Biotechnological Journal of
Environmental Microorganisms
"BJEM"

Biotechnological Journal of Environmental Microorganisms(BJEM) 3(11) 2024 580-588

Increasing Thermal Resistance of the Exopolysaccharide Produced by *Xanthomonas campestris* in Presence of calcium cation: Biopolymer Enhancement for Application in Petroleum industry

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Received: 07 August 2024/ Revised: 20 August 2024/ Accepted:23 August 2024

Abstract

Xanthan is one of the well-known polysaccharides which its industrial production began in the last century and yet is used in water-based drilling fluids for drilling oil wells, as well as other environmental applications such as increasing the resistance of building materials in geotechnics, in biochemical composition of barriers to prevent the spread of water pollution and soil cover to combat air pollutant fine-dust. This microbial biopolymer reduces water mobility by increasing viscosity and decreasing permeability, and each xanthan molecule binds countless water molecules. Despite its resistance to various stresses, increased resistance to high temperatures is required in petroleum biotechnology and other man-made environmental infrastructure. In this study, the culture of *Xanthomonas campestris* was investigated in the presence of salts and the extracted biopolymer was treated with different concentrations of the salt. The experiments showed that sulfite, which is used as an antioxidant agent to increase temperature resistance, had no significant effect. However, calcium chloride solution with a final concentration of 10,000 mg/L increased the thermal resistance and at 120°C the viscosity of the treated xanthan was more than 1500 centipoise and higher than that of the untreated 1% xanthan solution. Calcium cation increased the thermal resistance of xanthan even at concentrations lower than 1000 mg/L. The time of exposure to calcium cations was effective in increasing the thermal resistance. Addition of calcium ions to the culture of *Xanthomonas campestris* bacteria also had similar effects. This effect is promising for the environmental applications of xanthan, especially its use in the composition of water-based drilling fluids.

Key words: Drilling fluids, Environmental Applications, Thermal Resistance, Viscosity, Xanthan gum

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doi:10.71886/bioem.2024.1217414

Introduction

Xanthan is an anionic heteropolysaccharide produced by *Xanthomonas campestris*. This polymer is composed of glucose subunits linked by β1→4 glycosidic bonds (Petri, 2015) and is widely used as the most commercially successful polysaccharide to date, with annual global production more than 150,000 tons (You et al., 2025). Xanthan reduces water mobility by increasing viscosity and decreasing permeability in oil extraction. Stability against stress and suitable viscosity at high concentrations of salts (including calcium and magnesium ions) to control formation damage and fluid loss are among the advantages of xanthan over other polymers. In addition, high viscosity at low shear rate and low viscosity at high shear rate are among the special advantages of xanthan for oil recovery (Wellington, 1983; Howard et al., 2015; Zou et al., 2019; Martin- Alfonso et al., 2021; Soudi, et al., 2011); but biopolymers have problems like breakdown, oxidation and instability at high temperatures, which could limit their performance in extreme conditions (Tyagi et al., 2025). The viscosity stability of xanthan in the environmental conditions of oil extraction depends on several external factors such as temperature, salinity and porosity of rocks (Nasr, et al., 2007). Seawater contains various types of salts, the most common of which is sodium, followed by magnesium and calcium. Meanwhile, the viscosity stability of dilute xanthan solutions at temperatures of 80°C and above, the presence of high salt concentrations and high pressure, plays a very key role in the oil extraction process (Lambert and Rinaudo, 1985; Kierulf and Sutherland, 1988; Milas et al., 1988; Ash et al., 1983; Wellington, 1983). Various factors have an effect on increasing the thermal stability of xanthan. The presence of some salts in the xanthan solution could have a significant effect on increasing the thermal resistance. The reason for this may be partly due to the role of some salts in maintaining the regular structure of the xanthan molecule and preventing damage to the main chain from chemical agents at high temperatures (Martin- Alfonso et al., 2021; Cabot et al., 2015). The presence of oxygen is an important factor in the oxidation and decomposition of xanthan gum due to heat (Kierulf and Sutherland, 1988). Some studies have also pointed out the effect of pH on the thermal resistance of xanthan (Milas et al., 1988). In this study, we investigate the effect of Na_2SO_3 and $CaCl_2$ on xanthan viscosity before and after heat treatment.

Materials and methods

Effect of Na_2SO_3 on xanthan solution viscosity

In order to investigate the effect of Na_2SO_3 on xanthan viscosity, 3 samples of an aqueous solution of 5 g/L of xanthan was prepared and then Na_2SO_3 was added to each prepared xanthan solution at levels of 0.1 and 0.05 g/L, respectively (xanthan solution of 5 g/L with no Na_2SO_3 was considered as control). The viscosity of all 3 samples was measured before and after heat treatment by autoclave (15 min at 121°C) using a viscometer in a Brookfield system.

Effect of $CaCl_2$ on xanthan solution viscosity

In order to investigate the effect of $CaCl_2$ on xanthan viscosity, 3 samples of an aqueous solution of 10 g/L of xanthan was prepared and then a certain volume of calcium chloride stock solution was added to each prepared xanthan solution so that the final concentration of calcium chloride in each xanthan solution was 10, 2, and 0.5 g/L, respectively (xanthan solution of 10 g/L with no $CaCl_2$ was considered as control). The viscosity was measured using a viscometer in a brookfield system in 2 h and 24 h after the xanthan solution became uniform in room temperature, before and after heat treatment by autoclaving (15 min at 121°C).

Results

Heat treatment (autoclaving for 15 min at 121°C) of 5 g/L xanthan containing 0.1 g/L Na_2SO_3 and 5 g/L xanthan containing 0. 05 g/L Na_2SO_3 was observed in Figure 1. Xanthan solution (of 5 g/L) treatment with Na_2SO_3 at 0.05 g/L and 0.1 g/L did not differ in viscosity from each other. The viscosity of both solutions treated with Na_2SO_3 was decreased compared to the control, and no viscosity increase or stability was observed.

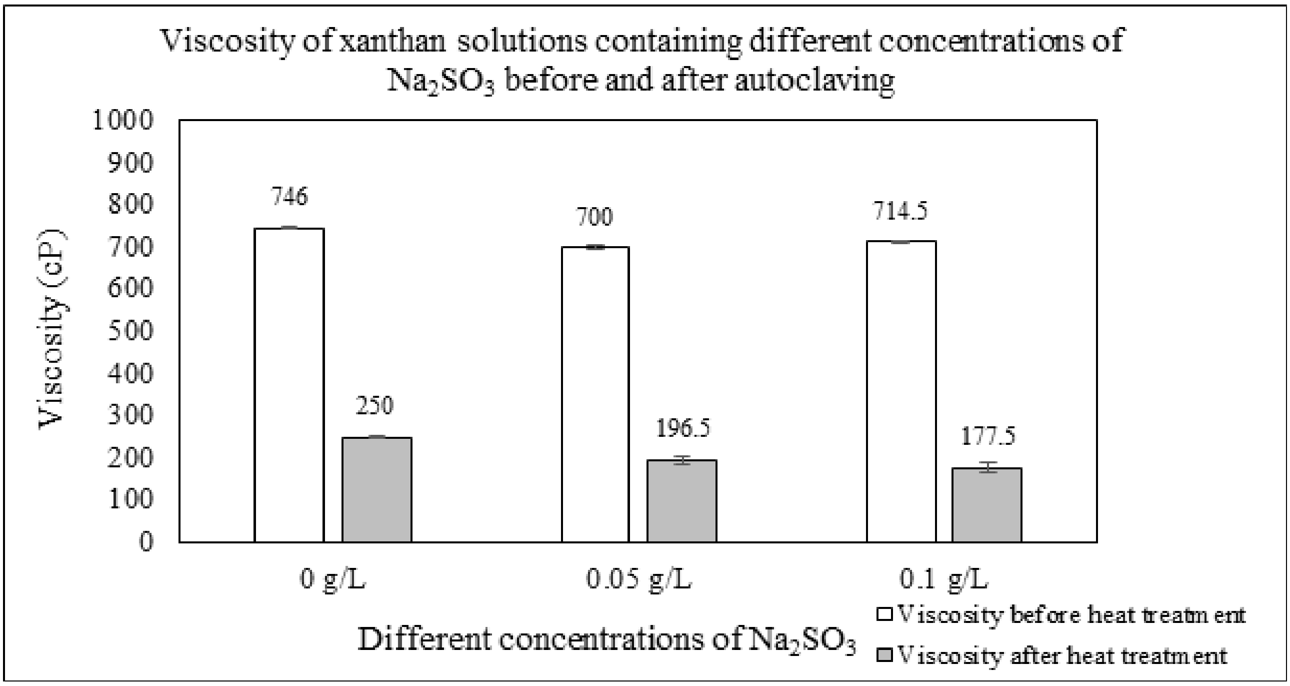

Fig. 1. Viscosity changes of different treatments of 5 g/L xanthan solution with Na_2SO_3 in different concentrations before (white column) and after (gray column) autoclaving

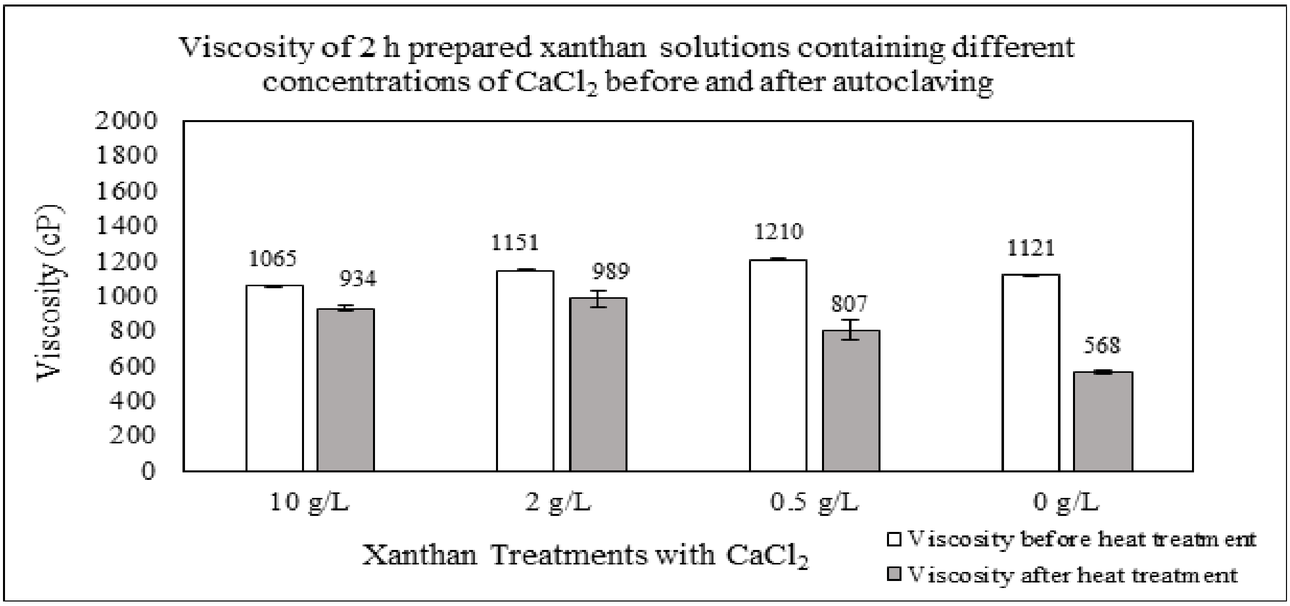

Fig. 2. Viscosity changes of different treatments of 2 h prepared of 10 g/L xanthan solution with $CaCl_2$ in different concentrations before (white column) and after (gray column) autoclaving

Heat treatment (autoclaving for 15 min at 121°C) of 10 g/L xanthan containing 10, 2, and 0.5 g/L $CaCl_2$ after 2 h that xanthan solution became uniform in room temperature, was observed in Figure 2. According to Figure 2, the viscosity of the 1 g/L xanthan solution (control) was significantly different from the viscosity of the 1 g/L xanthan solutions containing 10, 2, and 0.5 g/L $CaCl_2$. The viscosity of the 1 g/L xanthan solutions containing 10, 2, and 0.5 g/L $CaCl_2$, was significantly different from each other before and after autoclaving. The viscosity of the control solution decreased significantly (554 cP) after autoclaving, while the solutions containing 10 and 2 g/L $CaCl_2$ did not decrease significantly (about 131 cP and 162 cP, respectively). Meanwhile, the viscosity of the xanthan solution containing 0.5 g/L $CaCl_2$ decreased more than the other two samples after autoclaving (404 cP). Calcium chloride addition increases viscosity of xanthan gum solution and moves the solutions from a simple viscus ones toward jellified solutions (Figure 1). Heat treatment (autoclaving for 15 min at 121°C) of 10 g/L xanthan containing 10, 2, and 0.5 g/L $CaCl_2$ after 24 h that xanthan solution became uniform in room temperature, was observed in Figure 3. According to Figure 3, the viscosity of the two 10 g/L xanthan solutions containing 10 and 2 g/L $CaCl_2$ were increased by 174 cP and 83 cP, respectively, compared to 2 h prepared xanthan solution (Fig. 2). The viscosity of xanthan solution containing 0.5 g/L $CaCl_2$ did not show a significant increase in viscosity (11 cP). The viscosity of the 10 g/L xanthan solutions containing 10 and 2 g/L $CaCl_2$ were decreased slightly after autoclaving by155 cP and 147 cP, respectively and did not differ significantly from each other and were almost the same; however, the xanthan solution containing 0.5 g/L $CaCl_2$ was decreased in viscosity by 454 cP and was almost the same as the control.

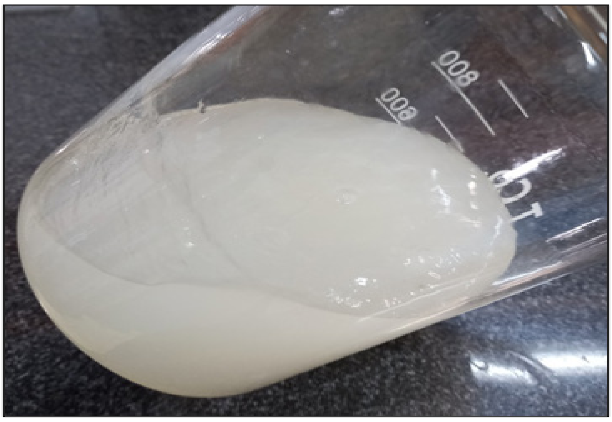

Figure (1): one percent xanthan gum solution dissolved in 1% calcium chloride aqueous solution. Jellified appearance of the solution can be easily observed.

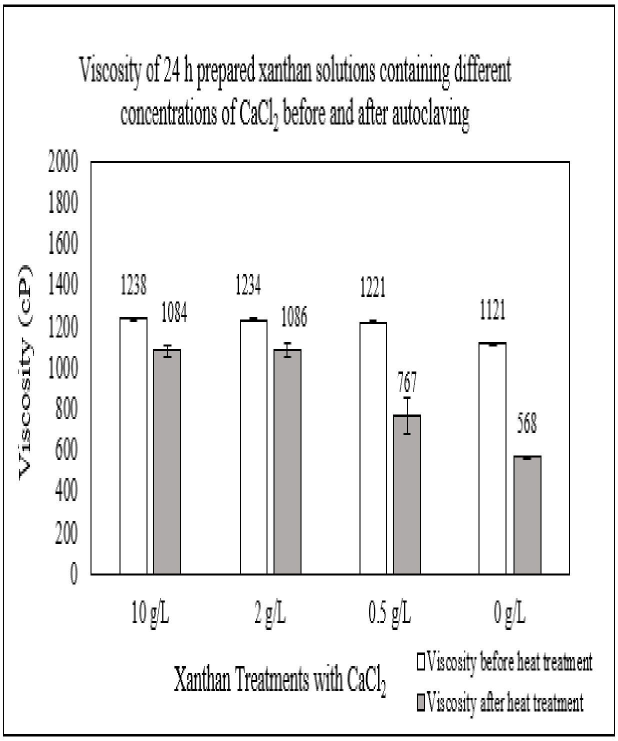

Fig. 3. Viscosity changes of different treatments of 24 h prepared of 10 g/L xanthan solution with $CaCl_2$ in different concentrations before (white column) and after (gray column) autoclaving

Discussion

The stability of xanthan depends on degree of salinity of the gum solution. Maintaining good viscosity over many months has been observed in sea water at 90°C. The successful application of xanthan at temperatures higher than 90°C requires an understanding of the factors that limit stability. A part of the high temperature resistance depends to intrinsic properties of the molecules, i. e. the nature of the gum. In addition to the nature of the polymer, other important factors include the concentration ot the gum, types and concentrations of the salts and overall physicochemical conditions, plus mechanisms governing the dissolving of the gum and its molecular degeneration. The stability features of a novel biopolymer are also described (Ash et al., 1983). Dilute solutions of polymers used to provide mobility control for EOR or making drilling fluids often lose viscosity, especially at higher temperatures. This loss of viscosity over the time results in questions on the feasibility of using polymers as mobility control agents (Wellington, 1983). It is clear that the properties of the xanthan gum are not limited just to its mobility. The thermal stability of xanthan gum in dilute aqueous solutions at 80°C or even greater temperatures is considered. The relative and apparent viscosity as a function of ageing time has been discussed by researchers, and it has been found to depend on the polymer concentration and molecular conformation as well as on the type and concentration of salt content (Lambert and Rinaudo, 1985). Divalent and trivalent metallic cations are much more effective than monovalent ones. For maximum stability, salts and protective agents were necessary, because the total absence of oxygen plays a key role in the gum stability. Chemical analysis of recovered polysaccharide indicated that whilst the carbohydrate structure was maintained, subtle changes has been occurred and for example acyl groups were very rapidly lost (Kierulf and Sutherland, 1988). *Xanthan gum* can be used in tertiary oil recovery because of its viscous and viscoelastic pseudoplastic properties (Milas et al., 1988).

It has been shown that the soluble calcium salts (including $CaCl_2$ and $Ca(NO_3)_2$) were able to induce the disordered state in the xanthan chains at concentrations between 1.0 g/L or 10 g/L, decreasing the flow consistency index (K) values. At great concentrations, as high as 100 g Ca-salt/L soluble salts K values were similar to those found for pure xanthan solutions, whereas at the same concentration of insoluble particles the K values increased 20% (Dario et al., 2011). Effect of the calcium chloride ($CaCl_2$ 1.5 mM) and pH (3, 5 and 7) on rheological properties of xanthan- carboxymethyl cellulose mixed solutions have been investigated. The biopolymer solutions in all of the tested ratios showed shear thinning flow behavior that was attributed and adjusted to the model of Herschel–Bulkley. Salt addition and pH decreasing both are the causative agents for apparent viscosity reduction (Hemmatzadeh et al. 2011). In over 40 years of use in this application, xanthan has built up a deserved reputation for reliable performance in drilling mud compositions and completion fluids, and for generally just negligible amounts of damage to reservoir formation. Its only peculiarity is its low transition or melting temperature (Tm) in low salinity fluids and in bromide brines (provided in sea salt solutions), meaning that the viscosity can collapse and degraded very suddenly at 70- 210 °C. Formate brines are capable of enhancing the thermal tolerance by increasing the *xanthan gum*'s meltinf temerature (Tm) and by providing antioxidant protection property. The extent to which the thermal stability of xanthan may be increased depends primarily on the type and concentration of the alkali metal formate salt in the biopolymer solution (Cabot et al., 2015). Xanthan solutions of different concentrations (1.0%, 2.0% and 2.5% (w/v)) were prepared in 0.1molar NaCl. in other experiments multivalent salts such as barium, calcium, magnesium, manganese, zinc, lead, as well as divalent and trivalent iron at different concentrations (0.025 M, 0.050 M and 0.075 M)) and pH values (3, 5, 7 and 9) and even aluminum salts were used.

The obtained results were very different and indicated that the success of crosslinking and the stability of the formed structures highly depend on the pH value of the salt solutions and the biopolymer concentration of the solutions. The most stable, solid structures were formed by dropping 2.5% xanthan solution into a 0.075 M solution of $FeCl_3$ at highly acidic pH values (Zahovic et al., 2018). Xanthan solutions have gained increasing interest for their use as an environmentally friendly microbial bioactive compound and biochemical agent not only in the oil industry, but other environment protection activities such as bio-cementation and so on (Hang et al., 2024). Xanthan is compatible with most concentrate brines used for controlling formation damage and fluid loss. we studied the effect of thermal aging on the rheological behavior of xanthan solutions as a function of the concentration in calcium chloride. Ionic strength below a threshold concentration does not prevent the degradation of the structure of xanthan after being submitted to aging at 121°C, the high temperature used in steam sterilization. Highly concentrated formate brines are necessary to maintain the strength of the rheological properties after exposure to high-temperature environments (Martin-Alfonso et al., 2021). Xanthan is a relatively cheap biopolymer and is suitable for oil recovery at limited temperatures and salinities. Many researches aim to modify xanthan gum to improve its viscosity for high-temperature and high-salinity reservoirs (Said et al., 2021). The presence of acetyl groups in xanthan gum can significantly influence the conformation and properties of xanthan molecules. The rheological data further illustrated that the gel strength and viscosity of deacetylated xanthan were higher than those of the intact xanthan. Collectively, acetyl groups help to maintain the ordered conformation of xanthan by the sustainable interactions between the backbone and side chains of xanthan molecules (Wang et al., 2021). Thermostability is an important indicator to evaluate xanthan applied in the oilfield industry. Besides reductive agents, salts, and pH, the inherent primary structure is also an important determinant of thermostability. Thermostability could be reinforced by adding Na_2SO_3 and saturated NaCl through improving Tm value or inhibiting degradation of the molecular chain and acyl groups (Wu et al., 2021). Our unpublished data does not necessarily similarly support those results obtained from antioxidant properties of Na_2SO_3. Xanthan is prone to thermal oxidative degradation, which limits its applications in heating processes. However, conformational changes in xanthan gum and appropriate stabilizers may improve its thermal stability. We modified the original strain used for xanthan gum production by genetic recombination through mutations and added stabilizers during the production process. According to literature pyruvyl deficiency, combined with the addition of sodium sulfite and glyoxal during the production process, was found to significantly improve the maintenance of viscosity. The apparent viscosity of the new xanthan gum solution remained above 100 mPa.s after being stored at 90°C for 48 days. Fourier-transform infrared spectra and scanning electron microscopy images showed that pyruvate free xanthan gum with added stabilizers had more extensive cross-linking than natural xanthan gum (Yuan et al., 2022). Since polymer flooding extends for over 6 months, it must retain its viscosity under reservoir conditions. Under ideal conditions (no dissolved oxygen, pH 7 to 8, and moderate-to-high salinities), xanthan solutions may exhibit half-lives of up to five years at temperatures between 75 and 80◦C. Xanthan's stability in seawater for 800 days at 80 ◦C and even better stability at 90 °C in a 50 g NaCl /L solution than 1 g NaCl /L have been confirmed (Hublik et al., 2024). In the solvent containing 5 g/L NaCl + 1 g/L $CaCl_2$ the intrinsic viscosity characteristics were the same as for the 50 g/L NaCl-solvent solution. The slight rise in intrinsic viscosity observed after ~3 months of heat treatment might be attributed to an extension of the backbone of the xanthan molecule (Lund, 1990). The latter research characterizes the flow behavior of low concentration xanthan solutions in calcium and potassium brines, evaluating the effect that potassium formate exerts on both the flow properties and the resistance to thermal degradation of xanthan solutions as a function of the biopolymer concentration. Xanthan in formate brine retains the pseudoplastic behavior up to 190°C, however, low concentrate solutions undergo a thermal degradation that decreases the recovery of pseudoplasticity after being exposed to high temperature (Reinoso et al., 2020). In Reinoso study, xanthan dispersions with KCl, LiCl, NaCl, $MgCl_2$ and $CaCl_2$ (ionic strength =150 mmol·L−1), or without any salt, were prepared. Cations from different chloride salts had no significant influence on the apparent viscosity of the dispersions (p- alue < 0.05). However, the presence of cations increased the density of dispersions, except in the case of that containing Li^+. the presence of cations induced an increase in intramolecular interactions of xanthan, especially through hydrogen bonds (H-bonds) between the backbone and the side chains. When considering the cations' net electric charges, xanthan side chains interacted more favorably with divalent cations (Mg^{2+} and Ca^{2+}) (Valente et al., 2023). The impact of divalent cations, such as Ca^{2+}, on the rheological behavior of xanthan is more complex. On one hand, divalent cations can form metal coordination bonds with the xanthan backbone, leading to an increase in solution viscosity. On the other hand, the electrostatic shielding effect of these cations reduces the repulsive forces between xanthan molecular chains, causing a decrease in viscosity. In practical water-based drilling fluid (WBDR) and EOR systems, salt concentrations are often high, sometimes reaching saturation levels. Under such conditions, increasing the xanthan concentration is necessary to maintain the desired viscosity. For instance, at xanthan concentration of 1%, increasing NaCl concentration results in an initial increase in viscosity, followed by a decline and eventual stabilization. This trend also contributes to an increase in the xanthan conformational transition temperature, with divalent cations exhibiting a similar pattern. Such measures are essential to ensuring the desired rheological performance in WBDF and EOR operations (You et al., 2025).

Conclusion

In conclusion, salinity plays a critical and intricate role in shaping the rheological behavior of *xanthan gum* solutions. Both inorganic and organic salts can significantly alter the viscosity and viscoelastic properties of xantha gum solutions by weak and strong bonds influencing intermolecular interactions, ion shielding effects, and hydrogen bonding associations. For WBDF and EOR applications, maintaining optimal viscosity and weak gel characteristics under harsh petroleum reservoir conditions requires a strategic approach. This can be achieved by carefully regulating salt and xanthan concentrations or selecting specific types of salts.

Acknowledgements

Authors would like to thank Mr. Mojtaba Badri-benam, CEO of Gum Tech Company (Azarshahr Road, Shahid Salimi Industrial Park, Tabriz, East Azarbayjan Province, Iran) for making financial support of the research to be carried out properly.

Conflict of interest

There is no conflict of interest.

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