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Xanthan Production by a Native Strain of *X. campestris* and Evaluation of Application in EOR

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Abstract: In this study, we used a native strain of *X. campestris* for xanthan production in lab-scale fermentor and the product was recovered with organic solvents and dried. Then we studied the potential usage of our products in different harsh conditions, including heat, pH and salinity treatments. Furthermore, we used 2D-micromodel for microbial oil recovery investigations. According to present experiments, temperature and salt contents did not have a significant influence on rheological behavior of xanthan solutions and these aqueous solutions maintained at least 80% of their primary viscosity. In addition, these solutions were resistant to a broad range of pH variations. Viscosity of the xanthan solution was increased as it was heated over 120°C. Micro-model experiments showed that the most efficient concentration of xanthan for Enhanced Oil Recovery (EOR) is 1000 mg L^{-1} and 53% of original oil in place was recovered, which showed remarkable increase comparing to original oil in place that was recovered (31%) from sole water flooding. The same or even better results were obtained from native xanthan, when its properties were compared to those of a commercial sample which was gifted by NIOC.

Key words: Enhanced oil recovery, polymer flooding, xanthan, *X. campestris*

INTRODUCTION

Xanthan gum is a complex heteropolysaccharide and an industrially important biopolymer of commercial significance (Garcia-Ochoa *et al.*, 2000; Leela and Sharma, 2000). The most efficient xanthan gum producer is *Xanthomonas campestris*, however, other species such as *Xanthomonas phaseolis, Xanthomonas carotae* and *Xanthomonas malvacearum* are known to be able to produce the same exopolysaccharide as well (Flores Candia and Deckwer, 2002).

Xanthan, widely used as a thickening or stabilizing agent in food, pharmaceuticals and oil recovery, represents the fastest growing segment of the polysaccharide industry. Xanthan gum is known for its distinctive rheological properties namely, high viscosity at low shear, shear thinning, stability over broad range of temperature and pH and high resistance to shear degradation in aqueous solution (Rosalam and England, 2003; Hsu and Lo, 2003; Flores Candia and Deckwer, 2002; Katzbauer, 1998).

The global demand for xanthan is increasing tremendously every year because of its wide applications; it becomes important to develop local strain of *X. campestris* (Leela and Sharma, 2000). Hence in present study an attempt has been made to use it in that regard. About 45% of the world's energy consumption is supplied by petroleum (Littmann, 1997). Conventional processes for extraction of oil from reservoir rocks are surprisingly inefficient. The proportion of the original oil in place which is extracted can vary from 5% to about 90%, but the average recovery is not much more than 30% (McInerney *et al.*, 1999). The petroleum industry recognized the problem of inefficient oil recovery by conventional (primary and secondary) recovery methods in early 1900s (Chang, 1978).

Flooding petroleum reservoir with water-soluble polymer may be regarded as the most economic tertiary chemical oil recovery method (Littmann, 1997). In polymer flooding, a water soluble polymer is added to the flood water (Flores Candia and Deckwer, 2002). This increases the viscosity of water. There are three potential ways in which a polymer flood can make the oil recovery process more efficient: Through affecting on fractional flow, by decreasing the water/oil mobility ratio and by driving injected water from zones that have been swept (Khachatoorian *et al.*, 2003).

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Polymer flooding is important in enhanced oil recovery because it is relatively simple to apply and the increased cost of production is moderate. It can be applied to reservoirs that are moderately heterogeneous and it requires less time and fewer steps from reservoir evaluation to field-wide development (Chang, 1978). The properties required for polymer flooding in EOR are fairly well understood. Water viscosity must be increased up to about the same value as that of oil being displaced and the solution must be resistance to shear degradation. In this study, xanthan was produced from a native bacterial strain and as a valuable biopolymer in EOR it showed a good viscosifying property and relative insensitivity to shear degradation and salt content.

MATERIALS AND METHODS

Microorganism: *Xanthomonas campestris* strain b82, a native strain previously isolated from soil (Soudi, 1990), is the bacterium used for xanthan production. It was maintained on YM agar slants at 4°C and was transferred every 14 days (Casas *et al*., 2000).

Cell culture and fermentation: Inoculum preparation was made by transferring the bacteria from the stock culture on YM-agar slant to 10 mL of YM-broth and incubated at 28°C over night. Six milliter of the last culture broth was used for inoculation of 54 mL YM-broth in 250 mL flask and aerated and mixed in a shaker incubator at 150 rpm and 28°C. This culture was used for inoculation of a bench-top lab-scale 2 L fermentor (Biostat B, B-Braun). The composition of the production medium was the same as synthetic medium introduced by Roseiro *et al*. (1992). The air flow rate was adjusted at 2 vvm and at different driving speed up to 400 rpm. The process was carried out at 28°C and runs were terminated after 72 h. All runs were replicated three times and average values calculated.

Extraction and partial purification: Fermented broth was harvested and immediately precipitated using methyl alcohol. Xanthan precipitate was collected by filtration and dried as previously described (Abd El-Salam *et al*., 1994).

Characterization of gum production: Xanthan yield in fermentation broth was determined by a simple modifications in the procedure described previously (Leela and Sharma, 2000). Viscosity measurements were performed using 1% (w/v) gum solution in distilled water and carried out using brook field viscometer with spindle number 3 (Nitschke and Rodriguse, 2000). All experiment repeated twice and the average presented in this study.

Effect of exogenous factors on xanthan gum: To assess the effects of important environmental factors, xanthan solutions went under three types of treatments including: Heat treatment (room temperature, 50, 70, 90 and 120°C for 15 min), pH treatment (1 to 14 using HCl and NaOH normal solutions) and salt treatment (1, 3 and 5% NaCl as final concentrations). The same tests were carried out with a sample of commercial xanthan gum gifted by NIOC (National Iranian Oil Company).

Glass micro-model experiments: We constructed and used a 2D-glass-etched micro-model. Details of the procedure described elsewhere (Shariatpanahi *et al*., 2005; Dastyari *et al*., 2005).

The micro-models were saturated with water and n-hexadecane (as a hydrocarbon model) was injected into the models at a high rate to evacuate the water. The remaining water at the end of experiment was served as connate water. The water colored with Methylene Blue and n-hexadecane colored with Sudan Red (Fig. 1A).

Polymer flooding conducted with a JMS syringe pump at flow rate of 0.4 mL h^{-1} (Fig. 1B). One pore volume of different concentrations of polymers (400-2000 mg L^{-1}) was injected. The results were obtained based on picture

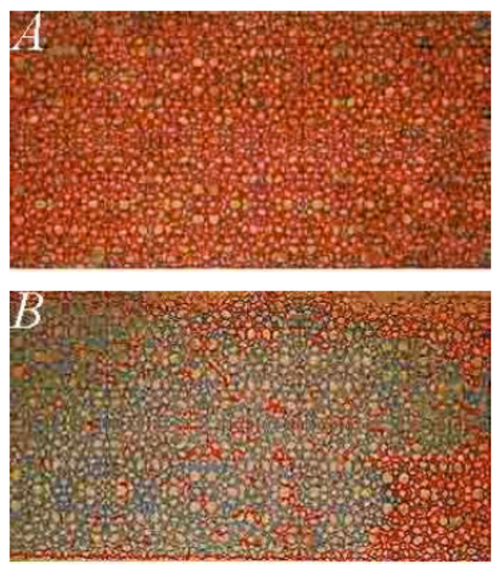

Fig. 1: Images of polymer flooding in 2D-micromodel saturated with hexadecane, A: before polymer flooding (dark color, because of red staining of hexadecane); B: After injection of 1 PV (pore volume) polymer solution in rate of 0.4 mL h^{-1} and replacement of hexadecane with blue-dyed polymer (light color)

processing using Matlab software. (Shariatpanahi *et al*., 2005; Dastyari *et al*., 2005). In addition to polymer flooding, water flooding test was performed using tap water under the same condition.

RESULTS

Xanthan production carried out using fed-batch fermentation. Considerable amount of xanthan was produced in 15.56 g L^{-1} concentration and yield of 51.86%. Also a small amount of biomass (0.918 g L^{-1}) was obtained. One percent solution of xanthan from this native strain showed a viscosity of 914.7 mPa.s.

The rheological properties of xanthan gum in salt-containing solutions and at increased temperature are of the great practical interest specially for its application in enhanced oil recovery during which xanthan solutions are exposed to increased salt concentrations and relatively high temperatures. We prepared 1% (w/v) solution of the xanthan gum and measured the variation of viscosity in different treatments. Viscosity flow curves present the dependence of the viscosity on these treatments (Fig. 2-4). It can be seen that increasing the temperature obviously maintained their viscosity in a wide pH range of 3 to 11. The same results were obtained for the commercial sample.

Present experiments focused on efficiency of polymer flooding. In this study we prepared different xanthan solutions and investigated the 1000 mg L^{-1}. We could recover 53 and 42% of

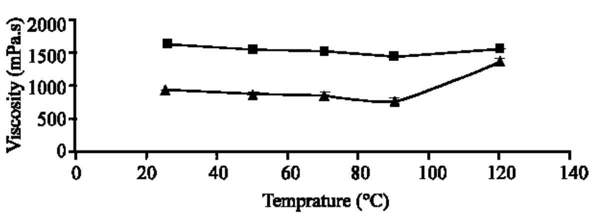

Fig. 2: Effects of different heat treatments on 1% (w/v) solutions of commercial xanthan gum (■) and gum from strain b82 (▲), in a constant time (15 min)

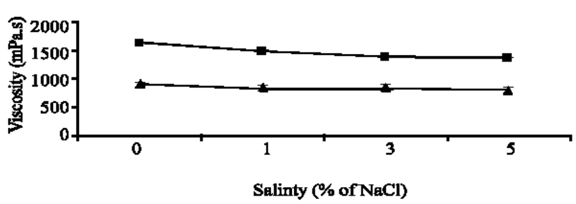

Fig. 3: Viscosity variations of biopolymer solutions in presence of different salt concentrations: commercial gum (■), gum from strain b82 (▲)

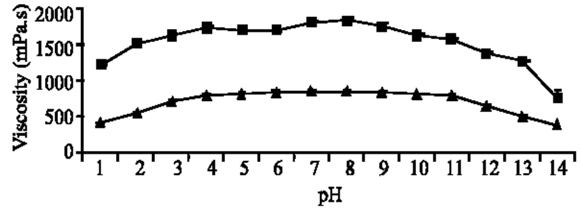

Fig. 4: Viscosity variations in a broad range of pH changes (1-14): commercial gum (■), gum from strain b82 (▲)

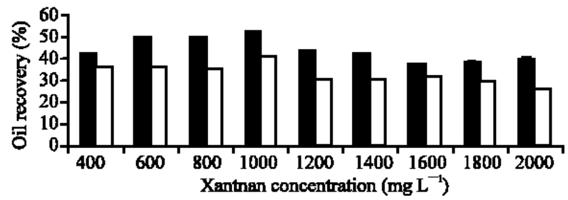

Fig. 5: Amount of oil recovery at different concentrations of biopolymer: commercial gum (□), gum from strain b82 (■). Errors bars are too small to be seen

original results in a slight reduction in viscosity. The same pattern was obtained when the influence of inorganic salt content on the viscosity investigated. Xanthan solutions are resistant to a broad range of pH values and they have percentage of oil recovery versus xanthan concentration. These experiments showed that the most efficient concentration of xanthan for EOR (in this system) was oil in place with native xanthan gum and commercial one, respectively. This indicates remarkable increase comparing to that recovered from sole water flooding (31%) of original oil in place (Fig. 5).

DISCUSSION

The fed-batch fermentation is a successful method for xanthan production. As suggested previously, achievable productivity and concentration for xanthan varies between 10 to 30 g L^{-1} (Garcia-Ocha *et al*., 2000) and we obtained an amount of 15.56 g L^{-1} in this experiments.

According to Jeremic *et al*. (1999) polymers that are most commonly found in petroleum operation must be resistant to the temperature and salt contained in reservoir. The present study showed that the biopolymer is scarcely sensitive to different salt concentrations up to 5% and relatively high temperatures and maintained more than 80% of its original viscosity under these conditions. In addition, the gum keeps its viscosity in various pH values which makes it an ideal biopolymer for enhanced oil recovery.

Present experiment showed that polymer flooding with the gum from strain b82 improved oil recovery and we obtained even better results in salt treatment experiment (remaining 88% of primary viscosity) comparing to that of commercial gum. Also in comparison, insignificant decrease in viscosity was observed.

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