Oilfield Biotechnology based 100% Biodegradable Enzyme Proteins Designed to Treat Oil & Gas Well Drilling Damages, Estimation and Design of Formulation based on Predictability Models

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Oilfield Biotechnology based 100% Biodegradable Enzyme Proteins Designed to Treat Oil & Gas Well Drilling Damages, Estimation and

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Debayan Ghosh, Dana Trono, Punit Bansal

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Epygen Labs FZ LLC, Dubai, UAE

Abstract

It is well established that the productivity of a horizontal well can be significantly impaired by residual filter cake following drilling and completion operations. Mitigation of drilling-induced formation damage remains an active area of research, with substantial developments over the past decade. The objective of this study is to develop a non-corrosive wellbore clean-up fluid comprising an enzyme system developed through site-directed mutagenesis and an organic acid, formulated both as an aqueous solution and in a buffered state, to remove external filter cake and reduce skin damage to near zero prior to well completion.

Biopolymer-specific genetically engineered enzymes targeting cellulose, xanthan, starch, and guar derivatives were developed to ensure compatibility with weak acid and buffer formulations used in the cleanup fluid. The dual-action mechanism of depolymerization and dissolution was designed to penetrate deeply into CaCO3–biopolymer-based nanodamaging drilling fluid filter cake in a controlled and systematic manner, rather than through aggressive, localized reactions typical of conventional acid or oxidizer treatments. This approach resulted in complete dislodgement and removal of the filter cake from the wellbore.

Weak organic acids such as acetic acid and corresponding buffer systems were formulated to

Corresponding author E-mail : debayan@epygen.com

dissolve carbonate particles while maintaining corrosion levels below the maximum acceptable limits. Compatibility tests between the weak acid–buffer systems and the protein-engineered enzymes were conducted to validate the formulation stability. The effectiveness of each organic acid and buffer system was evaluated by estimating filter cake removal efficiency under HPHT conditions, while corrosion rates were measured to assess the metallurgical advantages of the proposed system over conventional corrosive treatment fluids (e.g.: Hydrochloric acid and Hydrofluoric acid).

The formulated clean-up fluid was successfully tested on ceramic disks in HP HT system. The polymer-based drill-in fluid produced a very low-permeability filter cake (<0.1 mD), which significantly minimized internal filtrate invasion. Controlled deposition of this lowpermeability filter cake on carbonate core plugs resulted in reduced damaging invasion. Several ester hydrolysis reaction kinetics were evaluated, and a suitable acid precursor was selected based on its ability to generate the required acid concentration within three hours under downhole conditions. The selected precursor generated approximately 3% acid after three hours and maintained low acid concentrations for up to 12 hours.

Measured corrosion rates were significantly below industry-accepted limits, eliminating the need for acid corrosion inhibitors. Return permeability of muddamaged carbonate core plugs reached up to 96%

following exposure to the clean-up solution. A new drill-in fluid was developed, and its associated filter cake was completely removed using the recombinant enzyme–buffered acid. Unlike conventional wellbore clean-up fluids, which are highly acidic and require corrosion inhibitors, the proposed formulation effectively minimized corrosion of downhole tools without the use of corrosion inhibitors.

Keywords: Drill-in fluids; Non-damaging; Noncorrosive; Zero skin damage; Ideal packing theory, Oil & Gas Enzyme, Filter-cake clean-up

1. Introduction

Horizontal wells enable drainage over extended wellbore lengths, allowing lower drawdown rates compared to vertical wells.

The application of substrate-specific enzymatic wellbore clean-up fluids allows controlled and uniform reaction along the entire wellbore, as demonstrated in several studies conducted under simulated downhole conditions using systematic core-flood and HPHT filtration loss experiments (Ghosh et al., 2019). In addition, these systems are relatively mild on downhole equipment, as the reaction products are environmentally friendly and only mildly corrosive, remaining within industry-accepted corrosion limits (Al-Khaldi et al., 2011; Mehtar et al., 2012). Unlike strong acids (hydrochloric acid and hydrofluoric acid) and other chemical oxidants, Enzymes do not interact with formation or metals present in the downhole and hence does not allow undesirable secondary reactions.

Properly designed polymer-based drill-in fluids (DIFs) form a strong, nearly impermeable mud cake on the wellbore wall, thereby preventing or minimizing formation damage caused by particulate and fluid invasion. Common DIF formulations typically contain xanthan polymer, starch, or polyanionic cellulose, along with pore-bridging agents such as sized calcium carbonate particles. Xanthan

functions primarily as a viscosifier, while modified starches and cellulose polymers are used to control filtration loss. Together, these components form a near-impermeable flow barrier on the wellbore wall (Alcheikh and Ghosh, 2017). Following drilling operations and prior to well completion, removal of this flow barrier is critical to restoring well productivity or injectivity.

Conventional wellbore clean-up systems for water-based mud filter cake removal include hydrochloric acid (HCl), weak organic acids, chelating agents, oxidizing breakers (peroxides), and delayed-release acids (Brady et al., 2000; Harris et al., 2001; Al-Otaibi and Nasr-El-Din, 2005). These treatments primarily focus on dissolving calcium carbonate bridging particles within the mud cake. However, they are often ineffective at dissolving the polymeric components that form a protective coating around $CaCO_3$ particles, resulting in incomplete wellbore cleaning. Studies by Al-Kitany et al. (2008) have shown that HCl and oxidizing treatments tend to create wormholes and achieve only partial filter cake removal. Furthermore, concerns regarding high corrosion rates at elevated bottom-hole temperatures and rapid reaction kinetics limit treatment penetration, leading to localized degradation near the point of introduction and leaving large portions of horizontal sections partially or entirely unproductive.

The introduction of polymer- or substrate-specific bioderived enzymes has addressed several limitations associated with harsh chemical treatments (Brannon et al., 2003; Cobianco et al., 2007). Their controlled reaction with polymers—the primary binding components of the mud cake—combined with mild reaction kinetics and favorable HSE profiles, has encouraged many operators to adopt these systems for wellbore clean-up (Mehtar et al., 2012). The properties of enzyme's high specificity, which allows the activity to be accurately controlled with respect to specific polymeric substrate and the enzyme's catalytic efficiency. Enzymes degrade biopolymers by breaking polymer chains, enabling effective removal of filter cake and restoration of the well's natural flow capacity. Xanthan-specific enzymes cleave β-D-mannosyl-

β-D-1,4-glucuronosyl linkages (Hashimoto et al., 2003), while α-amylase hydrolyzes α-1,4 glycosidic bonds in starch backbones to produce water-soluble oligosaccharides containing 2–10 sugar units (Maureen, 2000).

Fig. 1a: Catalytic Domain of a typical

a-amylase enzyme

Fig. 1b: Schematic representation of Stabalize Enzyme Acid

formulation – Dual Action on Biopolymer and Calcium

carbonate on a water based mud filter cake

Similar studies involving galactomannanase enzymes applied to guar-based linear and cross-linked gels have demonstrated 94–98% clean-up efficiency with more than 99% viscosity reduction (Abdelrahim et al., 2021; Ghosh et al., 2021).

The gentle action of α-amylase-based clean-up fluids (Fig. 1a and 1b) on starch – and xanthan-based filter cakes

enable placement across the entire horizontal section prior to significant reaction onset, resulting in uniform dissolution and removal of the mud cake along the wellbore. Weak and dilute organic acids, such as acetic acid, are commonly incorporated to react with $CaCO_3$ bridging particles, thereby disrupting the mechanical integrity of the mud cake and enhancing clean-up efficiency (Stanley et al., 1999; Nasr-El-Din et al., 2005; Ghosh et al., 2020). This enzyme–acid combination acts synergistically: enzymes remove polymer coatings from $CaCO_3$

particles, exposing them to acid dissolution, while acid reaction and CO2 generation weaken mud cake structure and facilitate deeper enzyme penetration.

However, naturally occurring (wild-type) enzymes exhibit limitations that restrict their application under hightemperature, high-salinity reservoir conditions, particularly in acidic environments. Their stability and activity are confined to narrow ranges of temperature, salinity, and pH, as illustrated in Fig. 2 (Dutta et al., 2006).

Fig. 2: Stability and relative activity of wild strain

α-Amylase enzyme:2-A Effect of temperature, 2-B effect of pH, 2-C Effect of exposure time and

2-D Effect of salinity (From Dutta et al. 2005)

Enzyme denaturation at elevated temperatures and low pH, reduced activity in high-salinity carrier fluids, and potential formation damage due to precipitation have driven the development of genetically modified, chemically

robust, and thermally stable amylase enzymes (Cobianco et al., 2007; Samuel et al., 2010; Al-Khaldi et al., 2011).

In the present work, a hybrid amylase–carbohydrase enzyme was modified to withstand higher thermal stress by structurally reinforcing the β-helix layer to stabilize the catalytic center using preferential protein hydration techniques. Polyols were employed as co-solvents with water to enhance enzyme stability (Shimizu and Smith, 2004). The modified enzyme was evaluated for thermal and pH tolerance, as reported elsewhere (Al-Khaldi et al., 2011). The primary contribution of this study lies in the systematic experimental work conducted to stabilize the enzymatic clean-up formulation in the presence of various organic acid concentrations through optimization of buffer systems and evaluation of kinetic rate constants for enzyme–acid interactions during polymer degradation.

Kinetic rate constants for different formulations were determined by measuring reducing sugar release during the reaction between enzymatic clean-up formulations and starch-based drilling fluids. Laboratory investigations were undertaken to develop a solution capable of achieving the following objectives :

1. Effective removal of starch–biopolymer–CaCO3

damage, 2. Optimization of a single-step treatment for simultaneous

starch and calcium carbonate removal, 3. Consideration of environmental impact of back-flowed

fluids, 4. Minimization of corrosion to downhole equipment, and 5. Evaluation of practical placement options

2. Experimental Materials & Methods

2.1. EQUIPMENTS:

- Standard laboratory equipment & glassware
- Spectrophotometer, pH meter
- Water-bath, Hot Air Oven
- Centrifuge

2.2. REAGENTS:

- Drilling Grade Starch, Xanthan and CaCO3
- Modified thermally stable Hybrid Amylase Carbohydrase Enzyme (Develped by Epygen Labs FZ LLC, Dubai, UAE)
- Acetic Acid (99% Glacial) Sigma Chemicals
- Sodium acetate - Sigma Chemicals
- Sodium hydroxide – Sigma Chemicals
- 3, 5–Dinitrosalicylic Acid solution (DNS) – Sigma Chemicals
- Maltose – Sigma Chemicals

2.3. METHODS:

The development of cleanup fluid and their performance analysis were conducted through the following stages:

A. Optimization of solution pH and buffer composition: As discussed above, for effective dissolution of mud cake, it is essential to have the presence of enzyme as well as a hydrogen ion delivery mechanism, preferably a weak acid in the cleanup solution. However, most enzymes suffer a temporary disorientation of the 3D configuration, disengaging the active site, rendering a retarded substrate binding action and in some extreme cases irreversible denaturation (resulting coagulation and precipitation) at pH below 4.5. However, with equivalent acid loading, when the solution is buffered above pH 4.5, the cleanup formulation is stable for a long time. To quantify the requirement of buffering salt, different concentration of acetic acid (from 3% – 6% V/V) were mixed with enzymatic cleanup solution and their pH were adjusted with buffering salt added in small aliquots. This process was continued till the final pH of each solution has reached between pH 4.5 – 5.0. Two sets of experiments were conducted (with and without enzyme in the solution). The experimental data are presented in Fig 3 & 4.

Fig. 3: Buffering of acetic acid solution for pH optimization

(in absence of Enzyme)

B. Optimization of Acetic Acid concentration and minimum soaking time: Understanding the dissolution capacity of acetic acids and the dissolution rate of $CaCO_3$ is critical in optimizing the acid loading in the cleanup fluid formulation. This would depend among other factors, the $CaCO_3$ loading in the mud, bottomhole temperature and the interference of other ingredients (salt, enzyme etc.). The acid dissolution rate studies were conducted with two sets of acid formulations. The first set of tests were conducted with enzyme cleanup formulation, loaded with 1%

Fig. 5: $CaCO_3$ dissolution rate at different acid concentra-

tion in the cleanup solution

Table 4: $CaCO_3$ dissolution time under various acetic acid concentration

3% AA

CUF with 1%

CUF with 2%

CUF with 3%

Fluid composition

without Enzyme

6% AA Buffered Maximum Dissolution 99.80% 46.34% 89.50% 99.40% 81.40% 99.60% 97.60% 98.80%

AA

AA

AA

Time for dissolution 1.5 Hrs 40 Hours 20 Hrs 4.5 Hrs 40 Hrs 23 Hrs 4 Hrs 1 Hr

Fig. 4: Buffering of acetic acid solution for pH optimization

(in presence of 6.5% Enzyme)

– 3% acetic acid having their natural pHs. A second set of experiments were conducted with enzyme cleanup solution having acetic acid loading between 3% – 6% and buffered to raise their pH near 5.0. In 100 ml of these solutions, 1.5 gm of fine grade $CaCO_3$ was mixed and the remaining $CaCO_3$ in the solution were determined by measuring CO_2 released from the system. The details of the test method are described by Holm-Jensen (1960). $CaCO_3$ dissolution rate at different Acid and pH conditions are presented in Fig 5 & 6 and also in Table 4.

Fig. 6: $CaCO_3$ dissolution rate at diferent acid concentra-

tion in the Cleanup solution (buffered)

CUF with

CUF with

CUF with

CUF with

3% AA Buffered

4% AA Buffered

5% AA Buffered

C. Corrosion Test: The corrosion rates were measured using the weight loss on steel coupons. The steel coupons were rubbed against sandpaper to smoothen the surface and remove any contaminants. Coupons with smooth surface were selected for the study. Subsequently, coupons were cleaned with calcium chloride (CCl_4) solution, followed ethanol, and then dry in oven. Prior to corrosion test, the weight (in mg) and surface area (in cm2) of selected coupons were measured using precision weighing balance and steel calliper, respectively.

The steel coupons were immersed in the corrosion cell containing the clean-up solution. The cell was completely sealed to avoid evaporation loss and oxygen entry. The cell was then placed in container with different clean up solution (Table 1) with stirring to create minimum turbulence for 2 hours at desired temperature. Followed by static exposure (no stirring) for 8 hours at constant temperature. After treatment exposure, treated steel coupons were gently rubbed with green scrub to remove corrosion materials. Final weights of steel coupons were measured after drying.

□□□□□□□□□□□□□□□(□□

□□□□) = 87.6 □(□ □□□□□)

where, W = weight loss in mg D = metal density in g /cm3

A = total surface area of coupon in cm2

T = time of exposure of the metal sample in hours

Table 5. Corrosion rate of different cleanup fluids at

different temperatures

CUF-1 CUF-2 CUF-3 15% HCl

Temperature

(°C) Corrosion Rate in MPY

Corrosion rate of

50 1.77 2.31 3.29

15% HCl in deionized water at

70 2.3088 2.89 3.638

90 2.66 3.31 4.28

25°C is 78.82 Extrapolated Values

100 2.90888 3.5867 4.4835 NA

110 3.13088 3.8367 4.7315 NA

120 3.35288 4.0867 4.9795 NA

130 3.57488 4.3367 5.2275 NA

D. HPHT Filter Cake Efficiency: A high-temperature and organic acid-tolerant α-amylase enzyme (Ghosh et al., 2019) was developed through “directed mutagenesis” and “protein engineering” tools to minimize denaturation at high temperatures and the underlying chemistry. With the suitable modification of its macro-structure, α-amylase enzymes can achieve high temperature tolerance and maximum dissolution of the residual polymers (starch and xanthan) in the mud cake which disrupts the filter cake for the acid to attack uniformly.

Table 6. Composition and Cake Removal Efficiencies

of Clean-Up Solutions

Acetic

Test

No. Enzyme MF Acetic

2% KCl

Treatment

Clean-up Efficiency

Acid Surfactant

Acid Buffer

Duration

1 6% - 3% 1% - 90% 6 hr 89.70%

2 6% 15% - 1% - 78% 24 hr 90.40%

3 6% - - - 15% 79% 24 hr 91.10%

The filter cake clean-up fluid was prepared by using the modified α-amylase enzyme to degrade the polymeric matrix and a mild acid to react with the calcium carbonate bridging particles. The effectiveness of clean up solution was evaluated using an (American Petroleum Institute) API HPHT Filter loss equipment and 50 microns ceramic discs as porous media. The cleanup formulation was designed as per Table 6 varying recombinant enzyme and buffer proportions to evaluate cake removal efficiency. An overbalance of 100 psi and a temperature of 90OC were maintained for all tests. The cake deposition was allowed for 1 h, and the given exposure time for the cleanup treatment was for 6 and 24 h. The clean-up efficiency was calculated using the equation below:

□□□□*h*□□□□□□□□□□□□□□□□□−□□□□*h*□□□□□□□□□□□□□□□□□□□□□□□□

□□100

□□□□□□□□□□□□□□□□−□□□□□□□□□□□□□% = □

□□□□*h*□□□□*h*□□□□□□□□□□□□□□□

E. Reducing Sugar Assay:

Maltose standard: The maltose standard curve (Fig. 7) was prepared as follows. In numbered tubes, five maltose dilutions were prepared ranging from 1mM to 10mM. Distilled water was used as blank. One ml of each maltose dilution was pipetted into a series of corresponding

numbered tubes. Then, 1.2ml DNS solution was added into each tube. Reaction mixtures were allowed to stand at room temperature for 15 mins. Incubated at 95°C in a water bath for 5 minutes and cooled down in water bath for 10 mins and further allowed to stand at room temperature for 10 mins. An aliquot of 200µl reaction mixtures were diluted with 3ml distilled water in a cuvette. Absorbance was taken at 540nm.

Maltose Standard Curve

0.6

0.5

Absorbance (540 nm)

y = 0.0544x - 0.0029

0.4

R^2 = 0.998

0.3

0.2

0.1

0

0 2 4 6 8 10 12

Maltose Concentration (mM)

Fig. 7: Standard Reducing Sugar Optical

Density plot of Maltose solution

Preparation of DNS Solution: Suspended 20.0 g of 3, 5–dinitrosalicylic acid in 1000 ml of distilled water and gradually added with 300 ml of the 10.67 % (w/v) sodium hydroxide solution while mixing. The solution was heated to 50°C in water bath until it was clear. Gradually, 600 g of potassium sodium tartartetetrahydrate was added to the solution with continuous mixing. The final volume was made up to 2,000 ml with distilled water and filtered through a coarse sintered glass filter. Stored in a dark amber bottle at room temperature for further use.

Reducing Sugar Test Procedure:

A. 10 g of starch biopolymer DIF sample was weighed in

autoclave grade bottles and their pH measured.

B. The control, Blank and Test Clean up fluids (CUF)

were prepared.

C. The CUFs were gradually added to starch biopolymer

DIF samples with stirring while effervescence was observed. pH was recorded at 10 minutes after effervescence settled.

D. The final mixture of CUFs and mud samples were

incubated at 95oC for 2hrs, 4hrs, 6hrs, 8hrs and 20 hrs with mixing at intervals of 30 min.

E. For reducing sugar estimation 5 ml of sample was

collected from the reaction mixture.

F. Release of Reducing Sugar was estimated by DNS

method as follows :

• 5 ml of sample collected after reaction was centrifuged at 3000 rpm for 10 minutes.

• 1 ml of supernatant from each sample was taken for reducing sugar estimation.

• 1.2 ml of DNS-solution was added to 1 ml of sample, capped and vortex mixed. The solution was kept at room temperature for 15 mins.

• DNS reaction was carried out in water bath at 95oC for 5 minutes

• After DNS reaction, samples were kept in water bath for 10 minutes at room temperature, followed by room temperature for 10 minutes. The absorbance of the Control, Blank and Test was measured at 540 nm against distilled water blank and DNS reagent blank.

Calculation:

Reducing sugar (Maltose Equivalent) =

□

□

□ □ □ □

□ □ □

□ □ □

□ □ □ □ □ □

YIntercept Abs Abs dilution Final a 1 : 1

□

□

a

.....(Eqn 1)

EVALUATING KINETIC RATE CONSTANT K (Wang 2004 & Ghosh et al 2012):

Residual Biopolymer (substrate) after the enzymatic Hydrolysis can be calculated by: [St] = [So] – [RS]

Where [St] is the theoretical Biopolymer residue (mgs/ ml) at time ‘t’ of enzymolysis, [So] is initial Biopolymer concentration (in this case 473.16mgs/ml), and [RS] is

reducing sugar concentration (mgs/ml) in the supernatant of samples.

The net Hydrolyzed amount of Biopolymer can be determined by : [RS] = [So] - [St]..... (Eqn 2)

The relation between residual Biopolymer and treatment time: [St] / [So] = e-kt.....(Eqn 3)

Thus: [RS] / [So] = 1- e-kt.....(Eqn 4)

From the above equation : ...(Eqn 5)

Being a linear equation, the rate constant can be calculated by plotting ln(100 X [St]) / [S0]) values for each enzyme treatment against time for calculating the slope.

Determination of [S0]: Initial substrate concentration [S0] was determined by hydrolyzing the Starch Biopolymer drilling fluid with maximum concentration of enzyme (50% in CUF which is ten times of standard concentration, high % used to remove enzyme limitation factor in digestion) at 95ºC for 6 hrs. The release of reducing sugar was determined by DNS method and values were expressed in milligrams per milliliter. Experiment was carried out in triplicates. The average value of the reducing sugar was found to be 473.16 mgs/ml.

F. Composition of drilling and clean up fluids: Composition of the drilling & cleanup fluids are given in Table 2 & 3 respectively.

3. Results and Discussion

Experimental results presented in Figs. 3 and 4 demonstrate that the addition of buffering salts allows the clean-up formulations to be maintained at a pH of 4.5 or higher, which is a critical requirement for ensuring longterm stability of the enzymatic system. The amount of buffering salt required, however, varies depending on the organic acid concentration used in the formulation.

Table 1: Cleanup fluid compositions in deionized-

deoxygenated water

CUF Solution Petrolize HTA Plus Surfactant Acetic Acid HCl

CUF-1 6% 1% 1% --

CUF-2 6% 1% 3% --

CUF-3 6% 1% 5% --

CUF-4 -- -- -- 15% HCl

Table 2: Chemical composition of drilling mud used in

the study

Component Function Concentration

Fresh water Base fluid As required

NaCl Brine Base fluid & Weighting agent Upto 1.20 SG

Soda Ash Calcium remover 0.55 ppb

Caustic Soda Alkalinity control 0.55 ppb

Drilling grade xanthan gum Viscosifier 1.0 ppb

CaCO3 Fine grade Bridging Agent &

Weighting agent 4 ppb

CaCO3 Medium Grade Bridging agent 4 ppb

Starch Fluid loss control 5 ppb

Lubricant Lubricant 0.63 gal/ bbl

Biocide Bactericide 0.084 gal/ bbl

Table 3: Composition of different cleanup formulations

Sample No. Component Concentration Mixing order

Distilled water

As required to make up volume (100 ml)

KCl Buffering salt

CUF-1 For fast action

KCl Enzyme Acetic acid

2 wt% 5 wt% 3 vol%

Acetic acid

Enzyme

As required to make up volume (100 ml)

KCl Buffering salt

Distilled water

2 wt% 5 wt% 4 vol% As required to raise pH above 4.5

CUF-2 For delayed action

KCl Enzyme-A Buffered Acetic acid

Acetic acid Buffering salt

Enzyme

Subsequent optimization experiments focused on meeting operational time constraints. For effective wellbore cleanup, the acid concentration must be sufficient to completely dissolve the calcium carbonate particles present in the mud cake within the available treatment or soaking period. Acid dissolution data summarized in Table 4 and illustrated in Figs. 5 and 6 indicate that, for a treatment duration of 4–6 hours, a minimum acetic acid concentration of 3% is

required when no buffering is applied. At concentrations below this level, complete dissolution of $CaCO_3$ could not be achieved. In contrast, when the solution is buffered to pH 5, a higher acid concentration of approximately 5% is required to achieve similar dissolution within the same time frame. For delayed treatments targeting longer soaking periods of 18–24 hours, a buffered formulation containing 4% acetic acid was found to be optimal.

A comparison of Figs. 4 and 5 further reveals that the rate of acid-mediated $CaCO_3$ dissolution is significantly slower in buffered systems compared to unbuffered organic acid formulations. This slower reaction rate is advantageous for delayed and uniform filter cake removal along extended horizontal sections.

The acceptable corrosion rates of special-alloy tubulars within a temperature range of 275°F- 300°F (i.e., 135 to149°C) is 0.02 lb/ft2 which corresponds to 4.6 mils per year (MPY) (Kalfayan, 2008). The corrosion test results showed that cleanup solutions with 1% and 3% acetic acid (CUF-1 and CUF-2) exhibited corrosion rates well below the recommended limit, even at well-bore temperature of 130°C (Table 5). In contrast, CUF-3 (formulated with 5% acetic acid) reached the borderline corrosion limit above 100°C (Fig. 8).

Fig 8 . Corrosion rate of different cleanup fluids at

different temperatures

In comparison, hydrochloric acid (CUF-4) exhibited extremely high corrosion rates. Although the measurement was conducted at room temperature, the corrosion rate

of 15% hydrochloric acid was 20-25 times higher than that observed for the Petrolize-based clean up solutions. Moreover, the corrosion rate of hydrochloric acid not only exceeds the acceptable limit but is approximately 17 times higher than the maximum acceptable corrosion limit.

Fig 9. Filter cake clean up efficiency of different clean-up solutions: A) standard filter cake, B) Test 1 (6% enzyme: 3%

acetic acid:1% surfactant, C) Test 2 (6% enzyme:15% MF)

and D) Test 3 (6% enzyme:15% acetic acid buffer)

Based on the Fig. 9, all three clean-up solutions have nearly cleaned off the deposited filter cakes. Furthermore, all CUF solutions have effectively removed Starch-Xanthan Biopolymer based filter cake with >90% efficiency (Table 6). Notably, the Test-1 solution containing 6% enzyme:3% acetic acid;1% surfactant have effectively removed filter cake within 6 hours treatment compared to 15% methyl formate (Test2) and acetic acid buffer which required 24 hr exposure.

Fig.10: Net Reducing sugar generation by Enzyme action at different treatment time for different acetic acid and buffered

acid concentrations

Fig.11: Time Plot of Residual Substrate (in terms of Reduc-

ing sugar) by Enzyme action for different Acetic Acid

concentration. ln(100[St]/[So]) vs Treatment Time

Kinetic rate constant studies shown in Fig. 10 and 11, which track the time-dependent release of reducing sugars, demonstrate that the enzyme system in the presence of buffered acetic acid degrades starch macromolecules at a controlled, steady rate. This behavior is particularly critical for achieving uniform clean-up in long horizontal open-hole wells, where placement efficiency is inherently limited. Such controlled reaction kinetics help ensure consistent filter cake degradation along the entire wellbore, thereby maximizing well productivity.

The results also indicate that increasing acetic acid concentration leads to a reduction in enzymatic activity, as evidenced by lower reducing sugar production at equivalent reaction times. At a buffered acetic acid concentration of 4%, $CaCO_3$ dissolution occurs at a sufficiently slow yet exhaustive rate, while the enzymatic degradation kinetics remain marginally higher than those

Table 7: Kinetic rate constant of different clean up fluids

Kinetic Rate

Enzyme Acetic

Buffer to pH

Constant 20 Hours average

Acid

(W/V)

Control

-3.78E-02

5% 3% Not buffered pH 3.07 -1.84E-02

5% 3% pH 5.05 -3.24E-02

5% 4% pH 4.86 -2.93E-02

5% 5% pH 4.75 -2.36E-02

% 6% pH 4.69 -1.80E-02

observed with 5% buffered acid (Table 7). At buffered acid concentrations exceeding 4%, $CaCO_3$ dissolution accelerates, counteracting the objective of delayed and uniform filter cake removal.

Maximizing well productivity requires effective dissolution and removal of $CaCO_3$ bridging particles, particularly those preferentially located at pore throats and fracture apertures. These particles must be mobilized and removed during flowback to restore permeability. Therefore, careful optimization of buffered acetic acid concentration is essential to balance carbonate dissolution with controlled enzymatic polymer degradation.

Based on the experimental observations and statistical kinetic rate analysis, a final optimization of the clean-up fluid formulation was achieved by balancing $CaCO_3$ dissolution efficiency, acid–buffer chemistry, and enzymatic reaction kinetics, while accounting for operational constraints and target field requirements. The optimized formulations are summarized as follows:

a. For 4–6 hours treatment time:

Specially designed enzyme – 5%, acetic acid – 3.0% (unbuffered) in the clean-up fluid (CUF).

b. For 18–24 hours treatment time:

Specially designed enzyme – 5%, acetic acid – 4.0% (buffered to pH 4.5–5.0 in the (CUF).

4. Conclusions

• A hybrid amylase–carbohydrase enzyme was genetically modified to withstand elevated thermal stress by reinforcing the β-helix structure, thereby stabilizing the catalytic center through preferential protein hydration techniques. • Acid loading requirements for different treatment durations, along with long-term stability of the cleanup formulations, were successfully achieved through

systematic optimization of organic acid concentration and buffering salt dosage. • Kinetic rate constants of the clean-up formulations were determined using reducing sugar release measurements obtained via the DNS method, employing starch–xanthan gum–$CaCO_3$-based drill-in fluid as the reaction substrate. A clear correlation was established between system pH and the rate of polymer degradation into soluble sugars. • The derived kinetic rate constant index was incorporated into the final clean-up fluid design to enable application in high-temperature, long horizontal wells, allowing flexibility in soaking time to accommodate operational

constraints.

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