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INEEL Biotechnology for Oilfield Application – Microbial Enhanced Oil Recovery FY2003 Report

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ABSTRACT

The Idaho National Engineering and Environmental Laboratory (INEEL) Biotechnology for Oilfield Operations program supports development, engineering, and application of biotechnology for exploration and production. This continuing INEEL program also supports mitigation of detrimental field conditions. The program is consistent with the United States Department of Energy mission to "promote activities and policies through its oil technology and natural gas supply programs to enhance the efficiency and environmental quality of domestic oil and natural gas exploration, recovery, processing, transport, and storage." In addition, the program directly supports the focus areas of Reservoir Life Extension; Advanced Drilling, Completion and Stimulation Systems; Effective Environmental Protection; and Cross Cutting Areas. The program is enhanced by collaborative relationships with industry and academia.

For fiscal year 2003, the program focused on production and characterization of biological surfactants from agricultural residuals and the production and application of reactive microbial polymers. This report specifically details:

1. Use of a chemostat reactor operated in batch mode for producing surfactin, with concomitant use of an antifoam to prevent surfactant loss. The program achieved production and recovery of 0.6 g/L of surfactin per 12 hr.
2. Characterization of surfactin produced from agricultural residuals with respect to its ability to mediate changes in surface tension. Conditions evaluated were salt (as NaCl) from 0 to 10% (w/v), pH from 3 to 10, temperature from 21 to 70°C, and combinations of these conditions. When evaluated singularly, pH below 6 and salt concentrations above 30 g/L were found to have an adverse impact on surfactin. Temperatures of 70°C for 95 days had no effect. When the effect of temperature was added to the pH experiment, there were no significant changes, and, again, surface tension, at any temperature, increased at pH below 6. When temperature (70°C) was added to the experiments with salt, the impacts of salt up to 30 g/L were negligible. When all three parameters were combined in one experiment, no increase in surface tension was observed at 80 g/L NaCl, pH 10, and 70°C. The upper temperature limit of the surfactin was not determined in these experiments.
3. Impact of alkaline soluble, pH reactive biopolymers to alter permeability in Berea sandstone cores. The contributing effect of salt (as NaCl to 2%, w/v), temperatures to 60°C, and crude oil were evaluated. Residual resistance factors were increased 800 fold, compared to cores without biopolymer. This could lead to alternate technology for permeability modification, thus extending the life of a reservoir and preventing premature abandonment.

EXECUTIVE SUMMARY

The biosurfactant surfactin has potential to aid in the recovery of crude oil. However, high medium and purification costs limit its use in high-volume applications such as improving oil recovery. The Biotechnology for Oilfield Operations research program at the Idaho National Engineering and Environmental Laboratory (INEEL) has successfully produced surfactin from potato process effluents for possible use as an economical alternative to chemical surfactants for improving oil recovery. In previous work, we demonstrated that surfactin could be produced from an inexpensive low-solids potato process effluent with minimal amendments or pretreatments. Research also established that surfactin could be produced by *Bacillus subtilis* ATCC 21332 cultures and recovered by foam fractionation in an airlift reactor. Results using both purified potato starch and unamended low-solids potato process effluent as substrates for surfactin production indicate that the process was oxygen-limited and that recalcitrant indigenous bacteria in the process effluent hampered continuous surfactin production. The research reported here features the use of a chemostat reactor operated in batch mode for producing surfactin with concomitant use of an antifoam to prevent surfactant loss. The antifoam did not interfere with the surfactin's recovery (by acid precipitation) or its efficacy. Initial trials took about 48 hr to produce 0.9 g/L surfactin from potato effluent. Increasing oxygen mass transfer in the reactor by increasing the stirring speed and adding a baffle decreased production time to 12–24 hr and produced about 0.6 g/L surfactin.

Surfactin produced by *Bacillus subtilis* (ATCC 21332) was used to examine the effect of altering salt concentration, pH, and temperature on surfactin activity (as measured by reductions of surface tension). These parameters are some that define oil reservoir characteristics and can affect the application of surfactants. Surfactants enhance the recovery of oil through reduction of the interfacial tension between the oil and water interfaces, or by mediating changes in the wettability index of the system. Surface tension was determined using video image analysis of inverted pendant drops. Experimental variables included sodium chloride (0 to 10%, w/v), pH (3 to 10), and temperature (21 to 70°C). Each of these parameters, and selected combinations, resulted in discreet changes of surfactin activity, useful considerations for the application of surfactin.

Polymer injection has been used in reservoirs to alleviate contrasting permeability zones. Current technology relies on cross-linking agents to initiate gelation. Use of biological polymers is valuable because they can block high-permeability zones, are environmentally friendly, and have potential to form reversible gels without the use of cross-linkers. This research reports the production of a reactive alkaline soluble biopolymer from *Agrobacterium sp.* ATCC 31749, which gels upon decreasing the pH of the polymer solution**.** The focus of this study was to determine the impact of an alkaline-soluble biopolymer on permeability. Permeability modification was investigated by injecting the alkaline biopolymer into Berea sandstone cores and defining the contribution of pH, salt, temperature, and crude oil on gelation. The biopolymer is soluble at a pH above 11 and gels at pH below 10.8. The interaction of the soluble biopolymer with the geochemistry of a Berea sandstone core decreased the pH sufficiently to form a gel, which subsequently decreased permeability. Effluent

pH of control cores injected with 0.01M KOH (pH 12.0) and 0.1M KOH (pH 13.0) decreased to 10.6 and 12.7, respectively. Despite the reduction of pH in the control cores, permeability increased. In contrast, when biopolymer was injected, the buffering capacity of the core caused the biopolymer to form a gel and subsequently reduce permeability. Permeability of the sandstone core injected with biopolymer decreased greater than 95% at 25°C in the presence of 2% NaCl, and crude oil; however, permeability increased when the temperature of the core increased to 60°C. Residual resistance factors of Berea cores treated with biopolymer increased 800-fold compared to cores without biopolymer. Therefore, internal sandstone core buffering of an alkaline biopolymer yielding a stable gel could potentially lead to an alternate technology for modifying permeability, thus extending the life of a reservoir and preventing premature abandonment.

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ACRONYMS

ATCC	American Type Culture Collection
$CaCl_2$	calcium chloride
CMC	Critical Micelle Concentration
cp	centipoises
CSB	Coleville synthetic brine
DO	dissolved oxygen
EOR	enhanced oil Recovery
HNO_3	nitric acid
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
IFT	interfacial tension
KH_2PO_4	potassium phosphate monobasic
k_la	gas mass transfer coefficient
KOH	potassium hydroxide
L	liter
LS	low-solids potato process effluent
mD	millidarcy
NaCl	sodium chloride
rpm	revolutions per minute
RRF	residual resistance factor
SPE	simulated potato effluent
vvm	volume/volume/minute
w/v	unit weight/unit volume

INEEL Biotechnology for Oilfield Application—Microbial Enhanced Oil Recovery, FY 2003 Report

1. INTRODUCTION

The Idaho National Engineering and Environmental Laboratory's Biotechnology for Oilfield Operations research program comprised three thrusts during Fiscal Year 2003: (1) production of surfactants from agricultural residuals, (2) characterization of surfactants produced in airlift bioreactors from agricultural residuals, and (3) study of microbiologically derived reactive polymers. This report summarizes the research conducted in these three areas.

Biosurfactants, surface-active molecules produced by microorganisms, have numerous desirable properties for application as improved oil recovery agents, including a fairly broad range of pH and salt tolerance, low toxicity profiles, and potentially low production cost. Numerous reviews are available on the production and application of biosurfactants.[1,2,3,4,5] *Bacillus subtilis* produces surfactin, a cyclic lipopeptide antibiotic biosurfactant with an aqueous critical micelle concentration (CMC) of 25 mg/L that lowers the surface tension between water and air to 27 mN/m.[3] Surfactants enhance the recovery of oil by reducing the interfacial tension (IFT) between the oil and water interfaces, or by mediating changes in the wettability index of the system. In addition to applications for recovery of fossil fuels, biosurfactants could possibly replace costly and potentially toxic chemical surfactants in several industries.[6] Industries that can use biosurfactants include textile, environmental bioremediation, and fossil fuel recovery.[7]

High medium and purification costs limit the use of biosurfactants in many high-volume applications. In previous work, we showed that surfactin could be produced from an inexpensive low-solids potato process effluent with minimal amendments or pretreatments. Additionally, our research has also shown that surfactin can be both produced in *Bacillus subtilis* cultures and recovered by foam fractionation in an airlift reactor. Preliminary results using both purified potato starch and unamended low-solids potato process effluent as substrates for surfactin production indicate that the process was oxygen-limited and that recalcitrant indigenous bacteria in the potato process effluent hampered continuous surfactin production. Therefore, surfactin was produced in batch chemostat with the addition of antifoam. The antifoam kept the surfactin from foaming out of the reactor. In initial trials, it took about 48 hr to produce 0.9 g/L surfactin from potato effluent. Increasing oxygen mass transfer by increasing the stirring speed and adding a baffle reduced production time by about one-half. Later experiments produced about 0.6 g/ L of surfactin from potato effluent in 12–24 hr. Surfactin was recovered by acid precipitation.

Surfactin produced by *Bacillus subtilis* (ATCC 21332) was used to examine the effect of altering salt concentration, pH, and temperature on surfactin activity (as measured by surface tension reduction). These parameters are some that define oil reservoir characteristics and can affect the application of surfactants. Experimental variables included sodium chloride (0 to 10%, w/v), pH (3 to 10), and temperature (21 to 70°C). Each of these parameters, and selected combinations of them, resulted in discreet changes of surfactin activity, useful consideration for exploring surfactin as an enhanced oil recovery agent.

Polymers have been useful for enhanced oil recovery for water shutoff technologies, and plug flow conformance. Microbial polymers are of interest due to their potential cost savings, compared to conventional use of synthetic chemical polymers. Numerous microorganisms are known to produce extracellular polysaccharides. However, most require addition of divalent cations to increase the viscosity to a level useful for enhanced oil recovery technologies. One microbiological polymer of interest is curdlan, which has demonstrated gelling properties by a reduction in pH. Curdlan is a β–(1, 3) glucan

polymer produced by *Agrobacterium sp.* It has a molecular weight of 74,000 Daltons. We exploited curdlan's ability to gel upon reduction in the pH. Curdlan is soluble at pH 11.4 and becomes a gel when the pH is decreased to below 10.8.

2. MATERIALS AND METHODS

2.1 Microorganisms

Surfactant Production. *Bacillus subtilis* 21332 was obtained from the American Type Culture Collection (ATCC). *Bacillus subtilis* is a facultatively anaerobic, gram-positive spore-forming rod, about 0.7 × 2.0 μm in size. The organism is capable of growth to 55°C, salt tolerance (NaCl) to 10% w/v, and is negative for adherence to hydrocarbon. The organism produces surfactin, a cyclic lipopeptide surfactant.

Polymer Production. *Agrobacterium sp.* 31749 (formerly *Alcaligenes faecalis* subsp. *myxogenes*) was obtained from the ATCC. *Agrobacterium sp*. is an aerobe, gram-negative rod that produces an extracellular biopolymer, curdlan. Curdlan is an unbranched biopolymer composed of β–(1-3) glucose linkages. Curdlan is a water-insoluble but alkaline soluble biopolymer. Upon solubilizing curdlan at pH greater than 11 and subsequent reduction in pH to 10.8, an opalescent, firm gel is formed.

2.2 Maintenance and Growth Media

***Bacillus subtilis* 21332.** *B. subtilis* was cultured and maintained as previously described,[8, 9] except that simulated potato effluent (SPE) media was used [5.0 g/L potato starch, 0.5 g/L glucose, 1.0 g/L sucrose, 1.0 g/L maltose, 3.5 g/L peptone, 3.5 g/L tryptone, 0.2 g/L $MgSO_4$ $7H_2O$, 0.1 g/L yeast extract, 0.8 $(NH_4)_2SO_4$, 0.03 g/L $FeSO_4$, and 0.0022 g/L $MnSO_4$. H_2O]. For plating, 15 g/L agar was added. Surfactin used in characterization studies was obtained from a single bioreactor run to provide continuity for comparing each study.

***Agrobacterium sp*. 31749.** An ATCC lyophilized stock was cultured at 30°C in 5-mL of ATCC medium 3, Nutrient Broth. After 48 hours, the entire volume was transferred to 500-mL of fresh medium. Frozen stock cultures of log phase *Agrobacterium sp.* were prepared by resuspending the cell pellet in 1/20 the volume of medium 3 and adding an equal volume of 20% v/v sterile glycerol. The stocks were stored at -80°C. A new freezer stock was thawed and used to initiate growth in seed culture medium. The seed culture was grown at 30°C, 150 rpm, for 20 hours in 300 mL of medium. The seed inoculum contained 1.29×10^8 cells/mL and was used to initiate curdlan production in the basal fermentation medium. Seed culture and basal fermentation media have been previously described by Lee et al.[10] A 10% inoculum was transferred to a basal fermentation medium and incubated aerobically at 30°C, 250 rpm, for 5 days. All curdlan production experiments were conducted in 2-L Erlenmeyer flasks with 500-mL of broth. All media were pH adjusted to 7.2 before autoclaving. Phosphate stock solution was added after autoclaving. Sucrose and trace element solutions were filter sterilized using a 0.45-μm filter and aseptically added upon cooling. The culture pH was manually maintained at pH 7.0 for 24 hr using 5M KOH or 3N HCl.

2.3 Microbial Production of Surfactants and Biopolymers

Surfactant Production. Surfactin was produced from *B. subtilis* in an airlift reactor using low-solids potato process effluent obtained from a southeast Idaho potato processor, as described in Noah et al.[11] At the completion of a run, the culture fluid was centrifuged at 3,834 g for 30 min to remove cells and insoluble material. The supernatant containing surfactin was then stored in a sterile container at 4°C until needed.

Biopolymer Production. After 5 days, the culture was centrifuged at 6,816 g for 20 min, and 20°C in tarred 500-mL polypropylene centrifuge tubes. The supernatant was discarded, and the tubes were weighed to determine the wet weight of the cells and curdlan. The pellet was treated overnight at 4°C with 5M KOH. The alkaline-treated pellet was centrifuged at 6,816 g for 20 min at 20°C. Curdlan was

collected in the supernatant and the pH was adjusted to 7.0. The centrifuge tubes were weighed, and the differences in the weights were used to determine the wet weight of curdlan. Curdlan was transferred to tarred 50-mL polypropylene centrifuge tubes. The biopolymer was frozen, lyophilized to dryness, and weighed to determine the dry weight. All biopolymer batches were homogenized using a mortar and pestle. The dry biopolymer was stored at room temperature.

2.4 Production of Surfactants in Chemostat Reactors

2.4.1 Bioreactor

Surfactin production was performed in a New Brunswick BioFlo 3000. Dissolved oxygen (DO) and pH were monitored using sterile probes (0.61-m Ingold DO probe and 0.61-m Mettler Toledo pH probe, respectively), inserted through the head plate. A thermocouple was inserted below the liquid level within a thermowell and used to control temperature at 30°C. Two liters of potato process effluent were placed in the bioreactor and autoclaved for 60 min. The pH was then adjusted to ~ 7 with 5N KOH. The potato process effluent was inoculated with 200-mL of *B. subtilis* grown on simulated potato effluent media. Foam was suppressed by the addition of AF antifoam. The pH was automatically controlled by the addition of 5N KOH and 3N H_2SO_4. A batch run was conducted for at least 72 hours. Air was supplied at 0.5 vvm (1 L/min). Early batch runs were conducted at 250 rpm without a baffle. Later batch runs were conducted at 400–500 rpm with a baffle in place. Two Rushton impellers provide the agitation. Samples were collected over time and analyzed for cell numbers, starch, surfactin concentration, and surface tension.

After a run was completed, the surfactin was recovered by centrifugation and acid precipitation.[12] The reactor contents were collected, and the solids and cells removed by centrifugation at 10,000 *g* for 14 mins at 4°C. The supernatant was subjected to acid precipitation to recover the surfactin. Concentrated HCl was added until the pH dropped close to 2.0. The supernatant was then refrigerated for at least 24 hr. The precipitate contained the surfactant. The contents were shaken, then centrifuged at 11,000 *g* for 20 min at 4°C to recover the surfactin pellet. To place the surfactin back into solution, a known amount of nanopure water was added to resuspend the pellet, and the suspension pH was adjusted to 7.0. The surfactin suspension was analyzed for starch, surfactin concentration, and surface tension.

2.4.2 Antifoam Agents

Three antifoams were selected for evaluation: Antifoam T-H (Thompson Hayward Chemical Company, Kansas City, Kansas); Antifoam AF (Dow Corning Corporation, Midland, Michigan); and Antifoam B (Dow Corning Corporation, Midland, Michigan). All designations are as received from the manufacturer.

2.4.3 Mass Transfer of Oxygen

The purpose of these experiments was to get the oxygen mass transfer coefficient (k_la) as a function of rpm with and without the baffle in place in the BioFlo 3000. Two liters of 3N KCl were placed in the reactor. The air was turned on, and the DO followed over time until it reached 100%. The air was then turned off and nitrogen gas turned on, and the DO followed over time until the DO reached 0%. This was done at an airflow rate of 1 L/min at 125, 150, 200, 250, 375, 500, and 750 rpm with and without the baffle. One run was done at 2 L/min air and 250 rpm without the baffle.

2.4.4 Cell Counts

Plate counts using solid simulated potato effluent media were used to determine cell numbers. Contaminants were differentiated from *B. subtilis*, based on colony morphology.

2.4.5 Surfactin Concentrations

Surfactin was measured by high performance liquid chromatography (HPLC), as described by Lin and Jiang,[13] using a Supelco LC-18 column (250 × 4.6 mm, 5-μm particle size). Separation was achieved by elution on a gradient of 10 mM KH_2PO_4 (pH 6) and 100% methanol at 0.5 mL/min, as follows: (a) 0 to 30 min, 70.0 to 73.4 vol% methanol; and (b) 30 to 80 min, 73.4 to 95.4 vol% methanol. Samples were centrifuged for 10 min at 3,500 *g* and filtered through a 0.22-μm syringe filter before analysis. The injection volume was 500 μL. Surfactin was measured by absorbance at 210 nm. Purified surfactin (Sigma, Cat. No. S-3523) was used as a standard. All surfactin eluted from 34 to 80 min; thus, total surfactin was quantified in samples as the sum of the peak areas eluting in that time period.

2.5 Surfactant Characterization

2.5.1 pH

For each experiment, supernatant was placed in 15-mL conical tubes, and pH was adjusted with either 1N KOH or 1N HNO_3. The pH of the supernatant after the bioreactor run was 7.0. Simulated potato effluent media was used as a surfactin-free control, and pH was adjusted likewise. The simulated potato effluent contained the following per liter of nanopure water: 5 g potato starch, 3.5 g peptone, 3.5 g tryptone, 0.2 g $MgSO_4 \times 7H_2O$, 0.1 g yeast extract, and 0.8 g $(NH_4)_2SO_4$.

2.5.2 Salt Concentration

For each experiment, up to 10% (w/v) sodium chloride (NaCl) was placed in 15-mL conical tubes. Supernatant was added, and the tubes were gently stirred on a laboratory rotator for 2 hr. With NaCl concentrations above 3%, the supernatant became cloudy with a precipitate that interfered with surface tension measurements. The supernatant was therefore left overnight in an upright position to allow the precipitate to settle out, and readings of surface tension were taken the following day. The simulated potato effluent media was used as a surfactin-free control, and NaCl was added likewise. When pH was included as a parameter in this experiment, NaCl was added first, and the supernatant was stirred for 2 hr. Then, the pH of the supernatant was adjusted with either 1N KOH or 1N HNO_3.

2.5.3 Temperature

For each experiment, supernatant was placed in 15-mL conical tubes and then incubated at temperatures up to 70°C. Supernatant was incubated for a minimum of 1 hr. When experiments included NaCl and pH adjustments, supernatant was incubated overnight, or longer, to allow for the precipitate to settle out. For the surfactin stability experiment, supernatant was incubated at either 4 or 70°C for over 95 days. Surface tension was measured at temperature using a special heated cell made from stainless steel, with channels drilled in the cell to accommodate attachment to a heated circulating water bath (Polystat Water Circulator, Cole-Parmer Instrument Company, Vernon Hills, Illinois). Density was determined for the supernatant used in all experiments by placing a little more than 1 mL in a 1-mL volumetric flask and incubating it at 4, 21, 37, 51, and 70°C for 1 hr. After 1 hr, the excess supernatant was removed to the 1-mL mark. The flasks were then weighed to determine the density of the fluid. The average density (n=2) from this experiment was used in a quadratic equation to extrapolate density for each temperature in the range of 21 to 70°C.

2.6 Biopolymer Characterization

2.6.1 Solubility

Dry biopolymer was resuspended in 0.01M KOH, 1M KOH, and nano pure water in 10-mL glass test tubes and covered with parafilm. The solution was vortexed and set at room temperature for 30 min.

A block heater was used to determine the effect of temperature on curdlan solubility. An Accumet AB15 pH meter and pencil-thin pH probe were used to measure pH. The pH meter was calibrated before use with fresh pH 4, 7, and 10 buffers. Multiple biopolymer concentrations (1, 4, 10%, w/v) were tested to determine the solubility of the biopolymer at selected temperatures. Gelling characteristics of the biopolymer were visually noted. The pH of the biopolymer solution was decreased using 3N HCl.

2.6.2 Viscosity

A Wells-Brookfield Cone/Plate Viscometer model LVTDV-IICP (Stoughton, Massachusetts) with spindle CP-40 or CP-51 was used to measure viscosity. The viscometer was calibrated with Brookfield silicone standards; the sample volume was 0.5-mL.

2.6.2.1 Salt Concentration. The amount of salt in the modified CSB was varied to determine if salt concentration alters biopolymer gelling. The concentrations of salt tested were 0.0, 0.7, 1.5, 3, and 5% (w/v) NaCl. Dry biopolymer (10% w/v) was added to 0.1M KOH or 0.01M KOH brine. The pH was adjusted using 3M HCl.

2.6.2.2 Temperature. A Cole Parmer Polystat heated circulating bath was used to maintain temperature of the viscometer plate. The temperature range was 25, 50, 75, and 96°C. Initially, dry biopolymer (10% w/v) was solubilized in 0.01M KOH, and viscosity measurements were measured at the selected temperature. The biopolymer was then gelled using 3M HCl, and viscosity measurements were completed at the selected temperature.

2.6.3 Total Carbohydrate Analysis

Core effluent was monitored for total carbohydrate[14] to verify that biopolymer was injected through the core. Additionally, the carbohydrate content of the dry biopolymer was measured using a total carbohydrate analysis for quality control. Pure curdlan, obtained from Carbomer (San Diego, California) was used as the standard.

2.7 Core Preparation

2.7.1 Berea Sandstone Cores

Berea sandstone (Cleveland Quarries, Amherst, Ohio) was cut into cylindrical cores 1 in. in diameter by 6 in. in length, fitted with inlet and outlet endplates, and coated with Hysol epoxy (Dexter, Seabrook, New Hampshire). Pressure taps were prepared by drilling holes in the epoxy coating about 4 to 5 in. apart and fitted with stainless steel connectors. Each encapsulated core was evacuated and saturated with Modified Coleville Synthetic Brine (CSB). Modified CSB consisted of 7g of NaCl, 0.14g $CaCl_2 \times 2\ H_2O$ and 0.02g NH_4Cl per liter. Modified CSB was filtered and vacuum degassed before core saturation. Darcy's law was used to determine the brine permeability of each core. Porosity and pore volume were determined using dry and wet core weights, brine density, and core dimensions. Potassium iodide was injected into the cores as a tracer, and the effluent was measured using an UV-VIS spectrophotometer (Schimadzu, Kyoto, Japan). The biopolymer solution was injected into the core after the tracer test. Schuricht crude oil was pumped through four cores after the initial brine saturation and biopolymer solution were injected into an oil-saturated core.

2.7.2 Biopolymer Injection

Curdlan powder (10% w/v) was dissolved in modified CSB with the addition of 0.1M KOH or 0.01M KOH and filtered with 20–25 μm cellulose papers. The viscosity, total carbohydrate, and pH of the polymer solution were measured before core injection. Biopolymer was injected using a stainless steel accumulator (Temco, Tulsa, Oklahoma), an Isco syringe pump (Lincoln, Nebraska), and controller at a

constant flow of 1.0 mL/min for at least 5-pore volumes. Honeywell pressure transducers (Phoenix, Arizona) recorded the pressure. Effluent was collected in 15-mL polypropylene tubes and monitored for pH and total carbohydrate. Cores were shut-in at the desired temperature (23 or 60°C) for 6 to 11 days. Temperature was controlled using a sand-packed heat block. Initially, a hand syringe filled with modified CSB was used to obtain effluent to measure the pH. Flow was established with an Isco pump using modified CSB after the pH decreased below 10.8. Postpermeability measurements were calculated for each core. The cores were then heated to 60°C and cooled for 48 hr. Flow was established at 60°C and again when the core cooled. Postpermeability measurements were calculated at 60°C and again at ambient temperatures. When cores were heated to 60°C, the temperature of the injection brine was maintained using an Omega RTD controller, a variable autotransformer, a 1/8-inch thermocouple, and a Barnstead/ Thermolyne heat tape. Table 1 shows the experimental setup for the Berea sandstone cores. Permeability modification experiments were investigated by injecting the alkaline biopolymer into Berea sandstone cores and defining the contribution pH, salt, temperature, and Schuricht crude oil had on biopolymer gelation. Duplicate cores were conducted to measure the permeability modification, except single cores were used when the cores were saturated with Schuricht crude oil. Either 0.1M KOH or 0.01M KOH was injected into the control cores; biopolymer was not injected in the control cores. The buffering capacity of the control cores against 0.1M KOH or 0.01M KOH determined the molarity of the solvent used for the remaining cores injected with biopolymer. The residual resistance factor (RRF) was calculated for each core according to Equation (1):

$$RRF = \frac{K\ before\ treatment}{K\ after\ treatment} \tag{1}$$

where K is the permeability; *before treatment* is the permeability before biopolymer injection; and *after treatment* is the permeability after biopolymer injection.

Table 1. Experimental core description.

Core	Injection	Brine Molarity	Temperature	Pre-Injection Saturation
B12	Brine	0.01 M KOH	Ambient	Brine
B13	Brine	0.10 M KOH	Ambient	Brine
B14	Biopolymer	0.01 M KOH	Ambient	Brine
B15	Biopolymer	0.01 M KOH	Ambient	Brine
B16	Biopolymer	0.01 M KOH	60°C	Brine
B17	Biopolymer	0.01 M KOH	60°C	Brine
B18	Biopolymer	M KOH; 2% NaCl (w/v)	Ambient	Brine
B19	Biopolymer	M KOH; 2% NaCl	Ambient	Brine
B7	Brine	0.01 M KOH	Ambient	Schuricht crude oil
B9	Biopolymer	0.01 M KOH	Ambient	Schuricht crude oil
B6	Biopolymer	0.01 M KOH	60°C	Schuricht crude oil
B11	Biopolymer	M KOH; 2% NaCl (w/v)	Ambient	Schuricht crude oil

3. RESULTS

3.1 Production of Surfactants in Chemostat Reactors

3.1.1 Antifoam Screening

The three different antifoams, T-H, AF, and B, were screened in 500-mL chemostats. Three hundred mL of low-solids potato effluent was inoculated with 30 mL of *B. subtilis*. Antifoam was added by syringe. The screening criteria for selecting the antifoam was determined by how much antifoam had to be used and whether the antifoam hindered surfactin production or recovery. Only 400-ppm surfactin were produced when adding antifoam B, whereas 1585 ppm and 1793 ppm surfactin were produced when adding antifoam T-H and AF, respectively. AF antifoam was chosen over T-H antifoam because less AF antifoam was needed to control the foaming. Antifoam AF is composed of polydimethylsiloxane, stearate, emulsifiers, sorbic acid, and water.

3.1.2 Batch Chemostat Runs with Potato Effluent

Three trial runs were required to establish the correct parameters for automated pH and antifoam control. These runs confirmed that AF antifoam neither prevented reactor upset nor interfered with surfactin production. Starch utilization was noted at about 72 hours.

The next two runs were the same, except for separate batches of process effluent (both batches of potato effluent were between 3 to 3.3% solids). For the first run, surfactin concentration reached 0.8 g/l at 30 hr and 0.9 g/L at 72 hr (Figure 1). For the second run, surfactin concentration reached 0.5 g/L at 34 hr and 0.9 g/L at 72 hr (Figure 2). For both these runs, the dissolved oxygen (DO) dropped to 0% by about 7 hr and stayed at 0% the rest of the run (72 hr). By 42 hr, 90% or more of the starch was used. For the first run, the majority of surfactant production occurred during the first 30 hr and leveled off, whereas during the second run the surfactant production occurred over the course of the run. Also, during both runs other cell types appeared after 52 hr.

In an effort to maintain DO above 0% and decrease run time, reactors with increased stirrer speeds (400 from 250 rpm) were evaluated. This was hypothesized to prevent anaerobic contaminants and decrease run time. Results of this effort were successful, with surfactin concentration reaching 0.6 g/L at about 32 hr and 0.7 g/L at 68 hr. The DO did not drop below 30%. The soluble starch was depleted by 17 hr (Figure 3).

The addition of a baffle to reactors with stirring rates of 400 rpm was also evaluated, and proved to be more successful than increased stirring alone. With the resulting increase in oxygen mass transfer, the starch was depleted in 17 hr (Figure 4). The surfactin concentration was 1.1 g/L at 12 hr and at the end of the run at 66 hr. The DO dropped from 94% at the start of the run to 3.5 % at 17 hr. The DO then started to rise at 19 hr to 42% at 66 hr. In essence, the run was finished between 12 and 17 hr. The increased oxygen mass transfer cut the run time from around 48 hr to between 12 and 17 hr.

3.1.3 Mass Transfer of Oxygen

Increasing the flow rate to 2 L/min did not significantly increase the k_la (Figure 5). However, both an increase in the rpm and adding the baffle increased the k_la. At 500 rpm, the k_la was about 3.25 times greater than at 250 rpm without the baffle: 0.0235 versus 0.0072 1/sec, respectively. By adding the baffle at 500 rpm, the k_la was about double: 0.0447 versus 0.0235 1/sec, respectively. At 750 rpm, the chemostat entrains air from the headspace and mixing appears to be violent. By running the chemostat at 400 rpm with the baffle in place, the k_la was about 6 times greater than the previous runs at 250 rpm without the

baffle: 0.0447 versus 0.0072 1/sec. The increased mass transfer coefficient affects the run time (decreases), contamination (decreases), and production rate (increases).

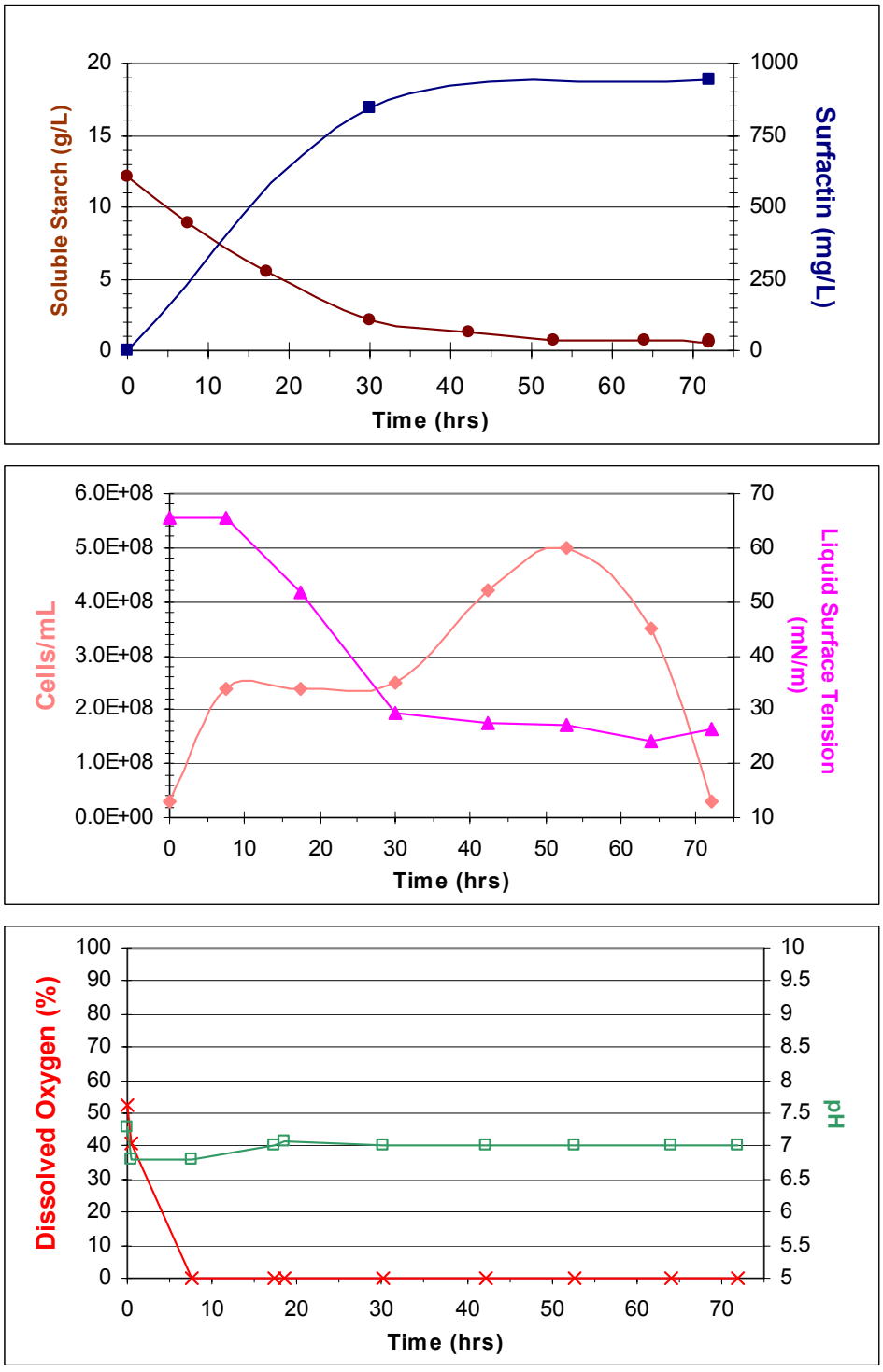


Figure 1. First-batch chemostat experiment. Potato effluent contained 3.25% solids: (top) Soluble starch and surfactin concentration over time; (middle) cell count and liquid surface tension over time; (bottom) dissolved oxygen and pH over time.

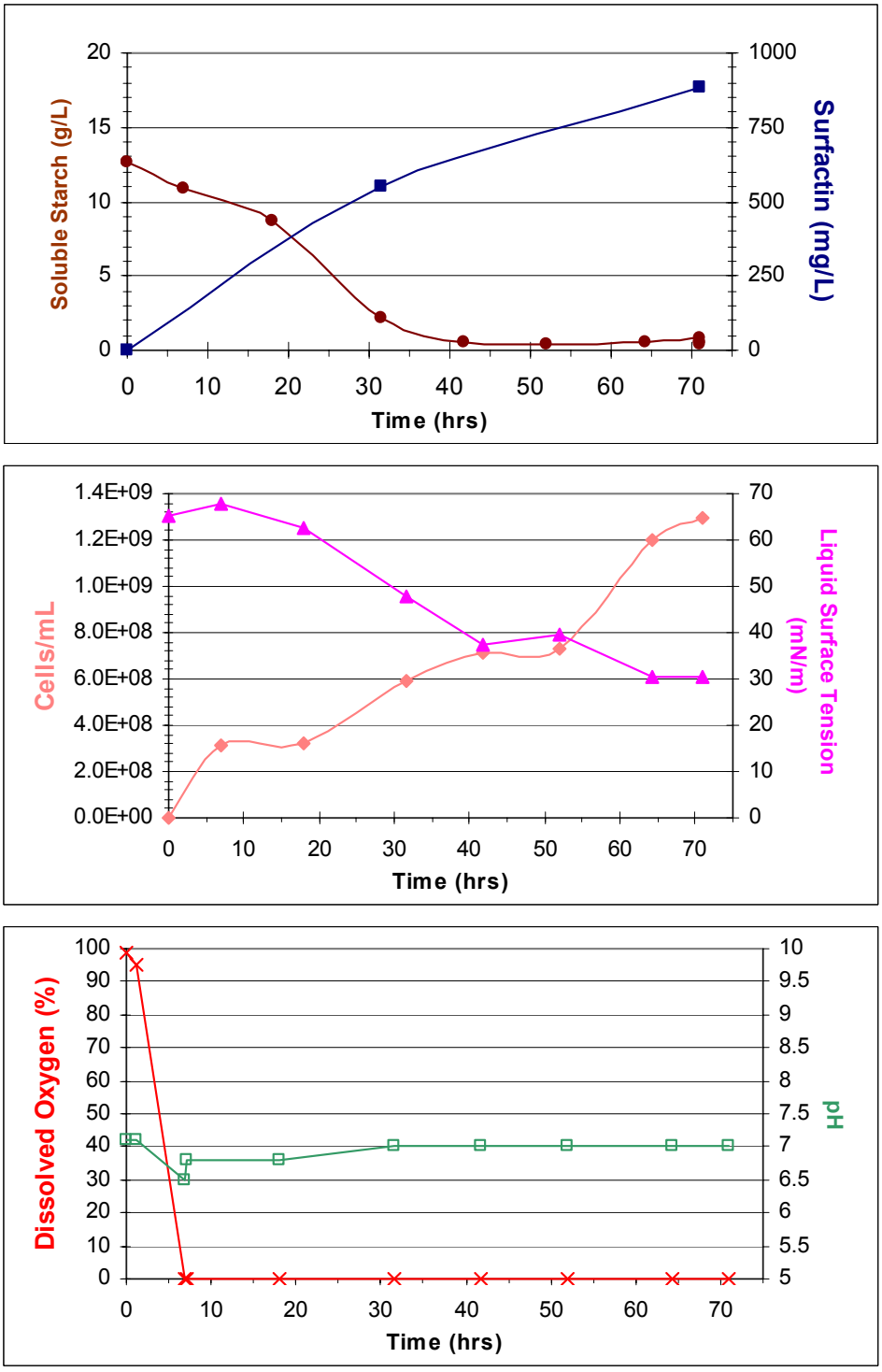


Figure 2. Second-batch chemostat experiment. Potato effluent contained 3.0% solids: (top) soluble starch and surfactin concentration over time; (middle) cell count and liquid surface tension overtime; (bottom) dissolved oxygen and pH over time.

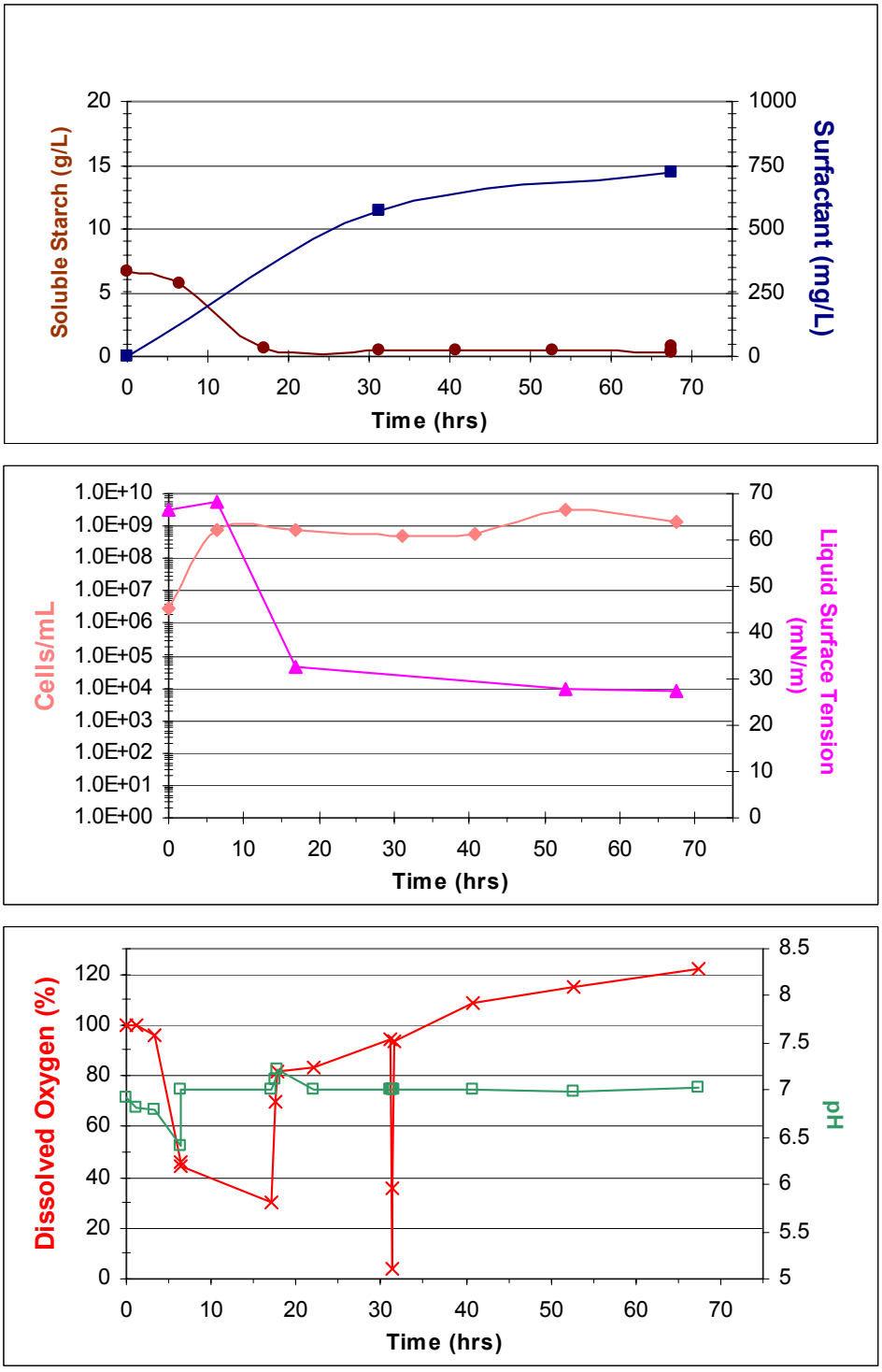
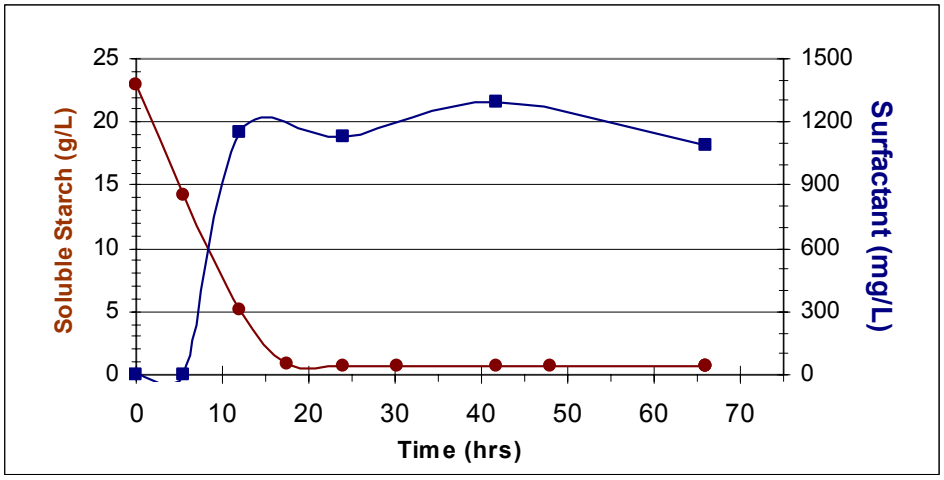
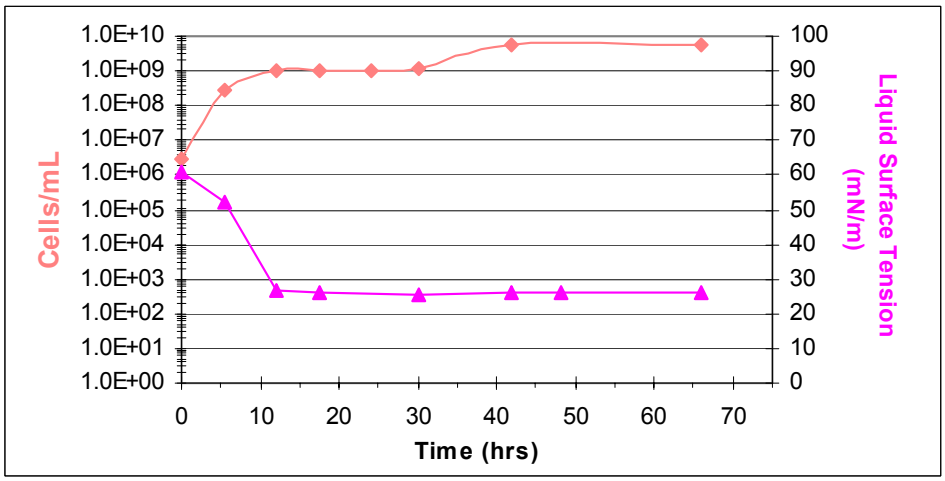


Figure 3. Chemostat experiment without baffle. Potato effluent contained 3.0% solids. Stirrer speed was 400 rpm: (top) soluble starch and surfactin concentration over time; (middle) cell count and liquid surface tension over time; (bottom) dissolved oxygen and pH over time.





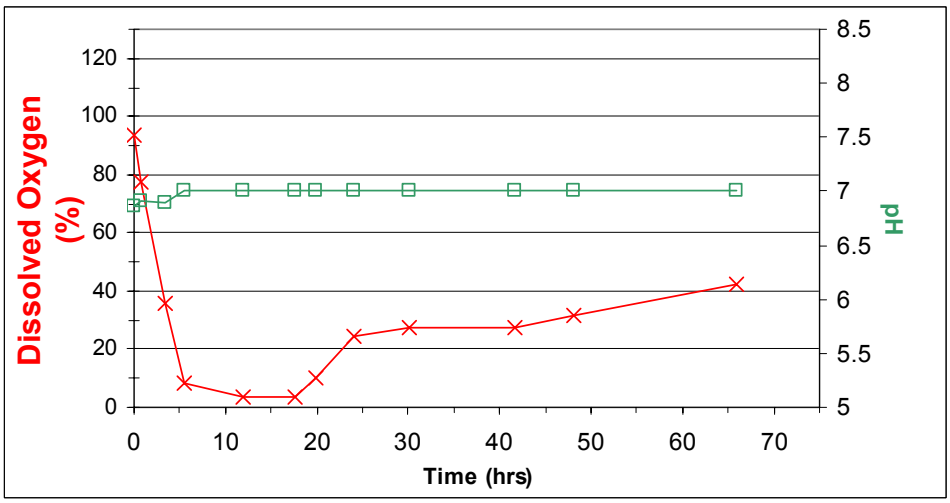


Figure 4. Chemostat experiment with baffle. Potato effluent contained 3.84% solids. During this run the baffle was in place and the stirrer speed was 400 rpm: (top) soluble starch and surfactin over time, (middle) cell counts and surface tension over time, (bottom) dissolved oxygen and pH over time.

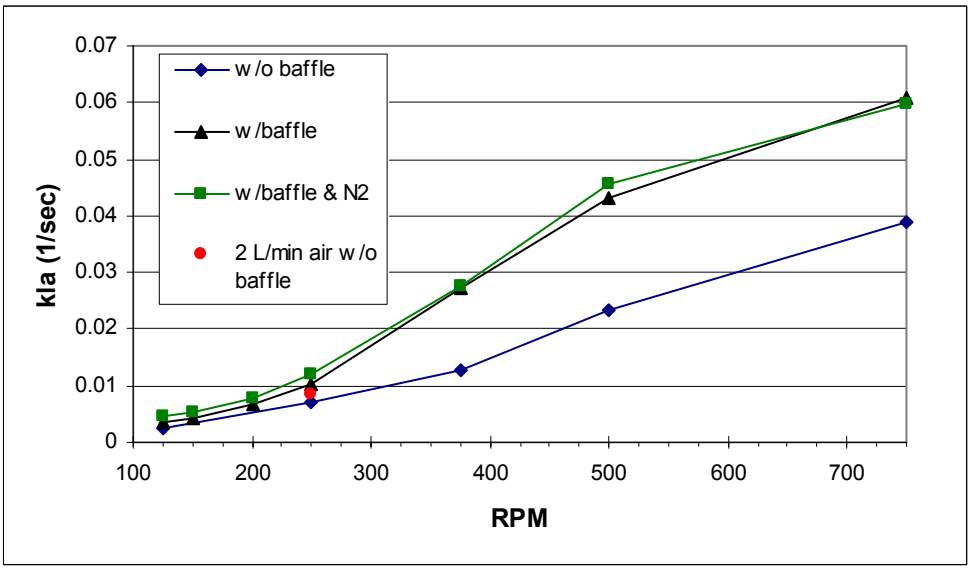


Figure 5. Determination of $k_l a$ in the batch chemostat, with and without a baffle at various stirrer speeds. Medium was 3N KCl.

3.1.4 Acid Precipitation of Surfactin

Surfactin was recovered by removing the cell mass and solids by centrifugation and adding HCl to the supernatant to a final pH of about 2. Evaluation of surfactin recovery was performed by distributing supernatant into five equal volumes of 200 mL each and adding HCl to a final pH between 4 and 2 to ascertain differences of surfactin recovery. The final pH for each fraction was 1.75, 2.10, 2.55, 3.14, and 3.9, which corresponded to a surfactin concentration in the final pellet of 10,000 g/L, 10,988 g/L, 9944 g/L, 8527 g/L, and 5088 g/L, respectively.

3.2 Characterization of Surfactants from Airlift Reactors

3.2.1 Effects of pH

Figure 6 shows the effects from altering the pH of the reactor supernatant containing surfactin. The starting pH of the supernatant produced from potato process effluents, without any pH adjustments, was 7.0, and the surface tension was 28.3 ± 0.1 mN/m. The pH was found to alter surface tension of the surfactin at values of less than 6. The sharpest transition in surfactin quality, as indicated by an increase in surface tension, occurred between pH 6 (30.7 ± 0.5 mN/m) and pH 5 (47.9 ± 0.4 mN/m). Between pH 6 and pH 10, surface tensions remained almost unchanged and had a range of 28.3 ± 0.1 to 33.0 ± 0.4 mN/m. Since isolation procedures use the precipitation of surfactin under acidic conditions, it was expected that a precipitate would form and surface tension would increase at pH<3. Indeed, a precipitate formed, and surface tension increased to 54.5 ± 0.3 mN/m. When the sample was centrifuged and the resulting pellet resuspended in nanopure water, the surface tension returned to 32.2 ± 0.3 mN/m.

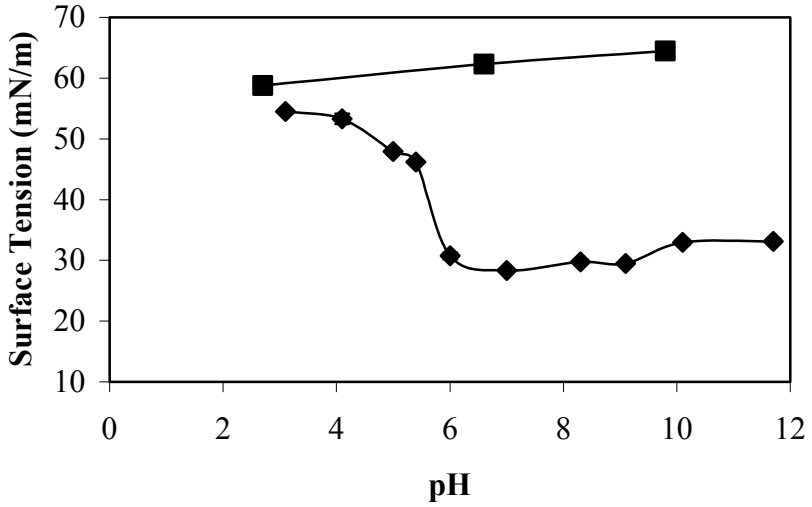


Figure 6. Effect from pH on surfactin activity. The pH of the supernatant without any alteration of pH was 7.0. (♦ = surfactin; ■ = surfactin-free control)

3.2.2 Effect of Salt Concentration

The surface tension of the surfactin without any addition of NaCl was 29.4 ± 1 mN/m. Experiments found NaCl concentrations above 30 g/L to increase surface tension of surfactin. Between 30 g/L and 50 g/L NaCl, surface tension increased from 29.4 ± 0.1 to 46.2 ± 0.4 mN/m. Between 60 g/L and 100 g/L NaCl, surface tension remained about 50 mN/m (Figure 7).

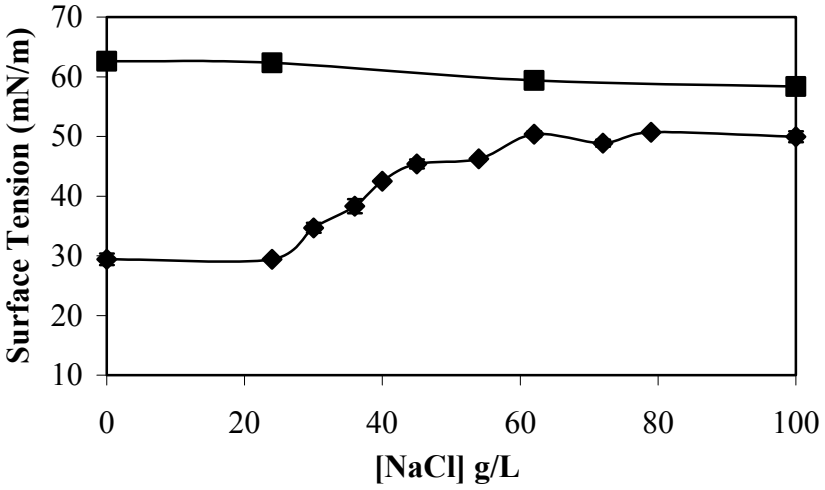


Figure 7. Effect from salt concentration on surfactin activity. (♦ = surfactin; ■ = surfactin-free control)

3.2.3 Effect from Temperature

Figure 8 shows the effects from incubating surfactin from 21 to 70°C. Although there is an apparent decrease in surface tension of surfactin at higher temperature, there is also a decrease in surface tension of water as temperature increases. The difference in surface tension between water and surfactin

remains the same, showing there is no effect from temperature on surfactin. Furthermore, a stability experiment indicated no change in surface tension of surfactin when surfactin was incubated at 70°C for over 95 days (Figure 9). There was also no change in surface tension of surfactin incubated at 4°C for over 95 days.

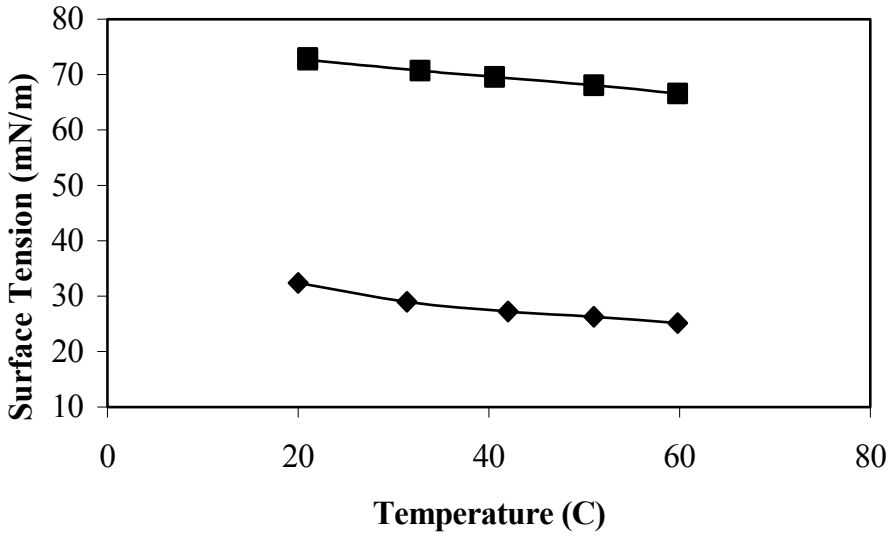


Figure 8. Effects from temperature on surfactin Activity. (♦ = surfactin; ■ = surfactin-free control)

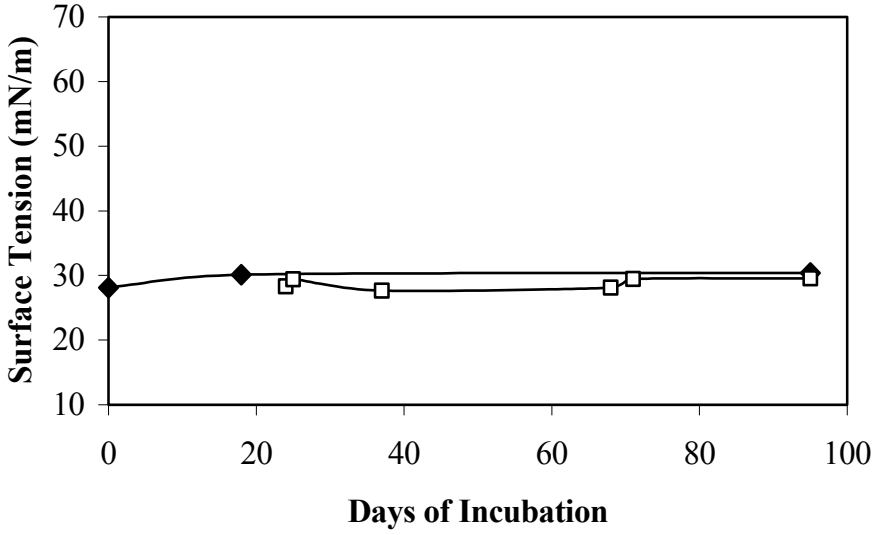


Figure 9. Temperature stability of surfactin over time. (♦ = surfactin incubated at 70°C; □ = surfactin incubated at 4°C)

3.2.4 Combined Effect of Temperature and pH

Temperature and pH effects were examined together to determine if there were synergistic interactions that were not seen when each was tested alone. Figure 10 shows the results from this experiment. Temperature alone did not alter surface tension of surfactin, nor did it alter the results seen

from pH alone. The combination of temperature at 31 and 56°C and pH 5 did increase precipitate in the supernatant, which interfered with measurement of surface tension. Attempts to centrifuge did not remove the precipitate and still did not allow for measurement of surface tension. However, visual observation of the pendant drop size indicated surface tension had increased relative to supernatant that had not been altered with KOH or HNO_3. At an incubation temperature of 70°C, pH 3 and pH 5 were cloudy, which interfered with surface tension measurements. Visual observation also indicated the surface tension was relatively high for these samples as well, since the pendant drop size was large compared to surfactin with surface tensions in the range of 27 to 28 mN/m.

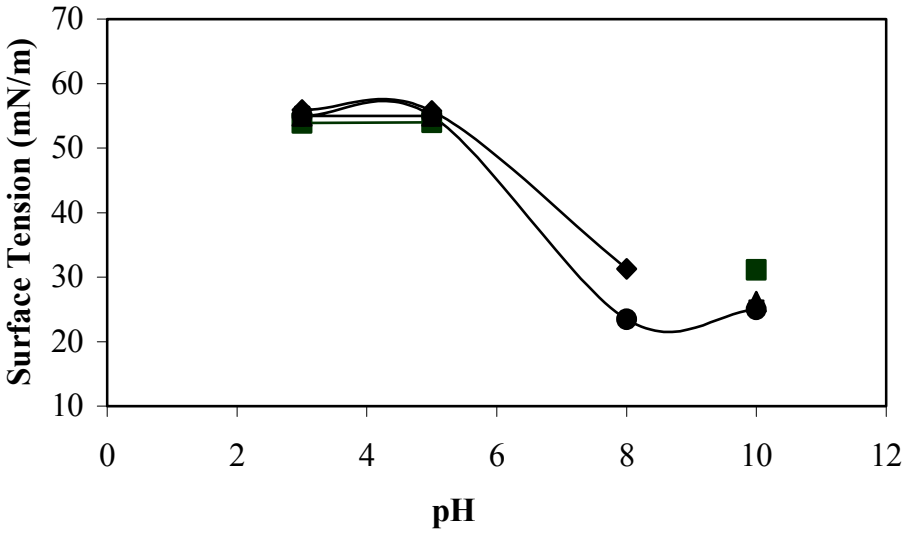


Figure 10. Effects from temperature and alterations from pH on surfactin activity. (♦ = surfactin, 21°C incubation; ■ = surfactin, 31°C incubation; ▲ = surfactin, 56°C incubation; ● = surfactin, 70°C incubation)

3.2.5 Combined Effect of Temperature and Salt

Figure 11 details the results from the experiment on the effects of temperature and salt concentration. Supernatant was prepared as described; however, there was a 9-day incubation instead of overnight, due to replacement of a burned out lamp in the interfacial tension instrument. There were no significant differences between all salt concentrations at 21 or 45°C. However, at 70°C, the higher salt concentrations did not increase surface tension of surfactin as those at the lower temperatures. At 50 g/L NaCl and 70°C, surface tension was 31.5 ± 1.3 mN/m compared to 50 g/L NaCl at 21°C and 45°C, with 50.9 ± 0.3 and 53.4 ± 0.2 mN/m, respectively.

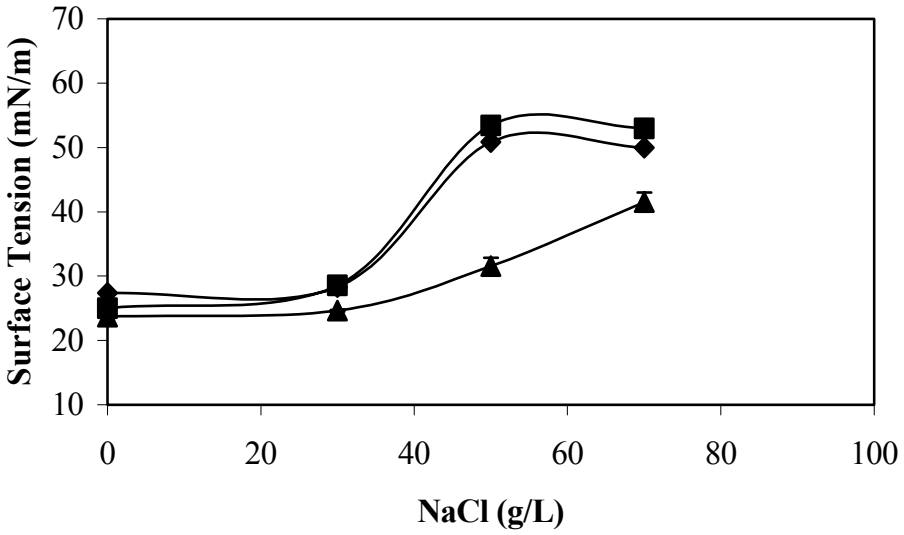


Figure 11. Effect from temperature and salt concentration on surfactin activity. (♦ = surfactin, 21°C incubation; ■ = surfactin, 45°C incubation; ▲ = surfactin, 70°C incubation)

3.2.6 Combined Effect of Temperature, Salt, and pH

Effects from the addition of NaCl (3 to 8%, w/v), alterations of pH (3 to 10), and temperature (21 and 70°C) were tested together to examine the effects of all three parameters combined. Figure 12 compares two experiments at 21 and 70°C. Samples were incubated overnight, except for those at 70°C, which were too cloudy to measure surface tension. These samples were incubated for 6 days until measurements could be taken. Incubation time was increased because a high-temperature centrifuge was not available to remove precipitate that had formed in the supernatant. We observed that the effect of increasing surface tension from increasing salt concentration is moderated at higher temperature and basic pH. The surface tension of supernatant at salt concentrations above 50 g/L at 21°C remained between 45.2 ± 0.4 mN/m and 51.8 ± 0.3 mN/m. The surface tension of supernatant at salt concentrations above 50 g/L and 70°C, however, was lower. This was observed most at pH 10, where surface tension of supernatant with 50 g/L NaCl was 26.6 ± 0.2 mN/m, and the surface tension of supernatant with 80 g/L NaCl was 25.8 ± 0.1 mN/m. The surface tension of supernatant with no addition of NaCl and no pH alterations at 70°C was 23.7 ± 0.2 mN/m, and 29.4 ± 0.6 mN/m at 21°C.

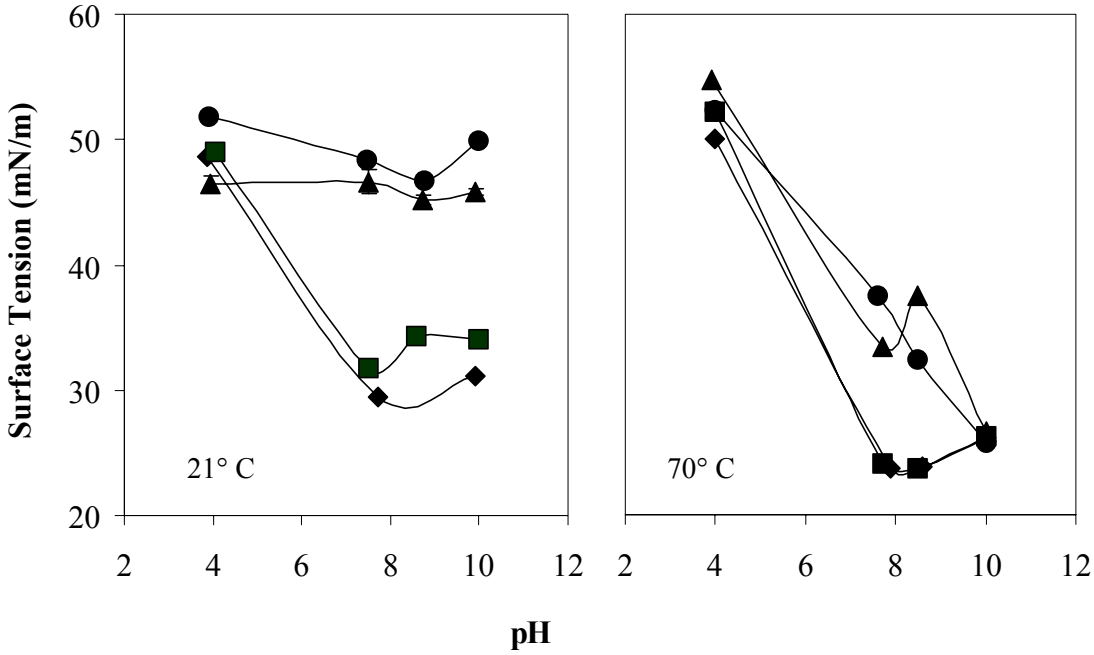


Figure 12. Effect from temperature, salt concentration, and pH on surfactin activity. (♦ = 0 g/L NaCl; ■ = 30 g/L NaCl; ▲ = 50 g/L NaCl; ● = 70 g/L NaCl at 21°C, 80 g/L NaCl at 70°C)

Supernatant containing surfactin, produced from LS-potato process effluents in an airlift reactor, was characterized under various environmental parameters to better define appropriate conditions for its use as an agent for enhanced oil recovery. By itself, pH was found to increase surface tension of the surfactin below 6. Concentrations of NaCl above 30 g/L were seen to increase surface tension, with the highest surface tension occurring about 50 g/L. When the effect of temperature was added to the pH experiment, there were no significant changes, and, again, surface tension, at any temperature, increased at pH below 6. Temperature alone, up to 70°C, did not alter surface tension of the surfactin. However, when temperature was added to experiments with salt concentration, increases of surface tension seen at 50 and 70 g/L NaCl appeared to be somewhat moderated at 70°C. This was further verified when all three parameters were combined in one experiment and no increase in surface tension was observed at 80 g/L NaCl, pH 10, or 70°C. Temperature experiments did not go beyond 70°C, so the high-temperature limit of surfactin has yet to be determined.

Surfactin is an anionic, amphiphilic, lipopeptide compound. These properties are the reason for its ability to lower surface tension so effectively.[15] Surfactin is also an effective antimicrobial, and antiviral, able to induce formation of ionic pores in phospholipid bilayers,[16] and transport cations across membranes.[17] Its cation-complexing property, due to two negative charges on the aspartyl and glutamyl residues,[17] is probably fully utilized in our system. which contains ubiquitous amounts of Ca^{+2} and Na^{+} ions in the low-solids potato process effluent.[9] The increase of surface tension of surfactin with higher NaCl concentration and lower pH is more likely due to a precipitation processes commonly seen with proteins and used in protein purification. Precipitation of proteins can be achieved by adding salts, organic solvents, altering the pH, or altering the temperature.[18] In this study, we altered three of these variables. Furthermore, an increase in temperature to 70°C, and an increase of pH to 10, probably decreased the hydrophobic effects caused from high salt concentration on the surfactin in the solution, so that protein aggregation by association of hydrophobic surfaces did not occur.

These are favorable results for the application of this surfactin as an agent of enhanced oil recovery, since high salt concentration, high temperature, and high pH describe the conditions of many oil reservoirs. This surfactin was also produced cheaply with potato process effluents, adding to its desirability. Previous experiments with surfactin produced in minimal salts media containing potato starch have shown similar results. However, note that changes in the process from which the feedstock is derived could have an impact on these results.

Future production of surfactin from potato process effluents will be used in corefloods to further characterize its potential application as an agent for enhanced oil recovery.

3.3 Biopolymer Characterization

3.3.1 Solubility

On average, 50 g/L of dry biopolymer was produced by *Agrobacterium sp*. ATCC 31749. The dry biopolymer was soluble when the pH was greater than 11.4, insoluble in nanopure water, and soluble when heated to 50°C. When the pH of the soluble biopolymer solution decreased to 10.8, a viscous free-flowing gel formed. When an insoluble biopolymer mixture was rendered soluble with heat, a semisolid gel formed upon cooling.

3.3.2 Viscosity

3.3.2.1 Salt Concentration. Figure 13 shows the affect of pH and salt concentration on the viscosity of a biopolymer solution. The viscosity of the biopolymer depends on the pH, but not necessarily on the salt concentration. Initially, the biopolymer solution was below 50 centipoises (cP). The biopolymer solution became viscous when the pH dropped below 10.8, then any additional reduction in pH below 6.0 caused the viscosity of the biopolymer solution to decrease. The addition of salt does not affect the biopolymers ability to form a gel; however, a delay in gelling was observed when a 3% salt concentration was used. The biopolymer was not soluble when 5% NaCl was added to the 0.01M KOH solvent.

After the pH is dropped below 10.8, a gel forms; any additional reduction in pH causes the viscosity to decrease, as shown in Figure 7. The biopolymer solution can then be increased to a pH above 11.4, and when the pH drops below 10.8, a gel reforms (data not shown).

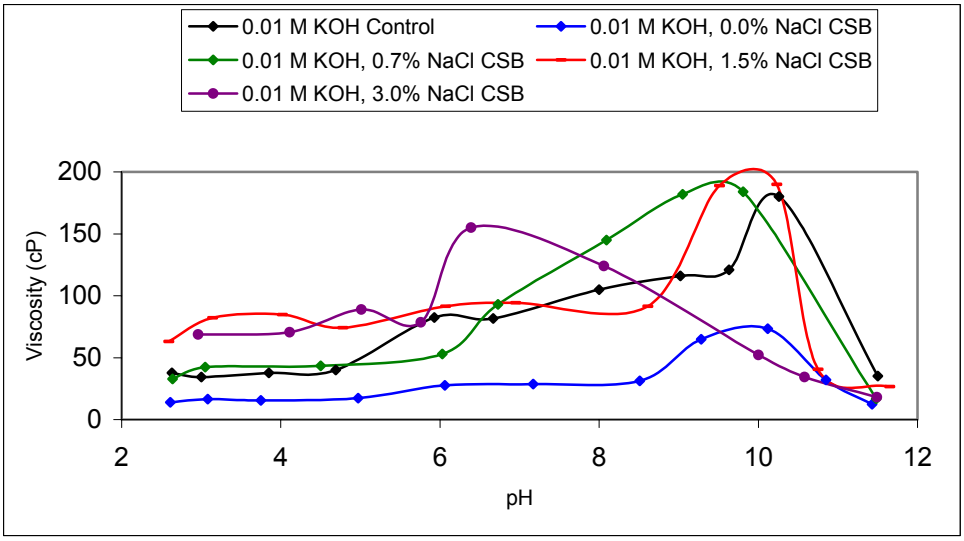


Figure 13. Viscosity of 0.01M KOH biopolymer solution as a function of pH and salt concentration (w/v).

3.3.2.2 Temperature. Figures 14 and 15 show the reduction of viscosity as a function of temperature. Figure 14 displays the relationship of viscosity and temperature of a 10% biopolymer solution with an initial viscosity of less than 20 cP and an initial pH of 11.36. Figure 15 shows the correlation of viscosity and temperature of the gelled biopolymer with an initial pH of 8.08 and a viscosity of greater that 300 cP. The data demonstrate a reduction in viscosity as the temperature of the biopolymer increases, regardless of the initial viscosity of the biopolymer. The results also show that when 0.01M KOH is used as the solvent compared to 0.10M KOH, higher viscosities are obtained using 0.01 M KOH. The results indicate that an elevated temperature is detrimental to biopolymer gelation.

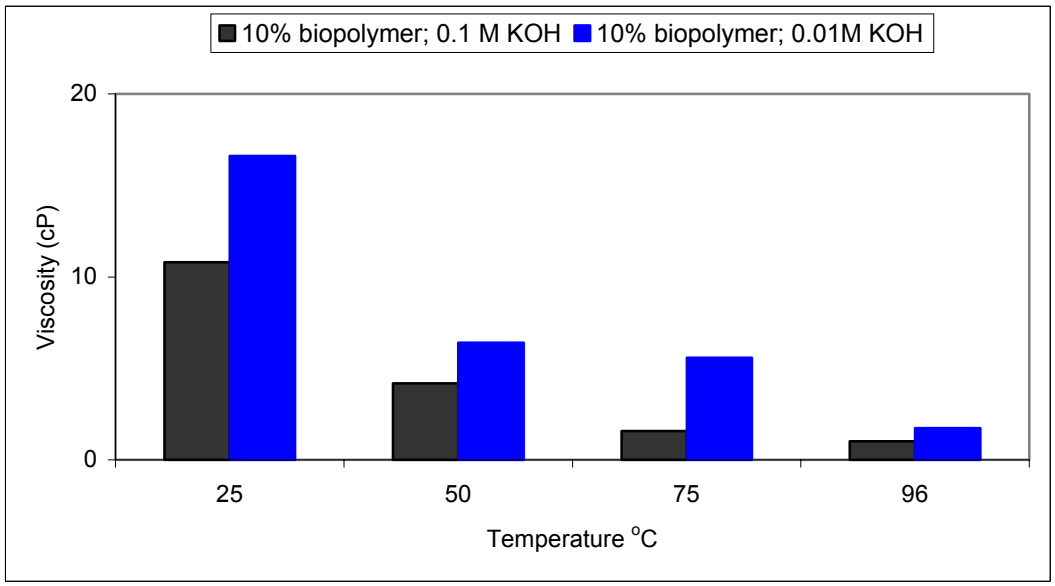


Figure 14. Viscosity of biopolymer solution as a function of temperature.

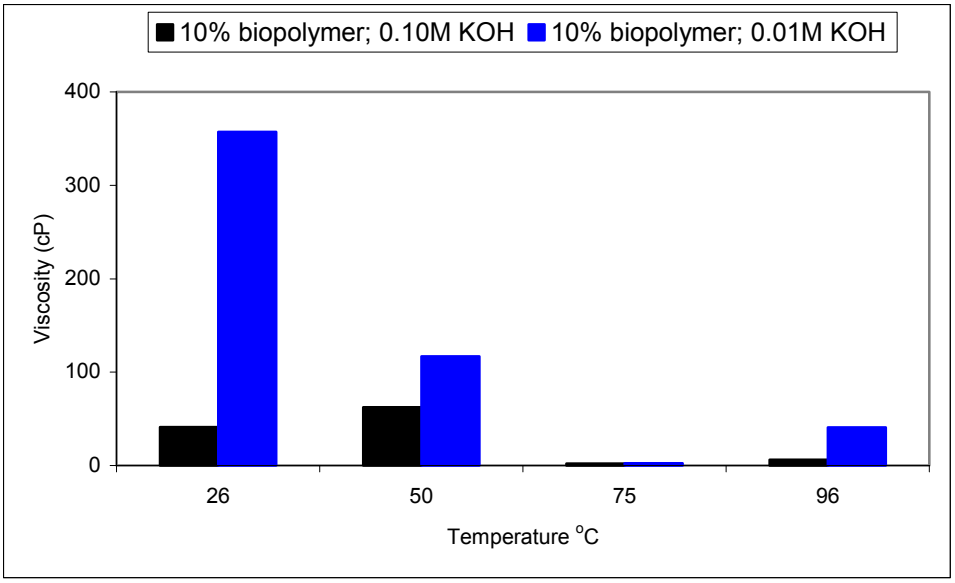


Figure 15. Viscosity of gelled biopolymer as a function of temperature.

3.3.3 Carbohydrate Analysis

The total carbohydrate results from each pore volume of core effluent are shown in Figure 16. The results demonstrate that at least 2 to 5 pore volumes of biopolymer solution need to be injected for biopolymer breakthrough. Results of the total carbohydrate injected into the core are compiled in Table 2. The results show that 9,991 to 13,986 ppm of carbohydrate was injected. Cores B14, B17, and B18 showed a higher effluent concentration of carbohydrate than what was injected. The carbohydrate analysis was completed to ensure that the biopolymer solution was an acceptable concentration range for the core studies. No attempt was made to vary the concentration of the biopolymer solution injected.

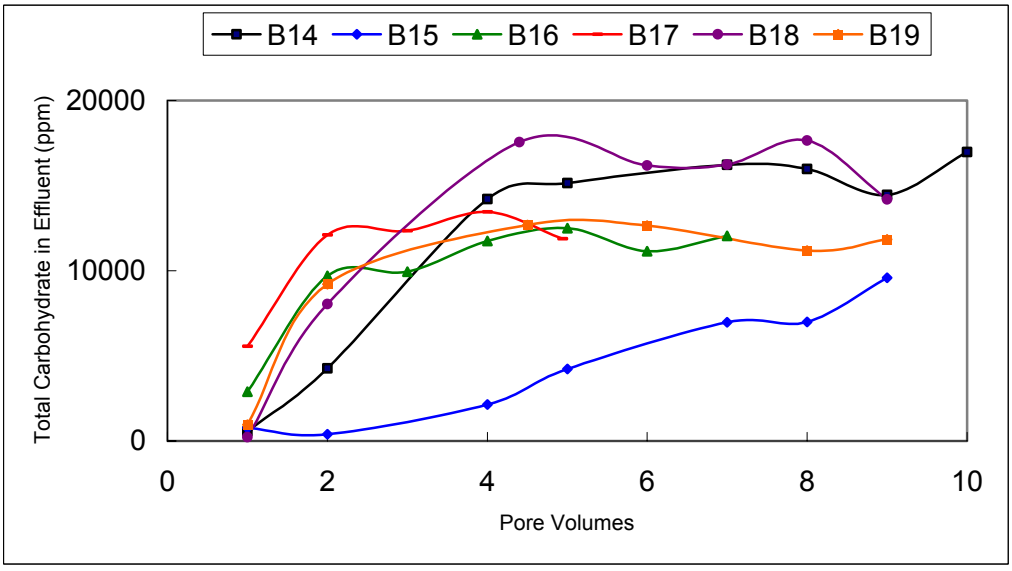


Figure 16. Viscosity of 0.01M KOH biopolymer as a function of pH and salt concentration.

3.4 Permeability Modification by Biopolymers

3.4.1 Berea Sandstone Cores

Table 2 shows the physical parameters of the Berea sandstone cores used for experimentation.

Table 2. Core characteristics.

Core	Length (cm)	Diameter (cm)	Pore Volume (mL)	Porosity (%)	Total Carbohydrate injected (ppm)	Viscosity of Injection Fluid (cP)
B12	13.30	2.53	12.81	19.18	0	1.02
B13	13.30	2.53	12.90	19.31	0	1.13
B14	13.26	2.53	12.84	19.28	11,185	7.21
B15	13.34	2.53	12.99	19.39	13,386	11.70
B16	12.64	2.53	12.19	19.20	12,478	10.40
B17	13.40	2.53	13.10	19.47	9,991	2.92
B18	13.40	2.53	12.93	19.21	12,871	8.22
B19	13.30	2.53	12.73	19.06	12,871	8.22
B7	13.30	2.53	13.22	19.77	0	1.02
B9	13.30	2.53	13.37	20.00	12,725	5.96
B6	13.30	2.53	13.31	19.91	13,986	7.98
B11	13.34	2.53	12.84	19.15	12,627	3.62

The results of all the Berea sandstone cores tested are shown in Table 3. The permeability of both control cores, which were injected with a different molarity of alkaline brine, increased. The pH of core B12 dropped from 11.93 to 10.68, whereas the pH of core B13 slightly decreased from 12.88 to 12.71. Despite the fact that the permeability increased, the results indicate that Berea sandstone could sufficiently buffer 0.01M KOH brine, but not 0.1M KOH brine. Therefore, 0.01M KOH brine was used for further core experiments. The remaining Berea sandstone cores injected with 0.01M KOH biopolymer solutions were effective at triggering gel formation and reducing the permeability. Previous reports indicate that 0.1M KOH or 1M KOH was not sufficient to cause gelation.[19] In contrast, our studies clearly indicate that 0.01M KOH can be buffered by the internal core matrix and is adequate for gel formation. The specific mechanism of gelation within the core is unknown and no attempt was made to investigate the gelation mechanism. Test tube results show that the biopolymer solution gels when the pH drops below 10.8. The Berea sandstone core buffers the alkaline brine, causing reduction in the pH and subsequent gel formation.

Table 3. Permeability modifications.

Core	Pore volumes injected (mL)	Pre injection Permeability (mD)	Post injection Permeability (mD)	Permeability Reduction (%)	Residual Resistance Factor (RRF)
B12	10.00	122.69	186.80	-52.257	0.66
B13	9.95	129.72	196.54	-51.51	0.66
B14	10.03	112.00	0.32	99.71	350.00
B15	9.48	134.65	0.17	99.87	792.06
B16	6.68	167.89	2267.46	-1250.56	0.07
B17	4.94	161.94	249.43	-54.03	0.65
B18	9.23	152.93	64.97	57.52	2.35
B19	8.84	146.70	5.13	96.50	28.60
B7	9.59	150.20	8.98	94.01	16.72
B9	6.46	136.60	6.41	96.31	21.31
B6	6.04	122.60	2.20	98.73	55.73
B11	6.87	169.00	0.98	99.42	172.45

All cores injected with alkaline brine displayed a reduction in pH. Figures 17–20 show the correlation of pH with permeability reduction. The effluent pH was measured before accumulating one pore volume so that the injection brine did not alter the pH. The effluent was clear after shut-in. The pH of core B14 dropped from 11.45 to 10.21, and core B15 decreased from pH 11.28 to 10.04. The addition of heat catalyzed a sharper decrease in the pH. Core B16 initially had a pH of 11.73, and decreased to pH 9.35. The pH from core B17 decreased from 11.73 to 9.17. The pH of core B18 dropped from 12.04 to 10.67, and core B19 decreased from 12.04 to 10.46. A further decrease in pH was observed in all effluent; however, this reduction can be attributed to brine introduction, since the pH was measured after more than one pore volume was injected.

The postinjection permeability increased in the control cores; however, the permeability decreased greater than 94% when biopolymer was injected. For example, core B15, which had biopolymer injected and shut-in at ambient temperature decreased the permeability from 134.65 to 0.17 mD. Like the control cores, the postinjection permeability increased when the cores where heated. These results correlate with the data described above in this document about a reduction in biopolymer viscosity at elevated temperatures.

The magnitude of the residual resistance factor (RRF) varied from 0.03 to 792.06. A residual resistance factor of 792.06 was calculated for core B15, which was injected with biopolymer at ambient conditions. The control cores and cores heated to 60°C had low RRFs; whereas the cores injected with biopolymer had higher RRFs. Lower RRFs were observed when 2% sodium chloride was added to the brine.

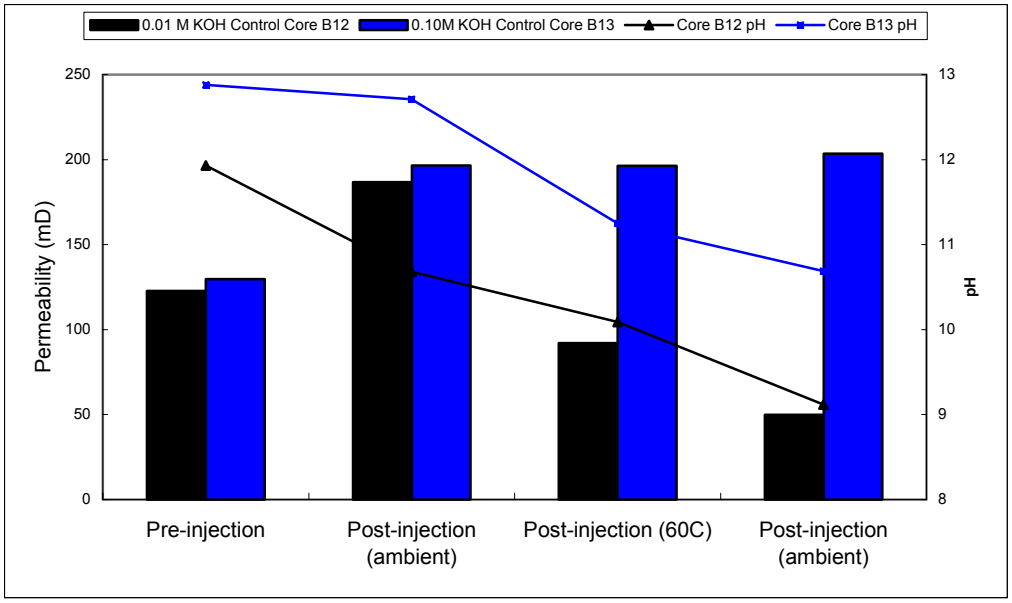


Figure 17. Permeability modification and pH control of Berea sandstone core.

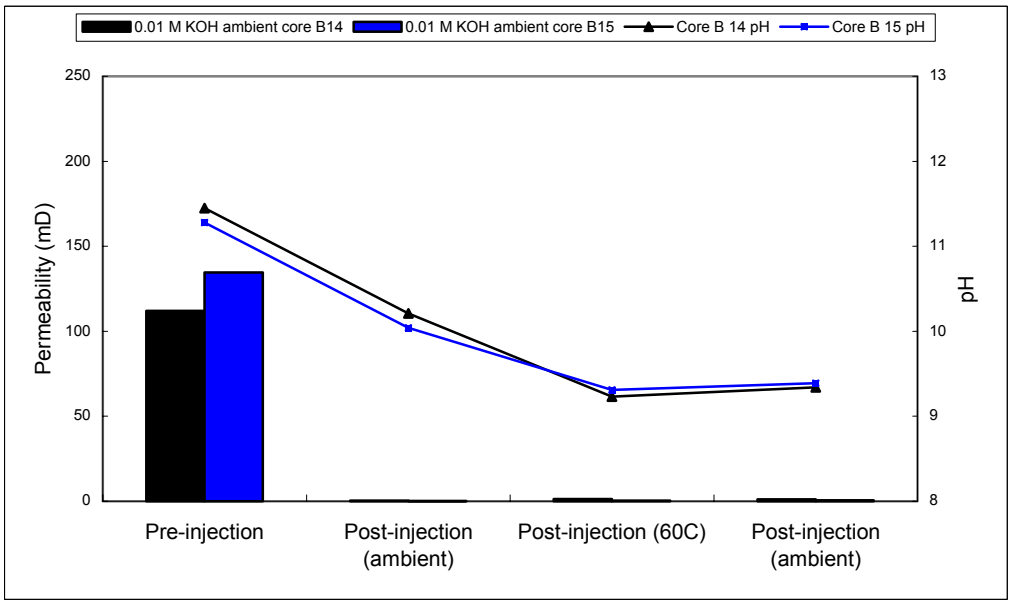


Figure 18. Permeability modification and pH control of Berea sandstone core at ambient temperature.

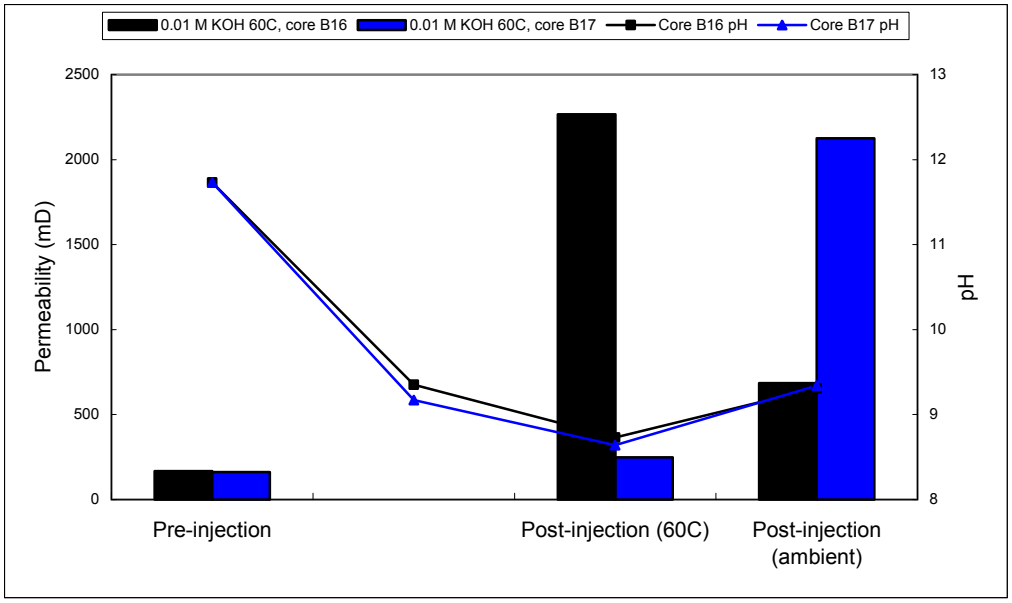


Figure 19. Permeability modification of Berea sandstone at 60°C.

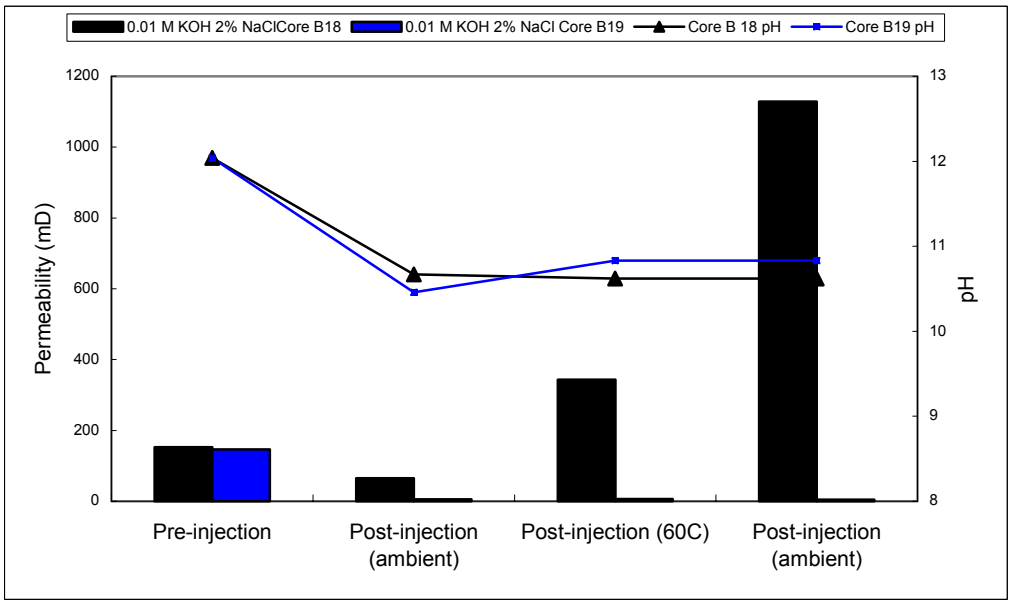


Figure 20. Permeability modification and pH control of Berea sandstone core with 2%w/v NaCl brine.

The effects heating and cooling had on permeability are shown in Figures 17–20. The postinjection permeability (60°C) represents the permeability calculated at temperature, whereas the postinjection permeability (ambient) shown on the far right represents the permeability after the cores were heated and cooled to room temperature. A decrease in permeability was noted for the 0.01M KOH control core after the core was heated and then cooled, whereas the permeability remained steady during the heat/cool treatment for the 0.1M KOH control. Cores B14 and B15, which were injected with biopolymer, maintained low permeability after the heat/cool treatment, indicating that the heat/cool treatment did not alter biopolymer gelation. However, the remaining cores show mixed results. The variability is attributed to the fact that more that one pore volume of brine was injected to determine the permeability at 60°C . At

elevated temperatures, the biopolymer becomes less viscous; hence, it is possible that the biopolymer washed out from the core.

3.4.2 Schuricht Oil Cores

All cores saturated with Schuricht crude oil displayed a reduction in pH when 0.01M KOH was injected. The results are shown in Figure 21. Control core B7 had an initial pH of 11.93 and dropped to 10.76. The pH of core B9, which had biopolymer injected, dropped from 11.62 to 10.59. The effluent pH of core B6 decreased from 11.65 to 9.32 with the addition of heat. Core B11 initially had a pH of 10.62 and decreased to 9.85. These results are consistent with cores tested without Schuricht crude oil and confirm that the presence of Schuricht crude oil was not detrimental to the buffering capacity of Berea sandstone cores.

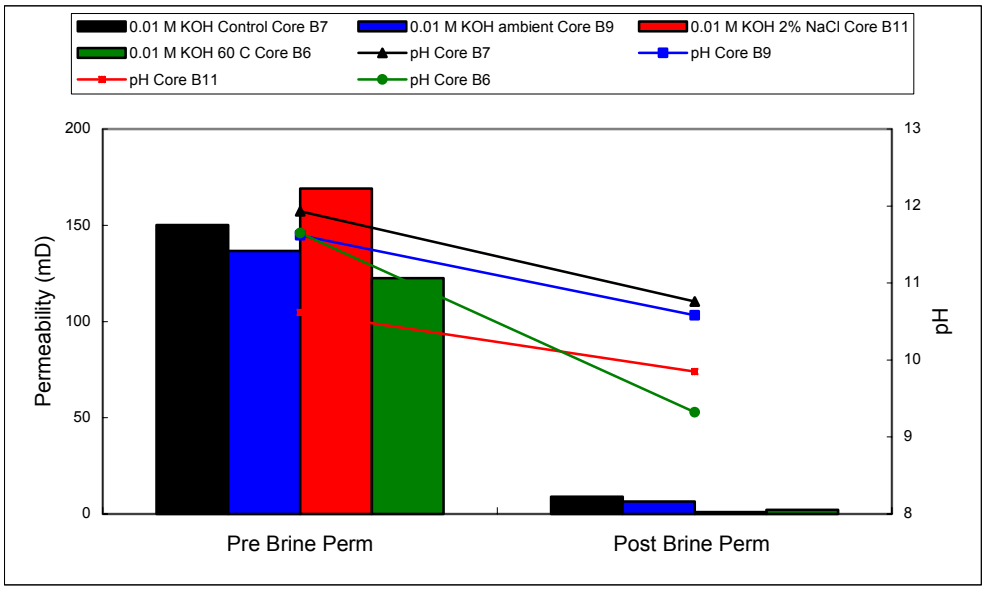


Figure 21. Permeability modification and pH control of Berea sandstone core saturated with Schuricht crude oil.

The postinjection permeability was reduced in all cores, including the control core, which had no biopolymer injected. The permeability decreased 94% in the control core contrary to the increase in permeability observed in the control core without oil. This indicates that Schuricht crude oil prevents the permeability from increasing. The postinjection permeability decreased from 122.60 to 2.20 mD when the cores were shut in at 60°C. When comparing core B6 to core B16 and B17, which had biopolymer injected and were shut-in at 60°C, the permeability decreased in core B6 and yet increased in core B16 and B17. The only difference between the cores was the presence of Schuricht crude oil. The results indicate that the presence of Schuricht crude oil reversed the permeability increase in the cores that were heated to 60°C.

The residual resistance factors for cores with Schuricht crude oil are reported in Table 3. The RRFs ranged from 16 to 172 in the cores with Schuricht crude oil. All cores injected with biopolymer showed an increase in the RRF compared to the control core. The highest RRF was 172 when 2% salt was injected with the biopolymer.

4. CONCLUSIONS

The results of the research described in the report can be summarized as follows for each of the major topics.

4.1 Production of Surfactin by Bacillus Subtilis from Potato Processing Effluent in Chemostat Reactors

- Refinement of airlift reactors led to the design and operation of chemostat reactors operated in batch mode with the addition of antifoam to prevent surfactant loss.
- Mass transfer of oxygen was doubled by increasing the rate of stirring and adding a baffle. This reduced microbial contamination and decreased production time by about one-half. This represents an important improvement over previous reactor designs because of the oxygen limitation noted in airlift reactors.
- The selected antifoam did not interfere with surfactin production, efficacy, or separation.
- Surfactin production of about 6 g/L was obtained in about 12 hr. This is an improvement compared to about 9 g/L produced over a time of about 72 hr in airlift reactors.

4.2 Characterization of Surfactin Produced from Potato Processing Effluents

- The dry biopolymer was soluble at pH greater than 11.4, insoluble in nano pure water, and soluble when heated to 50^{o}C.
- A 0.01 M KOH and 0.1 M KOH solution dissolved the biopolymer.
- The addition of sodium chloride did not affect the biopolymers ability to form a gel.
- The viscosity of the biopolymer solution increased when the pH dropped below 10.8.
- An increase in the temperature caused the biopolymer solution to become less viscous.
- Berea sandstone cores can effectively buffer a 0.01 M KOH solution, but not a 0.1 M KOH alkaline solution.
- Residual resistance factors were increased by a factor of 792 when biopolymer solution was injected to Berea sandstone cores.
- The 0.01 M KOH biopolymer solution reacted with Berea sandstone cores with and without Schuricht crude oil, causing pH reduction, gel formation, and subsequent reduction in permeability.

4.3 Permeability Modification Using a Reactive Alkaline-soluble Polymer

- The dry biopolymer was soluble at pH greater than 11.4, insoluble in nanopure water, and soluble when heated to 50°C.

- A 0.01M KOH and 0.1M KOH solution dissolved the biopolymer.
- The addition of sodium chloride did not affect the biopolymers ability to form a gel.
- The viscosity of the biopolymer solution increased when the pH dropped below 10.8.
- An increase in the temperature caused the biopolymer solution to become less viscous.
- Berea sandstone cores can effectively buffer a 0.01M KOH solution, but not a 0.1M KOH alkaline solution.
- The 0.01M KOH biopolymer solution reacted with Berea sandstone cores with and without Schuricht crude oil, causing pH reduction, gel formation, and reduction in permeability.
- Residual resistance factors were increased by a factor of about 800 when biopolymer solution was injected to Berea sandstone cores.

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