Rheological Properties of Guar Galactomannan Solutions during Hydrolysis with Galactomannanase and α-Galactosidase Enzyme Mixtures

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Guar galactomannan, a naturally occurring polysaccharide, is susceptible to hydrolysis by three enzymes: β-mannosidase, β-mannanase, and α-galactosidase. The β-mannosidase cleaves a single mannose unit from the nonreducing end of the guar molecule, the β-mannanase cleaves interior glycosidic bonds between adjacent mannose units, and the α-galactosidase cleaves the galactose side branches off the guar. In this study, hydrolysis of guar solutions using hyperthermopilic versions of these enzymes together in different proportions and combinations are examined. The enzymatic reactions are carried out in situ in a rheometer, and the progress of the reaction is monitored through measuring the variation in zero shear viscosity. We find the presence of α-galactosidase to affect the action of both β-mannanase and β-mannosidase with respect to solution rheology. However, this effect is more pronounced when the α-galactosidase and β-mannanase or β-mannosidase enzymes were added sequentially rather than simultaneously. This likely is the result of debranching of the guar, which facilitates attack on β-1,4-linkages by both the β-mannanase and the β-mannosidase enzymes and increases hydrolytic rates by the individual enzymes. A rheology-based kinetic model is developed to estimate the reaction rate constants and interpret synergistic effects of multiple enzyme contributions. The model fits the experimental data well and reveals that both the native and the debranched guar have the same activation energy for β-mannanase action, although debranching considerably increases the frequency of enzyme−guar interactions.

1. Introduction

Galactomannans are naturally occurring hetero-polysaccharides of galactose and mannose monosaccharide units. The molecular architecture of all galactomannans consists of a linear backbone of β-1,4-linked mannopyranosyl units with α-1,6-linked galactopyranosyl branches, as shown in Figure 1.[1] Although all naturally occurring galactomannans possess this general structure, they differ in their mannose/galactose (M/G) ratio. Among the naturally occurring galactomannans, guar gum is the one used most often industrially and has an M/G ratio of ∼2.[2]

The widespread use of guar galactomannan stems primarily from its structural properties that can lead to high viscosity polymer solutions.[3] Native guar can also be modified by both chemical and enzymatic methods to extend its range of applications.[4,5] Guar and its modifications have thus spawned a variety of commercial and industrial uses in hydraulic fracturing,[6] food,[7] pharmaceuticals,[8,9] textiles,[10] cosmetics,[11] detergents,[12] and health care and personal care products.[13] In addition, guar is safe for human consumption and is commonly mixed with other biopolymers to thicken or bind food products.[14−17] Since guar has the lowest M/G ratio among commercially available natural galactomannans, galactomannans with a higher M/G ratio can also be obtained by debranching guar. These modified guars are expected to mimic the properties of other natural galactomannans.[16,18−20]

Many applications of guar require control of its molecular weight (MW), molecular weight distribution (MWD), and the intermolecular interactions to tailor its rheological and microstructural properties. For instance, partially hydrolyzed guar gum can be used as a substitute for dietary fibers because of its low viscosity and high solubility.[7,21,22] The biodegradability and the release characteristics of guar gum as a tablet matrix can be modulated by judiciously restructuring its architecture.[9,23] In applications of guar solutions as hydraulic fracturing fluids, the outflow of oil/gas is facilitated by controlled hydrolysis of the guar solution. Guar is often used in many food products along with other polysaccharides[24] and proteins.[25] The molecular interaction between guar and other food ingredients can be further controlled by debranching the guar and changing the M/G ratio[26] to optimize food composition without compromising functionality. The degradation of guar can also be used to obtain different galactose or mannose oligomers.[27]

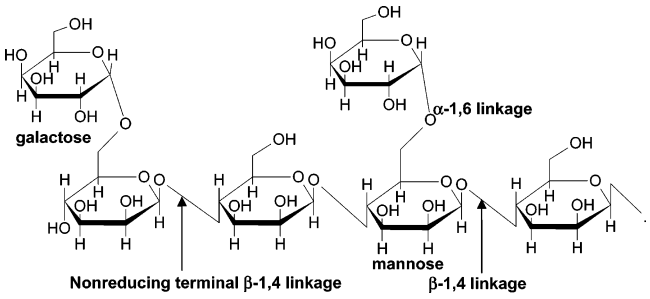

Figure 1. Structure of guar galctomannan showing the active centers of three different enzymes. β-Mannanase cleaves interior β-1,4-linkages; β-mannosidase cleaves only nonreducing terminal β-1,4-linkages; α-galactosidase cleaves α-1,6-linkages.

Guar can be depolymerized by several mechanisms including acid hydrolysis,[28] ultrasonication,[29] irradiation, and enzymatic hydrolysis.[30,31] Among all these methods, enzymatic hydrolysis

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of guar gum offers a powerful approach to restructure the molecular architecture because of its specificity. Three glycosyl hydrolase enzymes can hydrolyze guar into its individual sugars, galactose and mannose.[19] These three enzymes are β-mannosidase, β-mannanase, and α-galactosidase. The β-mannosidase cleaves a single mannose unit from the nonreducing end of the guar molecule. The β-mannanase cleaves interior glycosidic bonds between adjacent mannose units, and the α-galactosidase cleaves the galactose branches from the backbone. Figure 1 shows the structure of guar galactomannan with the locations of enzyme action.

The major focus on enzyme modification of guar and other galactomannans has been on hydrolyzing the mannose backbone to reduce solution viscosity[31,32] and/or on modifying the structure by cleaving off the galactose side chains.[16,18−20,33,34] These studies have been undertaken using a single type of enzyme. McCutchen et al.[35] showed that combined action of β-mannanase and α-galactosidase enzymes in guar solution results in enhanced viscosity reduction compared to the action of an individual enzyme for the same amount of time. However, no systematic investigations have been reported, specifically on the effects of multiple enzyme actions on the kinetics of guar hydrolysis and synergism between enzyme actions. In this research, we focus on hydrolyzing guar using multiple glycosidase enzymes in different combinations and proportions and examine if there is any synergy between the enzyme actions. Our notion is based on the premise that other polysaccharides have showed synergism between the glycosidase enzymes[36−41] and that the action of β-mannanase is affected by the M/G ratio and the substitution pattern of the galactose side chains on the mannose backbone.[42] A unique feature of this work is the combination of viscometry, rheokinetic modeling, and hyperthermophilic enzymes to examine this issue.

Enzymes synthesized by hyperthermophiles (microorganisms with optimal growth temperatures greater than 80 °C) show the same catalytic actions as their mesophilic counterparts but are highly thermostable and are optimally active at high temperatures.[35,43−45] We carry out the enzymatic reactions in situ in a rheometer and track the extent of hydrolysis by monitoring the changes in zero shear viscosity, which is known to be sensitive to polymer MW. We also develop a rheokinetic model to estimate enzymatic reaction rate constants and interpret synergistic effects of multiple enzymes in terms of variation of these rate constants.

2. Rheokinetics of Enzymatic Degradation

In a previous analysis,[46] we developed a kinetic model for the degradation of guar by the β-mannanase enzyme. We evaluated the model using changes in MWD during the enzymatic hydrolysis reactions, measured using gel permeation chromatography. In this analysis, we develop a rheometric method to estimate the kinetic parameters by measuring the changes in zero shear viscosity during the enzymatic hydrolysis.

The enzymatic reactions are assumed to follow behavior of Michaelis−Menten-type kinetics given by the following equation[46]

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P} \tag{1}$$

The enzyme (E) attaches to the substrate (S) (guar molecules) and forms an enzyme−substrate complex (ES). The enzyme then acts on the substrate to release the product (P) and free enzyme. On the basis of this mechanism, the rate of degradation of guar molecules of "i" monomer units (P_i) can be given by the following equation[46]

$$\frac{dP_i}{dt} = \frac{k'E_T}{K_M + \sum_{i=2}^{\infty} P_i}\left[-P_i + 2\sum_{l=i+1}^{\infty}\frac{P_l}{l-1}\right] \tag{2}$$

In the above equation, K_M is the Michaelis constant, k' is the catalytic rate constant, and E_T is the total enzyme concentration in the solution. We have shown that during the initial stages of the reaction when the total amount of substrates (guar molecules) containing hydrolyzable bonds is higher than K_M the reaction tends to follow zero-order kinetics.[46] For zero-order kinetics, the variation of the weight average degree of polymerization (X_w) or the weight average molecular weight (M_w) is given by[46]

$$\frac{{X_{wo}}^3}{{X_w}^3} = \frac{{M_{wo}}^3}{{M_w}^3} = 1 + k''t \tag{3}$$

where X_w is the weight average degree of polymerization and the subscript "o" refers to the conditions before the hydrolysis reactions begin. The apparent rate constant k'' is a function of the enzyme concentration (E_T), initial number average molecular weight (M_n), and polymer concentration (c_p) as given by the following equation[46]

$$k'' = \left(\frac{k'E_T M_n}{c_p}\right) \tag{4}$$

The relationship between the viscosity of a polymeric solution and its molecular weight can be represented as follows[47]

$$\eta = A{M_w}^{\alpha} \quad \text{for} \quad M_w \geq M_e$$

$$\eta = B{M_w}^{\beta} \quad \text{for} \quad M_w < M_e \tag{5}$$

where M_e is the entanglement molecular weight. The entanglement molecular weight and the parameters A and B are dependent on polymer concentration. For polymeric melts, the exponents α and β are 3.4 and 1.0, respectively. Graessley et al.[48] showed that the exponent α is 3.4 for polystyrene at concentrations between 25% and 100%. Weintjes et al.[49] have reported that α is 5.8 for guar solutions in the entangled solution regime. Robinson et al.[50] showed that the β value for a guar solution is approximately equal to 0.1. The unusually high value of the coefficient α for guar compared to that of other polymeric melts is attributed to the intermolecular interactions between the guar molecules. However, it was later found that these exponents are very sensitive to temperature[51] and polydispersity of the polymer.[52] Equation 5 can therefore be modified to take into account the effect of polydispersity

$$\eta = \sum_{i=1}^{\infty} K_i w_i M_i^a \tag{6}$$

where the subscript "i" represents the number of monomer units in a molecule. The coefficient K_i and the exponent "a" depend on the molecular weight of the molecule.

Guar is a polydisperse polymer with molecular weights in the range above and below the entanglement molecular weight. In the kinetic model derived below, we assume guar to be a mixture of two fractions, one having a weight average molecular

weight higher than M_e and the other having a weight average molecular weight lower than M_e. This assumption simplifies eq 6 to

$$\eta = w_e K_e M_{we}{}^{\alpha} + w_u K_u M_{wu}{}^{\beta} \quad (7)$$

where w_e and w_u represent the weight fractions of the entangled and the unentangled parts, respectively, so that

$$w_e + w_u = 1 \quad (8)$$

Further, the average molecular weight of the mixture can be given by

$$M_w = w_e M_{we} + w_u M_{wu} \quad (9)$$

During the initial stages of the reaction when w_e tends to unity, the rate of change of viscosity can be obtained by combining eq 3 with eq 7

$$\frac{\eta_0}{\eta} = (1 + k''t)^{\alpha/3} \quad (10)$$

or

$$\ln\left(\frac{\eta_0}{\eta}\right) = \frac{\alpha}{3}\ln(1 + k''t) \quad (11)$$

When $k''t \ll 1$, eq 11 reduces to

$$\ln\left(\frac{\eta_0}{\eta}\right) \approx \frac{\alpha}{3}k''t = k_0 t \quad (12)$$

where

$$k_0 = \frac{\alpha}{3} \quad \text{and} \quad k'' = \frac{\alpha}{3}\left(\frac{k' E_T M_n}{c_p}\right) \quad (13)$$

Equation 12 therefore provides an expression for the rate constant measurable in terms of viscosity change during the initial stages of enzymatic reaction. As the reaction progresses, the molecular weight will decrease in both the entangled and the unentangled fractions. This will cause some of the molecules from the entangled fraction to enter the unentangled fraction. Therefore, the weight fraction of the entangled fraction decreases continuously, and that of the unentangled fraction increases. Toward the late stages of the reaction when w_u tends to unity, the rate of decrease in viscosity of the guar solution can be obtained using eqs 3 and 7

$$\frac{\eta_0}{\eta} = \frac{K_e M_{wo}{}^{\alpha-\beta}}{K_u}(1 + k''t)^{\beta/3} \quad (14)$$

At the entanglement molecular weight M_e

$$K_e M_e{}^{\alpha} = K_u M_e{}^{\beta} \quad (15)$$

Using eq 15 in eq 14

$$\frac{\eta_0}{\eta} = \left(\frac{M_{wo}}{M_e}\right)^{\alpha-\beta}(1 + k''t)^{\beta}/^{3} \quad (16)$$

or

$$\ln\left(\frac{\eta_0}{\eta}\right) = (\alpha - \beta)\ln\left(\frac{M_{wo}}{M_e}\right) + \frac{\beta}{3}\ln(1 + k''t) \quad (17)$$

For guar, we know that $M_{wo} \gg M_e$ and $\alpha \gg \beta$.

Further, $(\alpha - \beta)\ln(M_{wo}/M_e) \gg \beta/3 \ln(1 + k''t)$ as $k''t \ll 1$ during the time interval of our experiments. Therefore

$$\ln\left(\frac{\eta_0}{\eta}\right) = \frac{\alpha - \beta}{3}\ln\left(\frac{M_{wo}}{M_e}\right) \approx \text{constant} \approx c \quad (18)$$

We thus have two scenarios. Equation 12 shows that during the initial stages of enzymatic degradation $\ln(\eta_0/\eta)$ increases linearly with time. During the late stages of the reaction, when the molecular weight of the polymer falls below M_e, eq 18 reveals that the variation in $\ln(\eta_0/\eta)$ with time is negligible. These two types of asymptotic behavior can be represented by a single equation of the following form where

$$\ln\left(\frac{\eta_0}{\eta}\right) = \frac{ct}{d + t} \quad (19)$$

$$\frac{c}{d} = k_0 \quad (20)$$

The parameter k_0 is the apparent rate constant that can be estimated from viscosity versus time curves and is proportional to the rate constant (k'') for the enzymatic reaction as given by eq 13.

In the above equation

$$c = \ln\left(\frac{\eta_0}{\eta_\infty}\right) \quad (21)$$

where η_∞ is the final viscosity that can be obtained on enzymatic degradation and d is the time required for $\ln(\eta_0/\eta)$ to reach a value $c/2$. The variation of rate constant with temperature can be represented by the Arrhenius equation[53]

$$k_0 = k_{00}\exp\left(\frac{-\Delta E}{RT}\right) \quad (22)$$

Here, ΔE represents the activation energy of reaction, and k_{00} is proportional to the frequency of enzyme−substrate interaction. Equations 19 and 22 are used in conjunction with experimental results to estimate reaction rate constants and interpret results.

3. Materials and Methods

Guar gum was purchased from Sigma-Aldrich and purified to remove all materials that are insoluble in aqueous media.[54] Following a previously established protocol, a solution of guar gum was prepared by sprinkling guar gum slowly into a vortex of water to a concentration of 10 mg/mL.[54] This solution was vigorously mixed for 2 h followed by low shear mixing for 24 h. A Fisher Scientific Dynamix mixture was used for the mixing. The solution was centrifuged at 10 000*g* for 30 min. The supernatant from the centrifuged solution was collected and mixed with 2 times its volume of ethanol. The guar precipitates in the ethanol/water mixture, and the precipitate was separated and lyophilized at 100 mTorr for 48 h. The dried guar was crushed to obtain a fine powder using a mortar and pestle. A 1.1% solution of the purified guar was prepared in a phosphate buffer at pH 7.0 using 0.2 mg/mL sodium azide as a bactericide and 0.05 M sodium thiosulfate to minimize thermal degradation of guar. For enzymatic reactions, the 1.1% solution was diluted to 1% using the phosphate buffer after adding the required amount of the enzymes.

For higher-concentration guar solutions, purified guar was digested in the desired quantity of pH 7.0 phosphate buffer containing 0.2 mg/mL sodium azide. The solution was heated to 80 °C and mixed for 15 min, followed by digestion at 50 °C to obtain a clear solution.

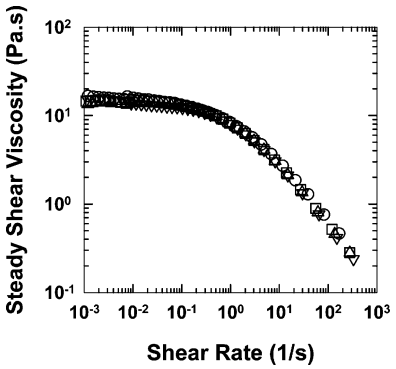

Figure 2. Steady shear behavior of a 1% guar solution. The figure shows the master curve obtained by time−temperature superposition of flow curves measured at 25 (○), 50 (□), 60 (△), and 75 °C (▽).

All three hyperthermophilic enzymes used in this study were produced at the North Carolina State University Hyperthermophile Laboratory from *Thermotoga maritima*.[55−58] Details on the enzymes, including thermostabilities and half-lives, can be found in these and other publications.[55−61] The enzymes were supplied in an aqueous solution form containing approximately 3 mg/mL protein. The activities of β-mannanase, α-galactosidase, and β-mannosidase were 0.106, 0.316, and 0.0341 U, respectively. One unit of activity (U) is equivalent to one micromole of glycosidic bonds hydrolyzed per minute per milliliter of enzyme solution at 75 °C and pH 7.0. The above enzyme stock solutions were stored at 5 °C in a refrigerator and diluted to the required activity when required. All enzyme reactions were carried out at 75 °C and at a pH of 7.0, unless otherwise noted.

The enzymatic hydrolysis reactions were carried out in situ in a TA Instruments (Newark, DE) AR-2000 stress-controlled rheometer using a Couette geometry. The reaction mixture was subjected to a shear stress within the Newtonian region of the guar solution, and changes in the zero shear viscosity of the guar solution were measured as a function of reaction time to track the extent of the reactions. TA Advantage data analysis and Sigma Plot 8.0 software (Systat Software Inc., Richmond, CA) were used for curve fitting and data analysis.

4. Results and Discussion

4.1. Steady Shear Viscosity of Guar during Enzymatic Degradation. We examine first the steady shear properties of 1% native guar solution at different temperatures. Figure 2 represents the master curve obtained by time−temperature superposition of steady shear viscosity curves measured at different temperatures ranging from 25 to 75 °C.[47] The steady shear behavior of 1% guar gum shows a zero shear viscosity region at low shear rates, followed by a non-Newtonian region with a slope of ∼0.7 at high shear rates. Figure 3 represents the changes in the steady shear viscosity of 1% guar solutions at different time intervals during enzymatic hydrolysis of the guar using the β-mannanase enzyme. The enzymatic reaction was carried out in 1% guar solution at 75 °C, and the samples were collected at different time intervals. The sample solution pH was adjusted to 12 using 2 N NaOH to terminate all enzymatic reactions (verified through observation of no change in viscosity with time following this treatment). Steady shear viscosity measurements were then carried out for the degraded samples at 25 °C and pH 12. We find from Figure 3 that viscosity drops by more than 2 orders of magnitude during the first 2 h. The biggest drop in viscosity occurs in the first 30 min, and the rate of reduction in viscosity decreases with increasing reaction time. We also find that, as the depolymerization continues, the critical shear rate at which shear thinning starts increases. The slope of the non-Newtonian region decreases during the depolymerization, and after 3 h of enzymatic reactions, the non-Newtonian region essentially vanishes. Although Figure 3 represents the changes in flow curves of 1% guar solution measured at 25 °C, the superimposibility of flow curves at different temperatures (Figure 2) indicates that similar changes in flow curves can be observed during enzymatic reactions conducted at any temperature.

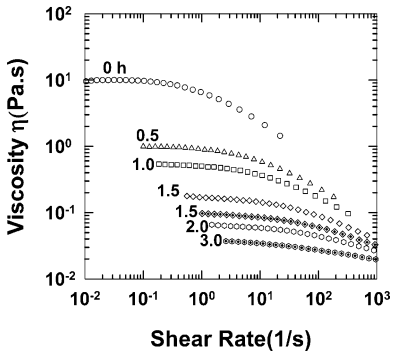

Figure 3. Changes in steady shear viscosity of guar gum during the degradation by the β-mannanase enzyme. The activity of the enzyme in the solution is 1.2×10^{-6} U/mL. The numbers in the figure indicate degradation time in hours.

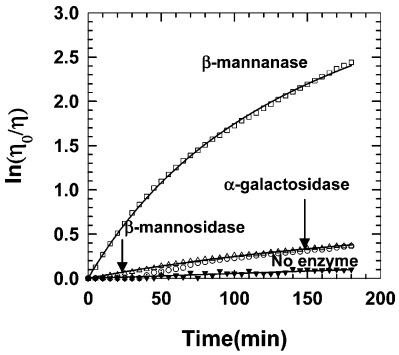

Figure 4. Degradation of a 1% guar solution by individual enzymes, β-mannanase (□), α-galactosidase (○), and β-mannosidase (△), monitored in terms of the viscosity ratio of undegraded guar (η_0) to degraded guar (η). The symbol (▼) represents the thermal degradation of guar. The activities of all of the enzymes are 1.2×10^{-6} U/mL.

In all subsequent experiments on enzymatic hydrolysis, the reaction mixture was subjected to a constant stress (τ) within the Newtonian region of the undegraded guar solution, and the viscosity of the guar solutions was measured at different time intervals during the reactions. Since the Newtonian region of the guar solution broadens during the degradation, as explained above, the measured viscosity corresponds to the zero shear viscosity during the entire degradation process.

4.2. Degradation of Guar by Individual Enzymes. Figure 4 compares the changes in viscosity of the 1% guar solution when reacted with different enzymes at 75 °C. During the reactions, the activities of all of the enzymes were maintained at 1.2×10^{-6} U/mL. In this and all subsequent figures, we plot the ratio of the viscosity of the native guar to that of the enzyme-hydrolyzed guar so that a larger number corresponds to more degradation of the guar. We find that the action of β-mannanase reduces viscosity by more than 90% within 3 h. This is because β-mannanase decreases the molecular weight substantially by cleaving the backbone of the guar molecule. The α-galactosidase cleaves off the galactose branches from the backbone. Pai et al.[62] showed that debranching of guar using α-galactosidase can result in more hydrogen bonding between the galactose-depleted mannose backbone and eventual gel formation. However, we do not observe any increase in viscosity during the reaction at

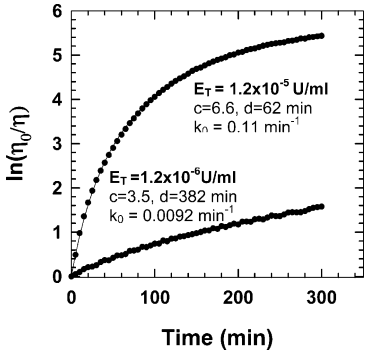

Figure 5. Rheometric estimation of kinetic parameters for the degradation of a 1% guar solution at 75 °C and pH 7 using two different concentrations (E_T) of the β-mannanase enzyme. The solid lines represent the model fit to the experimental data.

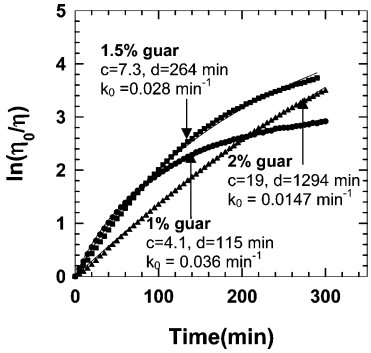

Figure 6. Rheometric estimation of kinetic parameters for the degradation of guar solutions with different concentrations of guar at 75 °C and pH 7 using β-mannanase enzymes at a concentration of 1.2×10^{-5} U/mL. The solid lines represent the model fit to the experimental data.

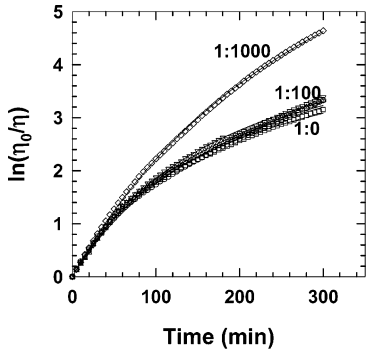

Figure 7. Degradation of guar by simultaneous action of β-mannanase and α-galactosidase measured in terms of the viscosity ratio of undegraded guar (η_0) to degraded guar (η). The activity of the β-mannanase enzyme in the solution is 3.6×10^{-6} U/mL, and the ratios shown in the figure are the ratios of β-mannanase to α-galactosidase activity.

75 °C; instead, the viscosity decreases slightly. We believe that no hydrogen bonds are formed in our case due to the high temperature of the reaction mixture. This is in contrast to the results of Pai et al.,[62] which were monitored at room temperature. Even in that case, the process was slow, taking 3 weeks to form a gel. The small decrease in viscosity observed in our case with α-galactosidase can be attributed to the slight change in molecular weight by debranching of the guar molecules. Finally, the β-mannosidase enzyme cleaves off mannose units from the nonreducing end of the guar molecule and does not result in any significant decrease in viscosity either. We conducted an experiment without any enzyme to check if there is any significant reduction in viscosity due to thermal degradation of the guar. High-temperature processing of guar gum can decrease solution viscosity;[63] however, addition of sodium thiosulfate reduces thermal degradation of guar,[64] and the changes in viscosity due to thermal degradation during the reaction are observed to be insignificant.

4.3. Rheometric Estimation of Kinetic Rate Constants for β-Mannanase Action. Figure 5 represents the variation in viscosity during the degradation of guar using two different concentrations of the β-mannanase enzyme. We find the rate of reduction in viscosity to increase with increasing concentrations of β-mannanase. The plot is fit to an equation of the form given by eq 19, and the fitting parameters are shown in the graph. We are able fit the data to the equation with a regression coefficient of $R^2 \approx 1$. The apparent rate constants (k_0) estimated from the plot are 0.11 and 0.0092 min^{-1} for the two enzyme concentrations, 1.2×10^{-5} and 1.2×10^{-6} U/mL, respectively. These values indicate that the rate constant increases linearly with enzyme concentration and are consistent with our model predictions (eq 13). Figure 5 also shows that the parameter d decreases with increasing enzyme concentrations; d values estimated from the graph are 62 and 382 min for β-mannanase concentrations of 1.2×10^{-5} and 1.2×10^{-6} U/mL, respectively. These values of d indicate that the time required for complete unentanglement of the guar molecules decreases with increasing concentrations of β-mannanase.

Figure 6 represents the changes in viscosity during the enzymatic degradation of guar at different polymer concentrations. For this experiment, a 2% guar stock solution was diluted to obtain concentrations of 1.5% and 1%. These solutions were subjected to β-mannanase action at an enzyme concentration of 1.2×10^{-5} U/mL. Figure 6 shows that the rate of degradation decreases with guar concentration. The apparent rate constants estimated from the graph are shown in the figure, and the rate constants decrease with increasing guar concentration as given by eq 13. The rate constant obtained for the degradation of 1% guar, prepared by diluting a 2% guar solution (Figure 6), is lower compared to that obtained directly from purified guar (Figure 5). Since the 2% guar solution was prepared by high shear digestion of the purified guar at 80 °C followed by digestion at 50 °C, the mechanical processes degraded the guar to some extent resulting in a decrease in the initial molecular weight. (This was verified by measuring the viscosity of guar before and after high shear mixing.) Nevertheless, these observations in Figures 5 and 6, on variation of the apparent rate constant (k_0) with enzyme concentration, polymer concentration, and the initial molecular weight, corroborate our derivation in eq 13, where it is shown that k_0 is directly proportional to the initial molecular weight and enzyme concentration and is inversely related to guar concentration.

4.4. Simultaneous Action of the β-Mannanase and α-Galactosidase Enzymes. Figure 7 shows the variation in viscosity of a 1% guar solution during the enzymatic degradation of guar with different concentration ratios of the β-mannanase and α-galactosidase enzymes. The β-mannanase enzyme concentration in the reaction mixture was kept constant at 3.6×10^{-6} U/mL, and the α-galactosidase concentration was varied from 0 to 3.6×10^{-3} U/mL. Figure 7 shows that as the α-galactosidase concentration is increased from 0 to 100 times the β-mannanase concentration there is no significant change in the viscosity reduction pattern during the initial stages of the reaction; however, the rate of viscosity reduction increases somewhat during the later stages of the reaction. With an increase in α-galactosidase concentration by another 10-fold,

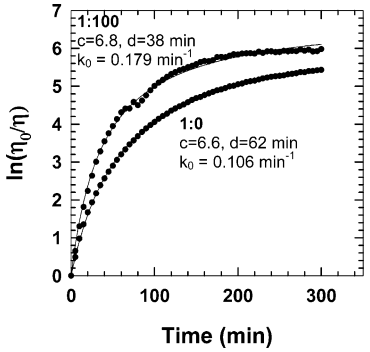

Figure 8. Degradation of guar by simultaneous action of β-mannanase and α-galactosidase. The activity of the β-mannanase enzyme in the solution is 1.2×10^{-5} U/mL, and the ratios shown in the figure are ratios of β-mannanase to α-galactosidase activity.

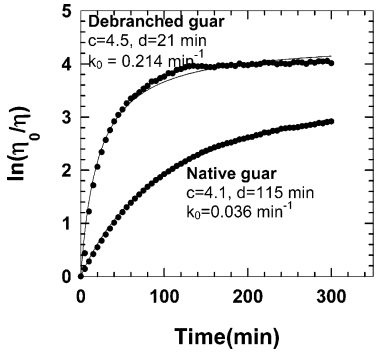

Figure 9. Degradation of guar by sequential action of the α-galactosidase and β-mannanase enzymes. The native guar is debranched by treatment with 1.2×10^{-3} U/mL α-galactosidase for 24 h. The debranched guar is then treated with 1.2×10^{-5} U/mL β-mannanase.

there is a significant enhancement in the viscosity reduction rate not only at long times but also at short times.

The effects of the simultaneous action of the α-galactosidase and β-mannanase enzymes are further probed by using different combinations of these two enzymes, and the results are shown in Figure 8. In this case, the β-mannanase concentration was maintained at 1.2×10^{-5} U/mL (10 times the β-mannanase concentration in Figure 7), and the α-galactosidase concentration was varied from 0 to 100 times the concentration of the β-mannanase enzyme. No significant change in the viscosity reduction pattern was observed when the enzyme concentration ratio was varied from 0 to 10, as observed in Figure 7; therefore, no data is shown for the ratio of 10. However, when the ratio is increased to 1:100, there is a significant change in the viscosity reduction pattern during the entire hydrolysis time. In this case, the apparent rate constant ($k_0 = 0.179$ min^{-1}) is higher for the mixture of the two enzymes compared to that with β-mannanase ($k_0 = 0.106$ min^{-1}) alone.

The β-mannanase enzyme acts on interior β-1,4-linkages on the mannose backbone, and the presence of galactose side chains may hinder the action of the β-mannanase enzyme. With the increase in concentration of the α-galactosidase enzyme more α-1,6-linkages are removed, making β-1,4-linkages more accessible for the β-mannanase enzyme. Consequently, the rate of degradation of guar increases with increasing α-galactosidase concentration.

In contrast to Figure 7, where the initial slope of the curve (the rate constant, k_0) essentially remains constant when the ratio of the enzyme concentrations was increased from 0 to 1000, Figure 8 shows that there is a significant increase in the rate constant when the α-galactosidase concentration is 100 times the concentration of the β-mannanase enzyme. In the latter case, we used a higher concentration of the β-mannanase enzyme. The results from Figures 7 and 8 show that the viscosity reduction pattern depends not only on the concentrations of individual enzymes but also on their composition in the mixture.

Our results from Figure 8 further show that there is a reduction in the value of d with increasing concentrations of α-galactosidase. This indicates that the time required for complete unentanglement of the guar molecule decreases due to simultaneous action of the two enzymes. Figures 7 and 8 also show that at a fixed β-mannanase concentration the final viscosity (η_∞) obtained is lowered with increasing α-galactosidase content. The action of β-mannanase on native guar results in blocks of galactose-substituted galactomannan oligomers; debranching of guar removes steric hindrances and allows β-mannanase to produce smaller oligomers of galactomannan, thereby resulting in a reduction in viscosity.

4.5. Sequential Action of the α-Galactosidase and β-Mannanase Enzymes. Figure 9 shows the viscosity reduction patterns of native and debranched guar. In this case, the guar solution was first treated with the α-galactosidase enzyme at a concentration of 1.2×10^{-3} U/mL and left overnight in a water bath at 75 °C. The solution was then treated with 1.2×10^{-5} U/mL of the β-mannanase enzyme, and the viscosity reduction was monitored in a rheometer. We observe that the rate of viscosity reduction increases considerably by prior debranching of the guar molecule. Figure 9 shows that the sequential action of the enzymes increases the apparent rate constant (k_0) to 6 times that for the native guar. Note that the above comparison of k_0 assumes the same initial molecular weight for the native and debranched guar. The debranching of guar at high temperature for 24 h may have reduced the molecular weight to some extent. Hence, the actual increase in the apparent rate constant (k') (eq 13) for debranched guar may be more than 6 times that of the native guar. This is in contrast to Figure 8, where the simultaneous action of the β-mannanase and α-galactosidase enzymes increases the apparent rate constant to twice that for the β-mannanase action alone. Although the data in Figures 8 and 9 correspond to guar treated with the same concentrations of the enzymes, the increase in the rate constant is more prominent in sequential action (Figure 9) than that in the case of simultaneous action (Figure 8). Figure 9 also shows that prior debranching of guar decreases the time required for unentanglement of guar molecules, as was observed in the simultaneous action of enzymes (Figures 7 and 8). Finally, Figure 9 reveals that the final viscosity achieved on complete degradation of debranched guar is smaller compared to that obtained after complete degradation of native guar.

To obtain further insight on how debranching affects enzyme action on the guar backbone, we conducted enzyme reactions at different temperatures with both the native and the debranched guar. The reactions were carried out in situ in a rheometer to develop viscosity versus time curves at each temperature. The apparent rate constant (k_0) was determined after fitting the graphs to the model in eq 19. Figure 10 shows the variation in the rate constant with temperature for both the native and the debranched guar. The data are fit to an Arrhenius equation as shown in the figure to estimate the activation energy and the frequency factor. The results show that the rate constant for debranched guar is higher compared to that of native guar at all temperatures. However, the activation energy of the reaction is essentially unaffected by debranching the guar. However, there is an order of magnitude increase in the frequency factor, indicating that debranching the guar facilitates guar−enzyme interaction, resulting in an enhanced rate of hydrolysis.

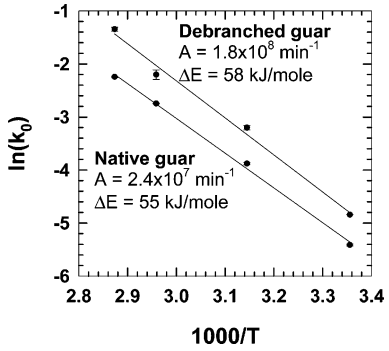

Figure 10. Effect of temperature on the hydrolysis of native and debranched guar by β-mannanase. The native guar is debranched by treatment with 1.2×10^{-3} U/mL α-galactosidase for 24 h. The activity of the β-mannanase enzyme in the reaction mixture is 1.2×10^{-5} U/mL.

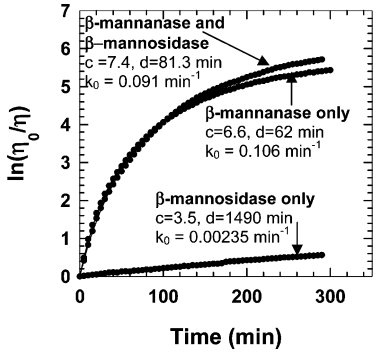

Figure 11. Degradation of guar using the mixture of β-mannanase and β-mannosidase. The activities of the β-mannanase and β mannosidase enzymes in the solution are 1.2×10^{-5} and 2.4×10^{-3} U/mL, respectively.

4.6. Simultaneous Action of the β-Mannosidase and β-Mannanase Enzymes. Figure 11 shows the simultaneous action of β-mannanase and β-mannosidase on guar hydrolysis in a 1% guar solution. For this study, a higher concentration of the β-mannosidase enzyme (2.4×10^{-3} U/mL) has been used as lower concentrations of β-mannosidase enzyme do not bring any significant changes in viscosity reduction patterns. The β-mannanase enzyme concentration is maintained at 1.2×10^{-5} U/mL. We observe essentially no change in the rate of reduction in viscosity during the initial stages of the reactions. However, the rate of reduction in viscosity increases during the later stages of the reaction. The final viscosity obtained by the combined actions of β-mannanase and β-mannosidase is lower than that obtained with the individual action of either the β-mannanase or β-mannosidase enzyme. The backbone cleavage by the β-mannanase enzyme creates more terminal β-1,4-linkages that can be degraded by the β-mannosidase enzyme. Consequently, the simultaneous action of these two enzymes results in lower molecular weight oligomers of mannose or galactomannans.

4.7. Sequential Action of the α-Galactosidase and β-Mannosidase Enzymes. Figure 12 represents the effect of debranching on the viscosity reduction pattern by the β-mannosidase enzyme in 1% guar solutions. The debranching of guar significantly enhances the extent and rate of the viscosity reduction by the β-mannosidase enzyme. The initial rate constant is higher for the debranched guar compared to that for native guar. These results indicate that the β-mannosidase enzyme may be cleaving only nonreducing terminal mannose units that are not attached with galactose side groups. If a β-mannosidase enzyme removes mannose units one by one from the nonreducing terminal of a chain, then the presence of galactose side groups obstruct the progress of the degradation further along the chain. Debranching facilitates further chain degradation resulting in a concomitant enhancement in the viscosity reduction rate and extent.

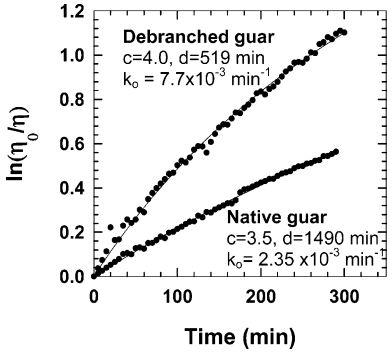

Figure 12. Degradation of native and debranched guar by β-mannosidase. The activity of the β-mannosidase enzyme in the solution is 2.4×10^{-3} U/mL. The native guar is debranched by treatment with 1.2×10^{-3} U/mL α-galactosidase for 24 h.

5. Conclusions

In this study, we examined the hydrolysis of guar galactomannan using three different hyperthermophilic glycosidases at different concentrations, ratios, and combinations. Synergistic hydrolysis is observed between different enzymes acting on guar galactomannan. The synergism depends on enzyme concentrations and ratios as well as the combination of enzymes used. The presence of α-galactosidase affects the viscosity reduction pattern of both β-mannanase and β-mannosidase. However, the effect is more pronounced when α-galactosidase and β-mannanase or β-mannosidase enzymes are added sequentially rather than simultaneously. The galactose branches attached to guar backbone obstruct the β-mannanase enzyme approaching β-1,4-linkages between the mannose units. The debranching of the guar makes the β-1,4-linkages easily accessible by the β-mannanase enzyme, increasing the rate of hydrolysis. The residual viscosity obtained on complete degradation of debranched guar is consequently lower when compared to that obtained on complete degradation of native guar by the β-mannanase enzyme.

A rheokinetic model developed to estimate reaction rate constants agrees well with the experimental data. The model predicts the rate constant to be directly proportional to enzyme concentration and initial molecular weight of the polymer and inversely related to the polymer concentration, consistent with experimental observations. The model further shows that both the debranched and the native guar exhibit the same activation energy for β-mannanase action. However, debranching considerably increases the frequency of enzyme−guar interactions. Debranching of guar also decreases the time required for complete unentanglement of guar molecules during the degradation of the guar by the β-mannanase enzyme.

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