enzyme transport into filtrate as well as augment the analysis filter cake removal efficiency.

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