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A Quantitative Method for Estimating α-Amylase-Based Enzyme Concentrations in Wellsite Field Samples and its Application on a Gravel Pack Completion

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Abstract

Enzymes are biological catalysts, or proteins that effectively speed up a chemical reaction without being consumed. There are many types of enzymes and like any catalyst, they are substrate or site specific. A starch-specific α-amylase enzyme was selected as the most effective means of cleaning up the filter cake deposited by a water-based mud on a well to be completed with a combination of premium sand screens and a gravel pack. Given the high cost and the importance of maintaining the programmed concentration of enzyme in the field, a rapid and independent means of accurately determining its concentration is potentially very useful.

This paper discusses a quick and relatively simple colorimetric method of approximating the concentration of an α-amylase enzyme solution in a mixed sodium chloride / potassium chloride brine. It was adapted for use at either the rig site or at an onshore laboratory from a technique originally devised by Ceska, *et al.*[1] and is based upon the reaction between α-amylase and a crosslinked, insoluble, starch derivative. Traditionally, enzyme assays would fall upon a third party onshore laboratory. However, this route invariably makes it an impractical proposition for "real-time" field operations. To prove the potential of this technique, regular samples were obtained over the course of a gravel-pack placement. Subsequent analysis of both samples and field data suggested that the cyclic filtration of solids-contaminated gravel-pack carrier fluid returns resulted in a loss of enzyme above programmed estimates. In some cases, this could potentially compromise the overall efficiency of the well cleanup.

Introduction

The *Gannet E* field is located in Block 21/30 in the Central North Sea, 100 miles east of Aberdeen. The well was completed in the *Forties Sand,* which is bounded by reactive shales. The 2060-ft horizontal interval was completed with a combination of premium sand screens and a gravel pack. Bottomhole Temperature (BHT) was 185°F (85°C).

A water-based drill-in fluid was used to drill the reservoir section. This consisted of a mixed sodium chloride / potassium chloride brine for density and inhibition, with added biopolymers and starch for viscosity and fluid-loss properties. Products for improved shale inhibition and sized calcium carbonate for bridging the formation were also included in the mix.

Cleanup of the filter cake trapped between the gravel and the screens is known to be difficult.[2, 3] This is particularly true when it contains clay-based drilled solids, not-to-mention sized carbonates.

In this situation, relying purely on filtercake flow-back is insufficient. Hence, a filtercake breaker is generally required. A number of cleanup options are available ranging from strong acids or oxidizers to polymer specific enzymes.[2-5] Each option has its advantages and disadvantages; the breaker selection is often dependent upon well conditions, the type of filter cake to be removed and health and safety concerns.

For this well, a starch-specific amylase enzyme, specifically an α-amylase, was selected as the most effective means of cleaning up the water-based mud filter cake. This was to be pumped with the gravel pack.

Hence, a key engineering component of this cleanup strategy was for a starch-rich water-based drill-in fluid to be used. Starch acts a binder for the calcium carbonate and other solids in the fluid, and, put simply, the α-amylase enzyme targets the starch in the filter cake for degradation. Once destroyed, the filter cake loses its integrity, allowing oil or gas to flow through it.[4] The process can be expensive. However, this is considered by many as one of the safest and most cost effective means of filtercake cleanup available.

Given the relative cost and the importance of maintaining the programmed concentration of α-amylase enzyme solution in the field, a rapid means of accurately determining its concentration both in the mud pits and during the gravel-packing process is deemed to be potentially useful. Normally, enzyme assays by onshore specialist laboratories would be the only means of

determining the concentrations of field samples. However, this process is invariably time consuming and therefore makes it an impractical proposition for on going rig operations.

To this end, a quick and relatively simple colorimetric method for estimating the concentration of α-amylase enzyme solution in a mixed monovalent brine based fluid was identified and adapted for this purpose. The results were then validated using established enzyme assay techniques by a third party laboratory.[6]

This paper details the adaptation of this method for potential field use and its application on a gravel pack completion. Based on the data obtained, a number of conclusions have been drawn that should be taken into consideration for any future wells of a similar nature.

Enzymes: Form and Function

Enzymes are biological catalysts, proteins that efficiently speed up a chemical reaction without being consumed or altered themselves.

Like any catalyst, reactions are substrate or site specific and many types of enzyme exist. For example, xanthanase enzymes can be used for catalyzing the hydrolysis of xanthan gum, whereas, amylase enzymes are used for breaking down starch and its derivatives. However, unlike many inorganic (or non-biological) catalysts, such as platinum or rhodium, enzymes work under less extreme environments. They are also restricted to specific chemical reactions involving biological molecules.[4, 7, 8]

Primarily due to their proteinaceous nature, enzyme function is dictated by physical parameters such as temperature and pH. Outside its normal operating window the enzyme will denature. That is, its protein structure will alter and it will cease to function effectively, if at all. Most importantly, enzymes demonstrate a high degree of specificity in terms of the reaction they catalyse, generally more so than any inorganic catalyst.[8]

For the *Gannet* well, the amylase selected was limited to a practical operating pH of between 8 and 9. Within this pH range, the upper temperature limits vary between 176 and 240°F (80 to 115°C), well within the operating temperature of this well of 185°F.

Enzymes function by a process known traditionally as the "lock and key" hypothesis as first devised by Emil Fischer in the late 19th Century.[7, 9] An enzyme substrate complex is formed by the alignment of matching substrate cleavage and active enzyme sites. When all sites are aligned, the substrate reacts to form an enzyme-product complex. This then splits (cleaves) into both enzyme and product, leaving the enzyme unaltered. This process continues rapidly until the substrate is degraded to a point where no further reaction can take place. This concept has since been further developed, notably, by Koshland with his "induced fit" theory.[9] However, the hypothesis of the matching-up of complementary structures more-or-less still prevails. These concepts are shown in Figures 1 and 2.

Starch and its Hydrolysis by Amylase

Starch, is a member of a group of compounds known as carbohydrates. Within this group occur simple sugars, known as monosaccharides. They are the basic building blocks of more complex oligosaccharide and polysaccharide structures.

Oligosaccharides are composed of only a few monosaccharide units, generally anything from two to ten. At the other end of the scale are the polysaccharides. These are carbohydrates of high molecular weight formed from a large number of monosaccharide units. Starch is a good example of a polysaccharide and one that is commonly found as a drilling fluid fluid-loss additive. It is normally present at relatively high concentrations making it a good candidate for enzyme hydrolysis.[3, 4] Structurally, starch is a mixture of linear (amylose) and branched (amylopectin) chains. Straight chain amylose makes up to 30% of most natural starches. It contains up to 1000 monosaccharide units. Amylopectin on the other hand is a branched form of amylose.[7]

There are two types of amylase, namely, alpha (α) and beta (β). They both hydrolyze (breakdown) starch although through different mechanisms. α-Amylase is the more effective of the two, and hence the enzyme of choice for this application. α-Amylase degrades starch polysaccharide into smaller oligosaccharide units, also known as dextrins, and simpler sugars.[10] (Figure 3).

Gravel Pack Operation and Enzyme Placement

The 8.5-in. reservoir section was drilled with a 10-lb/gal water-based drill-in fluid. A 7-in. pre-drilled liner was run to TD, the open hole displaced to clean filtered brine (10-lb/gal NaCl with 7% KCl) and a packer set. The pre-drilled liner was run to maintain both wellbore stability and to provide an additional Alpha Wave slurry path during the water packing process in the event that a shale section of the open hole collapsed during the pumping operation. After complete displacement to clean brine (inner liner and casing) the 4-in. premium sand screens were run-in hole and a packer set. The gravel packing process then commenced.

In long horizontal sections, a basic gravel pack consists of a two-step process.[11,12] The gravel is transported from the heel to the toe of the well as a slurry. As the slurry enters the annulus, its velocity decreases to a point where the gravel drops out of suspension, forming a dune on the low side of the well. As the dune builds in height a decrease in the cross-sectional annular flow area occurs causing the velocity to increase. This results in the sand along the top of the dune being transported further down the hole. This is a similar process as seen in riverbed sedimentation where

the gravel moves by hopping (saltation) of individual gravel grains along the top of the dune. This progressive movement of the gravel down the annulus is referred to as the Alpha Wave, generally filling 70 to 90% of the horizontal annulus.

As the sand reaches the end of the horizontal annulus it begins to back-fill the open space above the dune. This is known as the Beta Wave, which moves back towards the heel of the well, fully packing the annular space (Figure 4).

It was during the Beta Wave phase of the gravel pack that the α-amylase enzyme treatment was added to the slurry. This was deemed to be the best point at which to add the breaker. If added to the Alpha Wave, the enzyme would start to break down the filter cake, causing losses and the Alpha Wave to stall prematurely. When added to the Beta Wave, there was sufficient time to complete the gravel packing process, before any losses could occur.

For this operation, an enzyme treatment of approximately 3% by volume was required. Gravel pack brine returns were filtered over from the returns pit into the suction pit, with enzyme levels being maintained using a 40% concentrate. Viscosifying polymers were not used in the gravel packing process and the enzyme loss rate during the gravel pack operation was estimated at 30 to 40%.

Sampling

Samples were obtained over the course of the Beta Wave phase of the gravel packing operation from both the suction pit and the returns pit flow line. No sample was found to contain gravel-pack material. Icepacks were subsequently used to keep the samples cold until ready for analysis at an onshore laboratory.

The suction pit samples represent the mixture of filtered sodium and potassium chloride brine and the α-amylase solution prior to gravel addition. The flow-line samples represent the return pit samples immediately prior to filtration. This filtration step was performed between the returns and suction pits, at variable pump rates, using a combination of diatomaceous earth (DE) filters, in conjunction with both 10- and 2-micron filtration cartridges.

Samples were obtained from both the suction and return pits at roughly 15 minute intervals. Not all were analysed.

A Quantitative Method for Estimating α-Amylase Concentrations

A straightforward means of determining the relative concentrations of an α-amylase solution in monovalent brine was identified and adapted for potential use either offshore or at an onshore laboratory.

It has been adapted from a technique originally devised by Ceska, *et al*.[1] and numerous examples of its application can be found within the literature, particularly those in the field of biosciences.[13, 14]

The technique utilizes the enzyme hydrolysis of insoluble starch derivatives as a basis for determining amylase concentrations. For example, *Phadebas®* tablets (Pharmacia & Upjohn) can be used for this purpose. These contain a crosslinked, insoluble, blue-coloured starch polymer. After suspension in water the starch is hydrolysed by the α-amylase, giving soluble blue fragments. The absorbance of the resulting blue solution, measured at a wavelength of 620 nm using a visible spectrophotometer, is a function of the α-amylase concentration.

Preparation and solution development times can take up to two hours. Great care should be taken to avoid contamination from sweat and saliva. Experience has shown that only suitably trained personnel should run this test.

Sample Preparation

Initial filtration of all the enzyme samples was completed onshore as quickly as possible on their return using Whatman No.1 filter paper (retention size 11 microns). This step is designed to remove any residual gravel and formation solids.

Methodology

Apparatus:

- Water bath: 37°C ± 0.5°C
- pH meter
- Accurate pipette dispensers (0.000 – 1.000 mL and 1.0 – 10 mL)
- Pasteur pipettes and bulb
- Volumetric flasks
- Test tubes (disposable types are suitable)
- Accurate timer with seconds display
- Small plastic funnels and filter paper (Whatman No. 1 and Whatman No. 3 or equivalent)
- Spectrophotometer (Absorbance @ 620 nm ± 5 nm)
- Glass or plastic disposable cuvettes
- Vortex test tube mixer (optional)
- Tissue paper
- Disposable gloves

Reagents:

- Brine solution (field location sourced)
- Distilled water
- Same batch of α-amylase enzyme as designated for field operation
- *Phadebas tablets*

- Potassium Hydrogen Phosphate (K_2HPO_4)
- Sodium Hydrogen Phosphate ($Na_2HPO_4 \cdot 12H_2O$)
- Anhydrous Sodium Sulphite (Na_2SO_3)
- 1 Normal Sodium Hydroxide (NaOH)
- 4 Normal Hydrochloric Acid (HCl)

Buffer Preparation (to 500 mL with deionised water):

K_2HPO_4 = 1.30 g
Na_2HPO_4 = 7.21 g
Na_2SO_3 = 10.00 g

Adjust to pH 7.4 ± 0.05 with HCl. For 500 mL of buffer approximately 2.5 mL is required. Fresh buffer is required daily.

Test Procedure:

The analysis should be performed in duplicate, triplicate if possible. As this is a comparative test, it is important that the test procedure is performed as consistently as possible throughout, under clean conditions and that all assays within each series are run with *Phadebas* tablets from the same batch. Tablets must not become contaminated by human sweat. Therefore, clean gloves and tweezers must be used. If no vortex shaker is available then handshaking will suffice. All shaking should be kept as consistent as possible.

1. Ensure all glassware is clean and dry
2. Prepare calibration standards of *Enzyme* in field brine, e.g. 1, 3, 5 and 10 % v/v. [This will vary depending upon the concentration required at the wellsite.]
3. Further dilute these standards by taking 2.5 mL of each standard (or sample) and diluting with distilled water to 100 mL using a volumetric flask. [Please note that high concentrations of enzyme may require a higher dilution factor, e.g. 0.25 mL up to 100 mL]
4. Pipette 1 mL of each diluted standard solution into a clean test tube in duplicate.
5. Pipette 5 mL of fresh buffer into each test tube.
6. Shake each tube for 10 sec and then place in a water bath at 37°C ± 0.5°C for 10 min.
7. Remove the tubes briefly from the water bath and add a *Phadebas* tablet to each using tweezers. Start the timer.
8. Shake each tube consecutively for 10 sec and then place back into water bath.
9. Exactly 15 min after the addition of the *Phadebas* tablet, remove the tubes, timed consecutively, from the water bath and add 1 mL of 1 N NaOH solution. *[This stops the reaction.]*
10. Shake each tube consecutively for 10 sec.
11. Filter each solution through a Whatman No.3 filter paper (retention size 6 microns) into a clean and dry test tube.
12. Measure Absorbance @ 620 nm ± 5 nm within 1-hour period.
13. Plot a standard Calibration Curve of Absorbance versus Enzyme concentration.
14. Repeat Steps 3 to 12 using Whatman No.1 pre-filtered field samples. The same dilution used in Step 4 should be applicable. If not, adjustments may have to be made so the absorbance readings land on the calibration graph.
15. Read off the % v/v Enzyme concentration from the graph taking any dilutions into account. Discard values towards the end of the calibration graph that fall below the straight line.

Results and Post-Well Analysis

Field samples were measured at least three times. These were averaged and plotted against enzyme assay validation results obtained from a third party laboratory. Results are shown in Table 1 and Figure 5. Good reproducibility was obtained. However, the field results indicated a lower than expected enzyme concentration.

Predictive Modelling

Theoretical scenarios of predicted enzyme concentrations versus sample number were built. These models were then compared with the enzyme assay results as measured using both the field and third-party methods. These predictive models were based upon data supplied by the mud engineer at the wellsite and from the results of the third-party enzyme assays of each batch used. Theoretical and reported suction pit volumes pumped correlated to within ± 5%. (Figure 6).

Scenario 1: Assumes that the enzyme has not been retained on the filtration assembly and 100% enzyme activity.

Scenario 2: Assumes that the enzyme has been retained in its entirety on the filtration assembly and 100% enzyme activity.

Scenario 3: Assumes that the enzyme has not been retained on the filtration assembly and reduced activity based on batch enzyme assay results.

Scenario 4: Assumes that the enzyme has been retained in its entirety on the filtration assembly and reduced activity based on batch assay results.

The modelling assumptions have also taken into account the following:

- Pit volumes
- Pit maintenance volumes
- Down-hole pump rates
- Transfer pump flow-rate variability
- Homogenous mixing
- Returns rate of 65 %
- Accumulative enzyme concentrations (Suction Pit)

Study into the Effects of Drilled Solids on Enzyme Filtration

To further develop Scenario Numbers 2 and 4, which assumed that enzymes were being retained on the filtration medium, a simple experiment was performed that looked at the feasibility of drilled solids playing a part in the retention process.

Known concentrations of simulated drilled solids (Hymod Prima Clay) ranging from 0 to 4 % w/v were added to base brine together with 3% v/v α-amylase enzyme solution and rolled for 2 hours at ambient temperature. In this way, only the effects of the solids would then be considered rather than include any additional effects from thermal breakdown. The base brine used consisted of saturated NaCl with 3% w/w KCl and filtered to 0.45 microns. After rolling, the filtrate from each sample was collected with an API filter press using Whatman No. 42 filter paper (retention 2.5 microns).[15] The relative enzyme concentrations for each were then determined using both field and third party techniques. In this instance, enzyme depletion was noted with as little as 0.25% w/v drilled solids contamination, with 1.5 to 2 % w/v drilled solids resulting in the total stripping of enzyme from the base brine solution.

From these results, it is clearly evident that even small concentrations of drilled solids will have an effect on the filtration process. The exact mechanism for this could be the subject of further investigation. (Figure 7).

Discussion

A rapid and relatively simple method of determining the approximate concentration of α-amylase in a mixed monovalent sodium chloride / potassium chloride brine was successfully adapted for oilfield use. (Further work will be required if other types of brine systems are to be employed). This type of analysis would normally fall upon a third-party laboratory using automated enzyme assay techniques.[16] However, this process invariably makes it an impractical proposition for "real-time" field operations. The potential benefits of having a relatively fast test available, particularly at the wellsite, would be to provide a means of optimising enzyme concentrations in the pits, as a quality control tool, or as a way of highlighting improvement areas in the overall process.

Although enzyme concentration estimates can now be achieved in a more rapid manner than previously experienced, particular care and skill is required in order to obtain both accurate and consistent results. Good laboratory practice is essential. Water-bath temperatures and consistent timing between test stages are critical. Although the testing of unknowns can be completed within an hour or so, some time will be required beforehand in the preparation of fresh solutions and a calibration graph. It is equally important that the engineer or technician obtain some practice before conducting any critical testing at the wellsite.

When applied to the *Gannet* well situation, samples were obtained offshore with analysis being performed onshore as quickly as possible on their arrival. It was surprising how rapidly the enzyme concentrations appeared to deplete particularly after the first appearance of the "returns", with both methods confirming these trends.

At this juncture, in order to help explain this apparent rapid depletion of the enzyme as measured, numerical modelling was applied. Four predictive scenarios were modelled. One set assumed that there was zero retention of the enzyme by the filtration assembly during the operation (Scenarios 1 & 3), whereas, the remaining two models (Scenarios 2 & 4) assumed an accumulative retention of the enzyme on filtration. Other assumptions have also been made and these are detailed in the relevant text.

All four scenarios showed similar trends. Those modelled on accumulative enzyme retention gave the closest correlation. That is, the modelling suggests that the filtration assembly is systematically filtering out all or some of the enzyme before it has a chance to be recycled. At the time of the operation, knowing that the filtration cartridges were of a size that allowed enzyme material to pass through, this was not thought to be an issue. Therefore, the potential for drilled solids to affect the filtration efficiency with a resultant decline from programmed enzyme concentration was considered amongst other scenarios.

Further laboratory testing with simulated drilled solids confirmed this hypothesis, showing that as little as 0.25% w/v could have an effect on the efficiency of the filtration process. Specifically, the effect was on the ability of the enzyme to pass through the filtration assembly unhindered.

Another learning point is that in future operations, duplicate samples shall be obtained to allow concurrent analysis for both drilled solids and enzyme levels. With the advent of this quantitative method it is now possible to closely monitor "real-time" enzyme concentrations throughout the gravel-packing process.

Conclusions

- A rapid method of determining the concentration of α-amylase in a mixed sodium chloride / potassium chloride brine was successfully adapted for potential use offshore.

- Personnel will require specific training before conducting any critical testing at the wellsite. Good laboratory practice is essential.

- Calibration plots should be constructed using α-amylase batches specific to the operation. Enzyme batches of higher activity may require further dilution.

- In cases where more than one batch of α-amylase is programmed for use, calibration solutions should be mixed in the same proportions.

- Storage conditions, such as time and temperature, will affect enzyme activity.

- Post-well analysis identified that the presence of even small concentrations of drilled solids hindered the free passage of enzyme through the filtration assembly.

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Table 1 – Enzyme Field Method and *Validation Assay Results												
Field Sample No.	**Suction Pit (IN)** ("Field" Method)					**Suction Pit** (Validation)	**Return Pit (OUT)** ("Field" Method)					**Return Pit** (Validation)
				Average	**SD**					**Average**	**SD**	
1	2.05	2.05	2.05	2.05	0.00	2.64	0	0	0	0.00	0.0	< 0.30
5	1.40	1.20	1.35	1.32	0.10	1.45	0	0	0	0.00	0.0	< 0.30
9	1.75	1.80	2.20	1.92	0.20	2.64	0	0	0	0.00	0.0	< 0.30
13	1.00	1.05	1.00	1.02	0.03	1.36	0.65	0.65	0.70	0.67	0.03	1.55
17	0.35	0.40	0.45	0.40	0.05	0.60	1.25	1.22	1.20	1.22	0.03	1.45
21	0.35	0.45	0.45	0.42	0.06	0.55	0.85	0.85	0.85	0.85	0.0	1.09
25	0.30	0.30	0.30	0.30	0.00	0.37	0.40	0.45	0.45	0.43	0.03	0.50
29	0.25	0.20	0.20	0.22	0.03	0.40	0.20	0.20	0.20	0.20	0.0	0.60
* Third party procedure for enzyme analysis has been validated; internal variation (between replicates) has been reported at ±2%.												

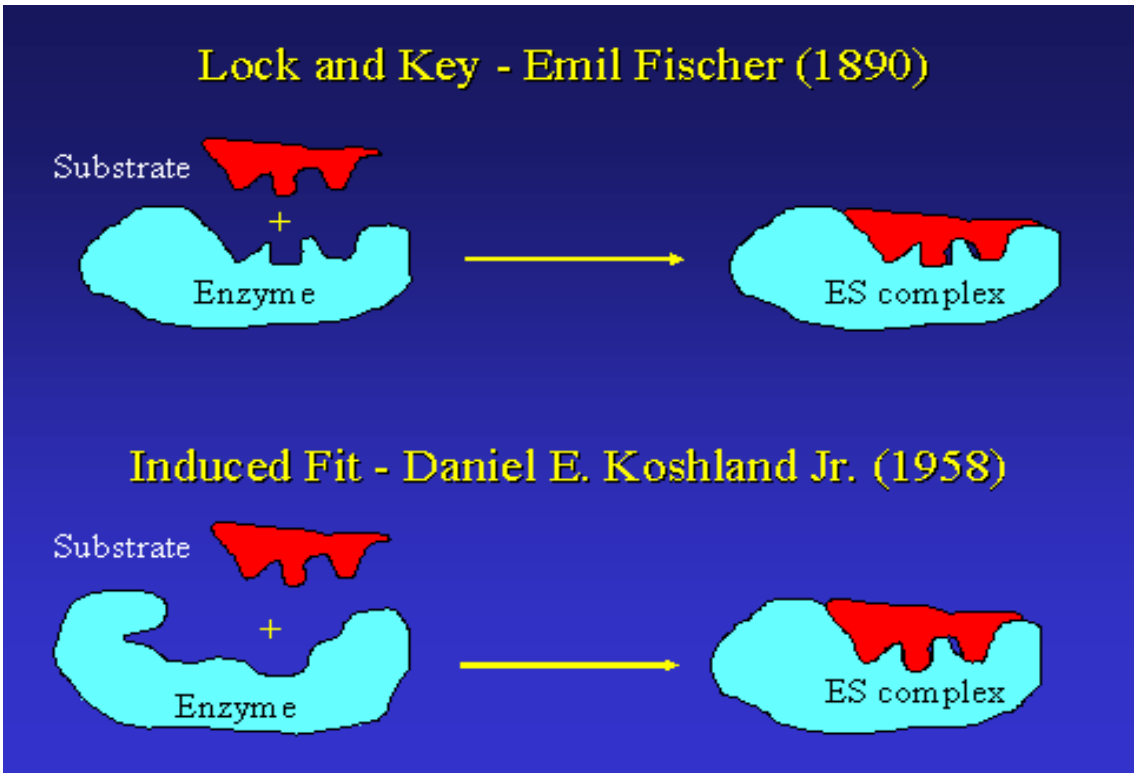

Fig. 1 – Schematic models demonstrating the theories of enzyme specificity through the matching-up of complementary structures; Fischer's "rigid" model of enzyme/substrate interaction versus Koshland's more flexible theory.

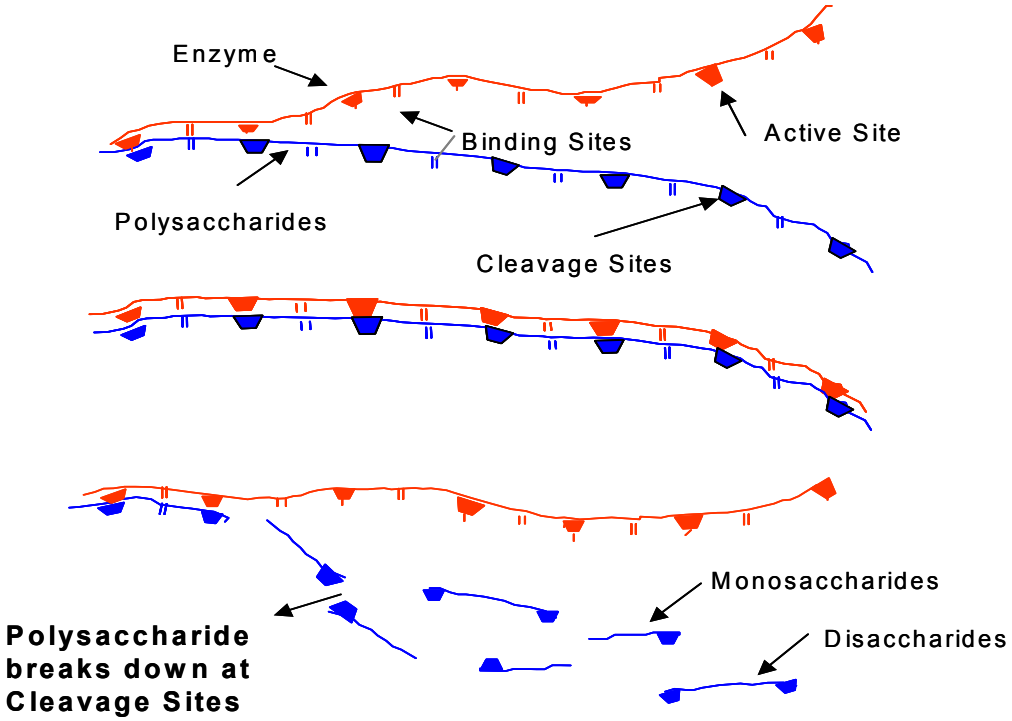

Fig. 2 – Schematic models demonstrating the alignment of matching substrate cleavage and active sites resulting in the breakdown of polysaccharide to smaller units.

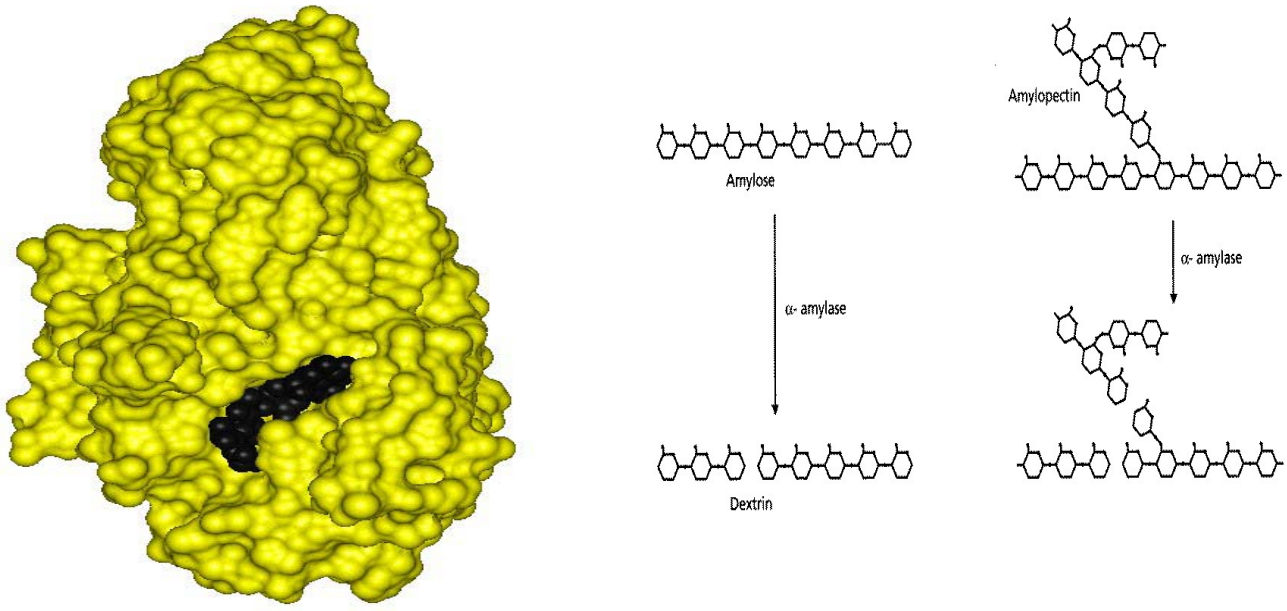

Fig. 3 (a, left) – Structure of enzyme-substrate complex for α-amylase and five monomer units of a starch polysaccharide (black). Fig. 3 (b, right) – Reaction mechanism for the degradation of amylose and amylopectin to oligosaccharides by α-amylase.

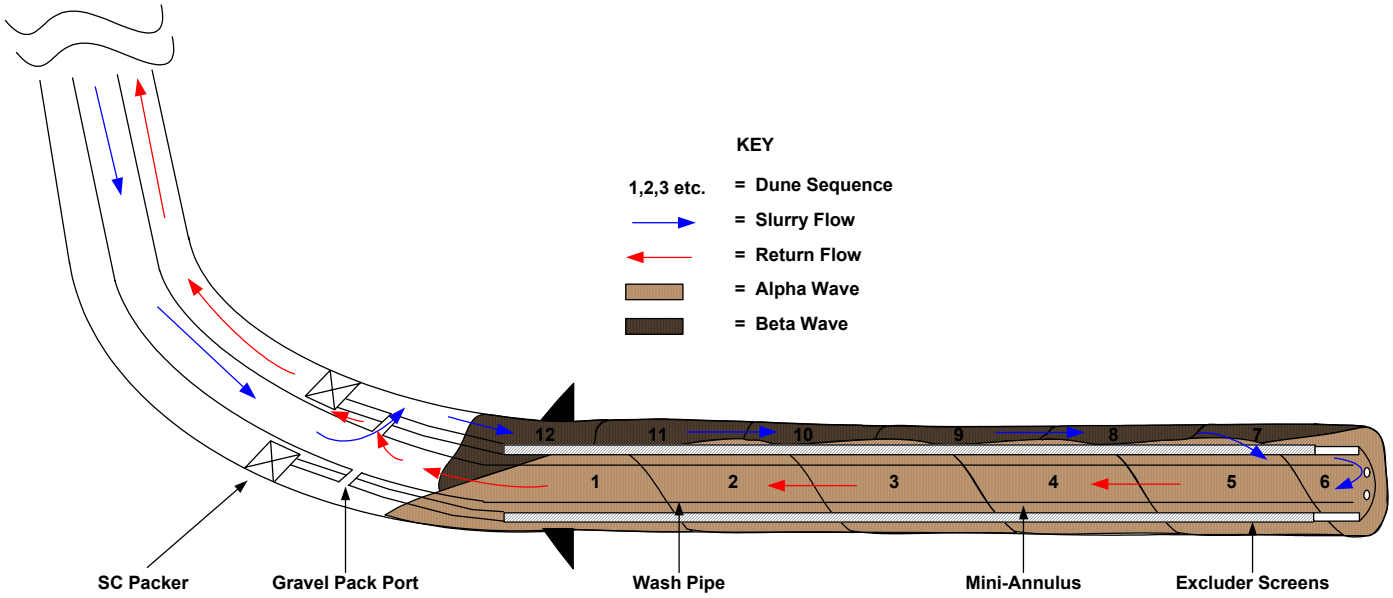

Fig. 4 - Alpha and Beta Wave progression during a horizontal gravel pack.

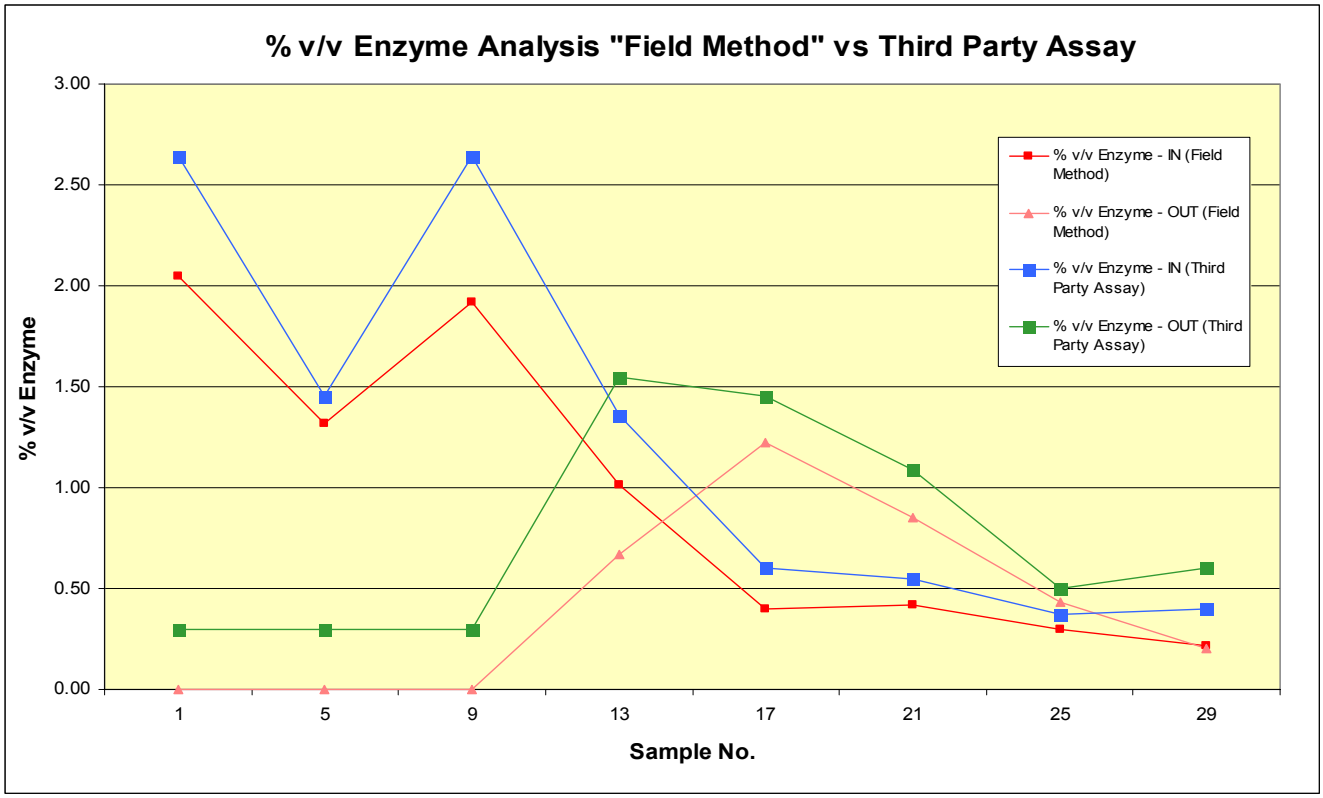

Fig. 5 – Enzyme analysis of suction pit (IN) and return pit (OUT) by "field" method and third-party assay.

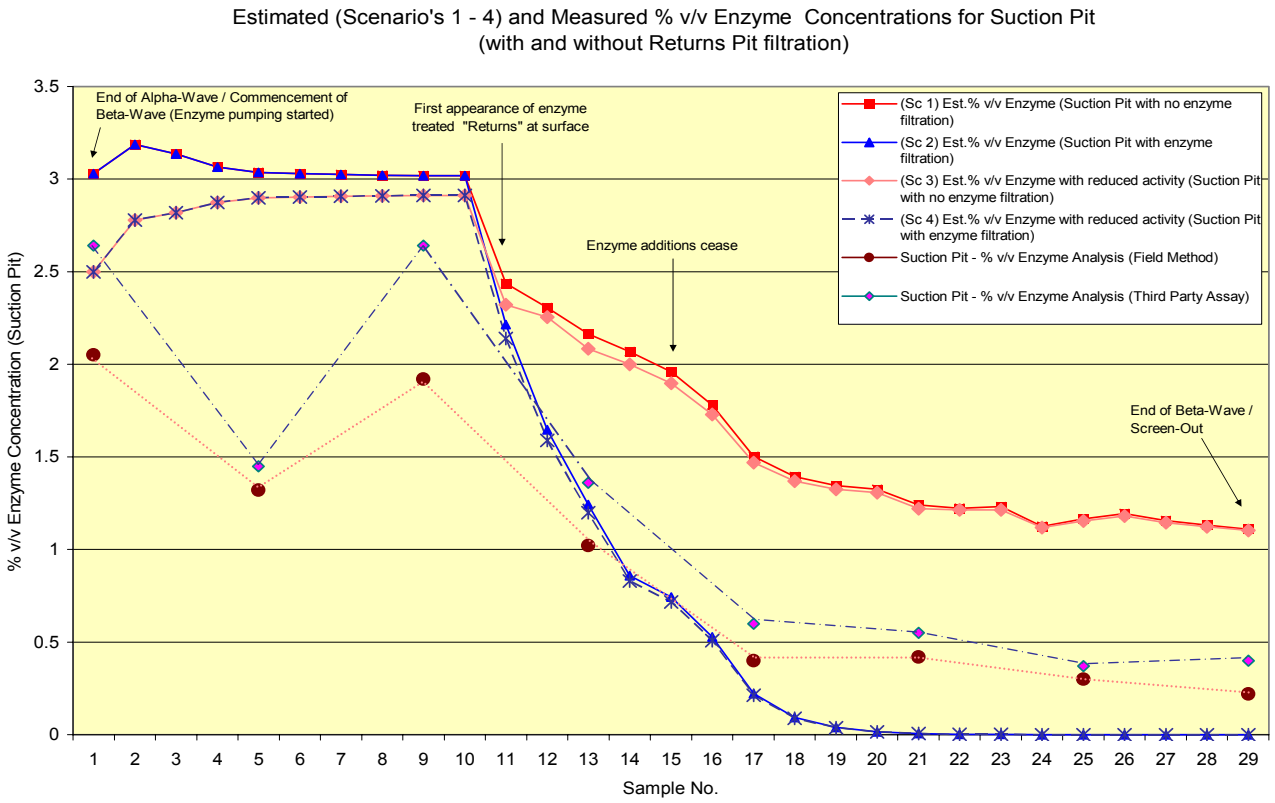

Fig. 6 – Predictive Scenarios 1 – 4.

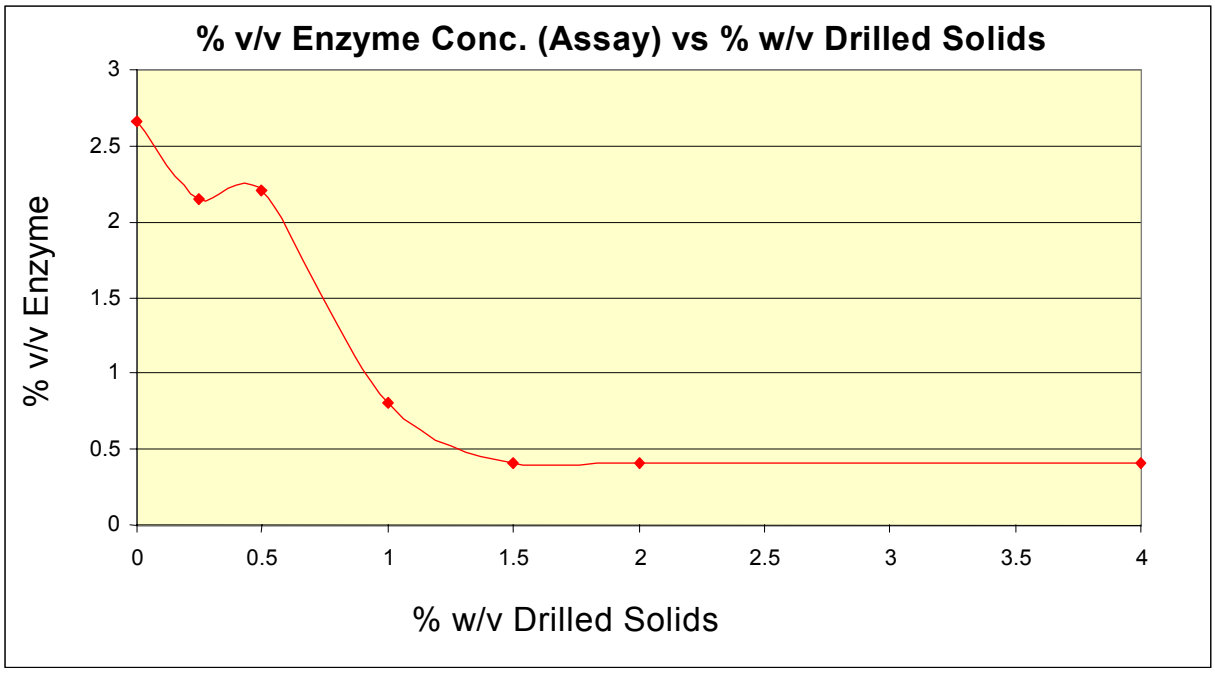

Fig. 7 – Study into the effects of drilled solids on enzyme filtration.