Effect of the Nitrogen Source on Pyruvate Content and Rheological Properties of Xanthan

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Batch and fed batch cultivations of Xanthomonas campesris with varying amounts of the N source (NH₄Cl) yield xanthan polymers which differ in pyruvate content. Analyzing the rheological properties of the xanthans produced exhibits that it is not only the molecular mass but also the pyruvate content that affects the viscosity of xanthan solutions. However, there is obviously no simple functional dependency but rather a step increase in viscosity when going from below 2 to above 3 wt % of pyruvate.

Introduction

The commercial interest and importance of xanthan gum largely stems from its specific and unique rheological properties in aqueous solutions. The solutions are highly pseudoplastic with an apparent yield stress and excellent stabilizing and particle suspending properties. The viscosity and pseudoplasticity can be maintained over a wide range of temperature, pH, and ionic strength. The exopolysaccharide, produced by cultivation of Xanthomona campestris, consists of a cellulosic backbone with thrisaccharide side chains linked to alternate backbone residues; acetyl and pyruvate substituents are carried in variable amounts on these side chains (bone residues; acetyl and pyruvate substituents are consistent with the hypothesis that pyruvate improves the viscosifying capability of the polymer. Whereas some reports in the literature (2–5) are consistent with the hypothesis that pyruvate improves the solution viscosity, other authors (6–8) reported that acetyl and pyruvate do not affect solution viscosity.

Xanthan samples from different strains of X. campesris having varying degrees of pyruvate content were analyzed by Sandford et al. (2), and the solution viscosities of samples with high pyruvate content (4.0–4.8%) were greater than those with low pyruvate content (2.5–3.0%). Similar studies using commercial xanthan samples indicated that those with high pyruvate content gave significantly larger increases in viscosity when KCl was added to salt-free solutions (4). In contrast to these reports, an evaluation of diluted samples, coming from partial removal of the pyruvate and acetate groups from a single polysaccharide sample, showed that these substituents do not affect solution viscosity (6). Equally, lyophilized xanthan samples from different strains of X. campesris having different acylation patterns show similar viscosities (7, 8). The methods, which were used to obtain these samples, raise the following controversial points: (a) The preparation of partially hydrolyzed samples may lead to some degradation of the polysaccharide; it then becomes difficult to directly compare native and pyruvate-free xanthans because of the differences in molecular mass. (b) Samples of xanthan produced from different strains may show changes other than those in the acyl content. Such changes may include differences in molecular mass and its distribution, as well as changes in the pattern of acyl substitution. From the results reported above, the role of acetyl and pyruvate groups on the rheology of xanthan is not clear. To elucidate this question, samples of xanthan having essentially the same or similar molecular masses with varied acylation degrees coming from the same strain and without pretreatment should be analyzed.

To assess the viscosity behavior of xanthan solutions, the model of Ostwald and de Waele (power law) is conveniently applied

\[ \tau = k\gamma^n \]  

or

\[ \eta_{eff} = k\gamma^{n-1} \]

The rheological parameters k and n, i.e., the fluid consistency factor and the flow behavior index n (≤ 1 for the pseudoplastic xanthan solutions) are dependent on xanthan concentration and quality. Often in the industry, the quality of the xanthan is just characterized by the viscosity yield; however, a more fundamental and direct measure is certainly the molecular mass. Herbst et al. (9) and Peters et al. (10) were the first to report quantitative relations between the Ostwald—de Waele parameters and the molecular mass

\[ k = 10^{-3} + (2.58 \times 10^{-6}) (\frac{M}{10^3})^{3.69} \]  

\[ \frac{1 - n}{n - 0.071} = 0.0227 (\frac{M}{10^3})^{2.08} (k - 10^{-3})^{0.47} \]

For most applications of xanthan, high values of k and low values of n are desired. Hence, the molecular mass (M) should be high. Peters et al. (10) also reported the following empirical correlation for M

\[ M = (4.9 \times 10^6) + (3.8 \times 10^6) \frac{Q_{O_2}}{Q^{*}_{O_2}} + (2.6 \times 10^7) \mu \]

which exhibits a dependency of M on the oxygen transfer.
rate (OTR) and the specific growth rate ($\mu$). Therefore, no-oxygen limitation ($\text{OTR} > Q_{O_2}^\ast$ = microbial oxygen demand) and high growth rates guarantee xanthans of high molecular mass and, hence, high viscosity yields. The significance of the eqs 3–5 has been exemplified by the scale-up studies reported by Herbst et al. (9).

However, with the investigations of Peters et al. (10) and Herbst et al. (9), an effect of the pyruvate content of the xanthan on the viscosity properties was not clearly recognized. Obviously, due to the applied cultivation method, the degree of pyruvulation of the product varied little and was above 3.5%. Thus, it was not possible to discriminate among the controversial findings of the effect of pyruvate on viscosity.

In a recent study (11) on the influences of the nitrogen source ($\text{NH}_4\text{Cl}$) on microbial growth and xanthan formation (12), a pronounced growth inhibition was observed accompanied by variations of the pyruvate content of the exopolysaccharide product. This offered the possibility to independently produce xanthans with different pyruvate contents by the same microbial strain. The results are given in this paper; in particular, it is elucidated how the pyruvate substitution of the xanthan influenced the viscosity properties.

Materials and Methods

Microorganism and Culture Media. Xanthomonas campestris NRRL B-1459 S4L-I was used in this study. Stock cultures were maintained on YMG (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L glucose monohydrate) broth at $-18 \, ^\circ\text{C}$. YMG medium was used in the first-stage preculture. In the second-stage preculture and in the main culture, a synthetic medium similar to that of Davidson (13) was used, (g/L): glucose monohydrate, 55 (sterilized separately); citric acid monohydrate, 2.3; $\text{KH}_2\text{PO}_4$, 5; $\text{Na}_2\text{SO}_4$, 0.114; $\text{MgCl}_2\cdot6\text{H}_2\text{O}$, 0.163; $\text{FeCl}_3$, 0.0014; $\text{ZnCl}_2$, 0.0067; $\text{CaCl}_2\cdot2\text{H}_2\text{O}$, 0.012; $\text{H}_3\text{BO}_3$, 0.006. $\text{NH}_4\text{Cl}$ in the range 0.62–8.6 g/L was used as the only varied nutrient.

Reactor and Experimental Setup. Fermentations were carried out in a 15 L stirred tank reactor with INTERMIG impellers ($D = 0.63$) made by EKATO (Germany). The working volume was 10 L in all the experiments. The temperature was controlled at 28 $\, ^\circ\text{C}$, and the pH was kept at 7.0 $\pm$ 0.2 by automatic addition of $\text{H}_2\text{SO}_4$ (4%) or NaOH (10%). Air was sparged at 0.33 vvm.

Analytical Methods. Biomass evolution was followed by optical density (OD) and cell dry weight. The culture broth was diluted with KCl solution (1 wt %) to reduce the viscosity and then centrifuged in 50 mL plastic tubes at 38000 g for 30 min to settle cells. The cell pellet was first washed with distilled water and then centrifuged again under the same conditions described for the sample centrifugation. After this second centrifugation, the washing water was discarded and the cell settled in the tubes were resuspended in KCl (1%) for OD measurements at 600 nm. Dry biomass concentration was determined after 48 h of drying at 80 $\, ^\circ\text{C}$. Ammonium concentration was measured using a gas-selective electrode (Orion).

Xanthan Determination. Xanthan was assayed in duplicate as ethanol-precipitated material from cell-free broth. Xanthan was precipitated in the supernatant by the addition of ethanol to 65% w/w, and then the precipitated xanthan was recovered by centrifugation. After centrifugation (30 min at 38000g, 20 $\, ^\circ\text{C}$), the precipitate was redissolved in KCl solution, precipitated again, centrifuged, and dried at 40 $\, ^\circ\text{C}$ under vacuum for 48 h.

Xanthan Molecular Mass Assay. The molecular mass of xanthan was determined by size exclusion chromatography on TSK PW 5000/6000 columns using a conventional HPLC system with a Beckman 156 differential refractometer detector. For the calibration of molecular mass as a function of the elution volume, xanthan standards were used with molecular mass distributions characterized by low-angle laser scattering. Details were described by Herbst et al. (14).

Xanthan Pyruvate Content. After dilution to a xanthan concentration of about 1–2 kg/m$^3$ with KCl solution (1 wt %), the cells were removed by centrifugation. The same volume of concentrated hydrochloric acid was added, and the hydrolysis was carried out for 90 min at 90 $\, ^\circ\text{C}$. The pyruvate concentration was determined by HPLC (Beckman, pump model 116). The operating conditions in the HPLC were as follows: 45 $\, ^\circ\text{C}$, 0.5 mL/min constant flow of 0.005 M $\text{H}_2\text{SO}_4$, and ion-exchange column (Supelco, ORH-801 organic acid column). Under these conditions 10 $\mu$L of hydrolyzed sample was injected and detected by a UV detector (Beckman, model 163) at 210 nm. Alternatively, after adjustment of the pH value with NaOH and phosphate buffer solutions, the pyruvate content was determined by oxidation of NADH, detected photometrically at a wavelength 340 nm (test kit: Sigma 726).

Rheological Determination of Xanthan. The flow behavior was investigated at 28 $\, ^\circ\text{C}$ with a concentric cylinder viscosimeter (Contraves 115) at shear rates of about 10 to 3600 s$^{-1}$. The rheological properties $k$ (consistency factor) and $n$ (flow behavior index) were calculated using the Mettler Control-Evaluation Software SWR 37 according to the Ostwald de Wale model (power law) (eq 1). Unfortunately, there was no low-shear equipment available to estimate zero-shear viscosity, which would possibly allow a more accurate characterization of molecular interactions.

Results and Discussion

Effect of the Nitrogen Source on Xanthan Quality. The results to be evaluated here correspond to batch cultures of X. campestris in synthetic media with varied initial concentrations of ammonium chloride ($\text{NH}_4\text{Cl}$ ($t_0$)), while other conditions were kept constant (see the Materials and Methods). The variations in the N source are as follows (g/L): 0.62 (F1), 1.01 (F2), 1.82 (F3), 3.5 (F5), 5.46 (F6), and 8.59 (F7). The viscosity yields of xanthan, expressed by the consistency factor ($k$) and the shear thinning behavior (flow index, $n$), are shown in Figure 1 as function of xanthan concentration for different initial $\text{NH}_4\text{Cl}$ concentrations. In the range of $\text{NH}_4\text{Cl}$ ($t_0$) = 0.6–3.5 g/L, $k$ and $n$ were not significantly affected and the values are in the ranges reported by Peters et al. (10) and Suh et al. (15). However, at high values of $\text{NH}_4\text{Cl}$ ($t_0$), negative deviations from the desired profiles, i.e., a lower consistency factor and a higher flow index, can be inferred. At $\text{NH}_4\text{Cl}$ ($t_0$) = 8.6 g/L the lowest values for $k$ and the highest values for $n$ were consistently obtained. These variations in the rheological properties of the culture media at identical xanthan concentrations clearly suggest that an initial concentration of $\text{NH}_4\text{Cl}$ above 3.5 g/L impairs the thickening ability of xanthan and should be avoided in batch culture.

Figure 2 shows the time course profiles of the mean molecular mass (M) of xanthan produced in a culture with a low (1 g/L) and high initial ammonium chloride
In the former culture, M showed a typical pattern as reported by Suh et al. (15, 16). It increased rapidly during the growth phase, reached a maximum (9.6 $10^6 \text{g/mol}$), and then fell during the stationary phase, reaching a relatively constant value (ca. 7.0 $10^6 \text{g/mol}$) after a prolonged cultivation period. In the culture with high cNH4Cl ($t_0$), 8.6 g/L, xanthan with steadily low molecular mass ((6–6.5) $10^6 \text{g/mol}$) was produced (after a lag phase of about 50 h).

The pyruvlation degree of xanthan was found to be a function of both the cultivation time and cNH4Cl ($t_0$) (Figure 3). With the exception of the culture with cNH4-Cl ($t_0$) = 8.6 g/L, the pyruvate content of xanthan increased during the growth phase and leveled off after prolonged cultivation in the stationary phase. A decline of the pyruvlation degree in the stationary phase was observed as cNH4Cl ($t_0$) increased beyond 1.8 g/L. The highest pyruvate content (7.2 wt %) was obtained in the stationary phase of the culture with the lowest cNH4Cl ($t_0$). This high level of pyruvilation is very close to the theoretical maximum (7.4 wt % for 100% pyruvilation). In contrast, at the highest cNH4Cl ($t_0$) applied (8.6 g/L), the pyruvulation level was steadily maintained at a very low level (about 1.5 wt %) throughout the whole fermentation. It is obvious that the effect of NH4Cl on the pyruvate content appears to be stronger than the effect on the molecular mass at the end of the cultivation phase (see Figure 2).

The ascending trend of the pyruvate content as found for most of the cultures (Figure 3) was also observed by Shu and Yang (17) while studying the effect of temperature. Flores et al. (18) also observed increased pyruvlation throughout the whole fermentation period when keeping the dissolved oxygen tension (pO2) above a level of 40% air saturation. However, the maximum level of pyruvate content in these studies was almost constant. Flores et al. (18) further showed that, when the pO2 was kept at 10%, the increase of pyruvate content leveled off during the stationary phase as well. So far, the pyruvate content of xanthan has been considered to depend mainly on the availability of oxygen (18, 19). The results here suggest that a variable maximum degree of pyruviation can also result from other operating conditions such as the NH4Cl level as shown by the results of Figure 3.

A comparison of the results shown in Figures 1–3 reveals that the high viscosity yield of xanthan produced at initial concentrations of NH4Cl in the range of 0.6–3.5 g/L coincides with high molecular mass and pyruviation degrees above 3 wt %. On the other hand, the low viscosity yield of xanthan produced at cNH4Cl ($t_0$) = 8.6 g/L corresponds with a low molecular mass and low degree of pyruvulation (about 1.5 wt %). This is also receivable from the data compiled in Table 1. Remarkable is also that the time course of the polymer properties largely depends on the initial cNH4Cl ($t_0$). As exemplified in Table 1 and Figure 2, for cNH4Cl ($t_0$) = 1.01 g/L, the molecular mass passes a maximum in the early fermentation (growth phase) while the pyruvate content and the consistency index rise particularly in the final stage of the fermentation run. In contrast, during the run with cNH4Cl ($t_0$) = 8.6 g/L, both the molecular mass and the pyruvate content are nearly constant and low.

**Effect of the Pyruvate Content on Xanthan Rheological Behavior.** From the batch runs it can be concluded that a high NH4Cl level diminishes both the molecular mass and the pyruvulation degree of xanthan, which in turn impairs the rheological properties of xanthan. However, it should be mentioned that, during
the stationary phase, the M in the culture with cNH₄Cl (t₀) = 1 g/L is not significantly lower than the M with cNH₄Cl (t₀) = 8.6 g/L (Figure 2). This may suggest that the pyruvilation degree rather than the molecular mass is a predominant factor in determining the rheological properties of xanthan under certain conditions.

Of course, such a conclusion is also received from the time course of run F2 alone. Indeed, the batch runs showed also that the best microenvironment which promotes maximum pyruvilation activity is the bacterial growth phase under conditions of low N concentrations (<0.6 g/L). Accordingly, a fed batch cultivation with intermittent ammonia feeding was performed. In this cultivation all media components were given initially while the N source was maintained at a low level (≤0.65 g/L) by four pulse additions of highly concentrated NH₄Cl solution (Figure 4A). A total of 2.4 g/L of NH₄Cl was used and the growth phase could be extended up to 56 h, in which approximately 16 g/L of xanthan were accumulated (Figure 4A,B). A gradual increase in the pyruvination degree (Figure 4B) is also observed; however, k displays a different behavior (Figure 4B), namely

1. Up to about 40 h of cultivation time, k remains at a low level which coincides with a pyruvate content of ≤2.5–3 wt %.

2. An increase of k at fermentation time of ≥40 h, where also pyruvate contents higher than 3 wt % are observed.

It could be argued that the increase of the rheological index k has to be attributed to the molecular mass (M), which is seen to change during the time course of the fermentation (Figure 2 and upper curve in Figure 4C). To separate the effects of molecular mass and polymer concentration, the empirical correlation (eq 3) of Peters et al. (10) is applied and the value of

$$k_{\text{pyr}} = \frac{k_{\text{exp}}}{(2.58 \times 10^{-6}) \left[ \frac{M}{10^{6}} \right]^{1.369} c_{\text{p}}^{0.77}}$$

(6)

is calculated therefrom. For the fed-batch run the values of k_{\text{pyr}} are given in dependence of the pyruvate content in Figure 4C. It can be discerned from Figure 4B,C that k and k_{\text{pyr}} are little affected in the first 40 h of cultivation, i.e., as long as the pyruvate content is low, say about 2–3 wt %. With increasing fermentation time (>40 h) k increases further, this rise being obviously a result of the polymer concentration and roughly independent of the molecular mass. This is also supported by the experimental data given in Table 1. During the last 20 h of cultivation, k increases mainly as the result of increasing product concentration and pyruvate content. As indicated by the time course of k_{\text{pyr}} in Figure 4C, the consistency factor improves three times if the pyruvate rises from 3 to 7%. Interesting to notice is the time course of the fed-batch run with regard to product quality. In contrast to the batch runs, both molecular mass and pyruvate content increase monotonically and contribute to improved viscosity yield.

The different rheological behavior of solutions of xanthans varying in pyruvate content observed in this study also confirms the findings of Sandford et al. (2). These authors showed that solution viscosity rises with increasing pyruvate content at 1 and 5 g/L xanthan in 0.12 M aqueous KCl. They also concluded that xanthan with high (>4 wt %) and low pyruvate content (2.5–3 wt %) behaves differently. However, the mechanism of the

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positive role of pyruvate was not clearly evaluated. Kleinitz et al. (20) later postulated that high pyruvate content may promote high M; however, no obvious correlation between the pyruvate content and molecular mass could be found. Considering that pyruvilation is not directly associated with the polymerization and biosynthesis of xanthan (21), the assumption of Kleinitz et al. (20) can be rejected. This is also evident from Figures 2 and 3, indicating that xanthan of high molecular mass and low pyruvate content is generated in the starting phase of the polymer production.

When studying aqueous solutions of xanthan above 0.2%, Smith et al. (4) demonstrated that the extent of macromolecular association is directly related to the degree of pyruvate substitution. The same authors have also shown that the unusual increase in viscosity and pseudoplasticity with increasing ionic strength, for xanthan samples highly pyruvilated (>3 wt %), arises from the high degree of macromolecular association. Recently, the lack of the external mannose (and pyruvate) in the side chain of xanthan produced by a Xanthomonas mutant has been associated with loss of side chain flexibility and impairment of viscosity (22).

A rapid increase in the extent of self-association of xanthan molecules with increasing pyruvate content above ~3 wt % may provide an explanation for the atypical increase in viscosity yield observed during the time course of fermentation, where high levels of product are accumulated. Increasing the polymer concentration leads to a replacement of polymer–solvent interaction by polymer–polymer interaction (23). The extent of such interactions, as shown by the results of this study, are determined by the degree of pyruvilation. High product accumulation does not ensure strong molecular interaction when the points of dynamic contact are diminished. A high degree of pyruvilation may lead to the situation where groups of segments of neighboring polymer molecules interact strongly, the result of which may be that their motions appear coupled and entanglements take place.

To further demonstrate the role of pyruvate on the intermolecular interactions, representative samples of xanthan from cultures with cNH₄Cl (t₀) = 1 g/L and 8.6 g/L were purified and resuspended in 0.12 M KCl solution; thus ordered forms exist and the same solution conditions are given. As shown in Figure 5, the viscosity yield of xanthan containing ~6 wt % of pyruvate (run F2 with cNH₄Cl (t₀) = 1 g/L) was increased by more than 100% in the presence of 0.12 M KCl as compared to its rheological ability in the fermentation broth. No significant effect was observed in xanthan containing ~1.5 wt % pyruvate (cNH₄Cl (t₀) = 8.6 g/L, F7). Similar yield improvement (~85%) was reported for highly pyruvilated xanthan solutions when water was replaced by 0.1 M KCl, whereas little difference was found in pyruvate-free xanthan (4). These results clearly show that the increase in viscosity yield due to salt addition (KCl) only occurs when the pyruvilation degree of xanthan is high enough to promote macromolecular association. The benefit of KCl indicates that polymer–polymer interactions become more significant when the degree of pyruvate substitution is high and the electrostatic repulsion between chains is minimized by high ionic strength.

To characterize this phenomenon further, the flow curves of 0.5 g/L xanthan solutions in 0.12 M KCl are represented in Figure 6. As evaluated by the fractional change in viscosity (δη/η), between xanthan with high and low pyruvulation degrees, xanthan coming from the culture with cNH₄Cl (t₀) = 1 g/L becomes more sensitive to shear rate because the high pyruvilation degree increases the pseudoplasticity of the solution so that δη/η is greater at lower shear rates. The enhanced pseudoplasticity of solutions containing associated macromolecules may derive from the progressive structure breakdown under the influence of increasing shear rate. The results shown in Figure 6 also demonstrate once again the large effect of the pyruvate content on the viscosity in salt solutions (0.12 M KCl). If one corrects for the small difference in the molecular masses of the xanthans used by (7/6.47)¹/³ = 1.33, according to eq 3, the effect of the pyruvate alone amounts to a factor of about 7 by which the viscosity increases (total effect as shown in Figure 6 is an increase by a factor of about 10).

The effect of pyruvate on macromolecular interactions can be also shown by the synergistic gelling capability of xanthan when it combines with other polymers, for example, locust bean gum and gum guar. Highly pyruvilated xanthans form a strong thermoreversible gel network, while depyruvylated xanthans or naturally pyruvate-free xanthans reduce interaction and depress gel formation (23–26). Deacetylation, on the other hand, has relatively little effect on gel formation when pyruvate is still present (8). These results support the observation that intermolecular association is promoted by pyruvate methyl groups, which are suitably situated near the
in the medium. As NH$_4$Cl primarily influences the growth of NH$_4$Cl on the degree of pyruvilation and its effect on experimental data are yet needed to clarify the impact be in agreement with the aggregational behavior as to high pyruvate as qualitatively shown in Figure 7 could be in dilute solution. Int. J. Biol. Macromol. 1987, 9, 291–293.

At high NH$_4$Cl concentration in the medium the pyruvate content is low (say ≤2.5 wt %) and the consistency factor $k$ is low as well and seemingly independent of the pyruvate content. However, the data of this study appear insufficient to develop an unambiguous correlation which properly accounts for the effect of pyruvilation.

In agreement with observations reported in the literature, the results of this study rather indicate a behavior as shown in Figure 7. At high NH$_4$Cl concentration in the medium (above 5 g/L), the pyruvate content is low (say ≤2.5 wt %) and the consistency factor $k$ is low as well and seemingly independent of the pyruvate content. At low ammonium chloride in the medium the pyruvate content increases (inversely proportional from about 3–7%) giving a higher level of $k$. Obviously the data used in the correlation of Peters et al. (10) are predominantly taken from this range as the xanthan products were usually obtained from cultivations with NH$_4$Cl concentrations of 2 g/L. The narrow transition range from low to high pyruvate as qualitatively shown in Figure 7 could be in agreement with the aggregational behavior as proposed for the polymer chains in solution. Further experimental data are yet needed to clarify the impact of NH$_4$Cl on the degree of pyruvilation and its effect on the rheological parameters. A metabolic flux analysis is under preparation to better understand the metabolic effect of NH$_4$Cl on product composition.

Summary and Conclusions

The development of the properties characterizing xanthan quality (viscosity yield) during the cultivation depends largely on the amount of the N source (NH$_4$Cl) in the medium. As NH$_4$Cl primarily influences the growth rate, it is obviously the growth rate of the microorganism which is the predominant factor influencing the polymer quality. Low concentrations of NH$_4$Cl give high growth rates and improved rheological properties. In addition to the correlation proposed by Peters et al. (10), the viscosity properties of xanthan solutions depend not only on the polymer concentration and its molecular mass but also on the pyruvate content. However, the data of this study appear insufficient to develop an unambiguous correlation which properly accounts for the effect of pyruvilation.

In agreement with observations reported in the literature, the results of this study rather indicate a behavior as shown in Figure 7. At high NH$_4$Cl concentration in the medium (above 5 g/L), the pyruvate content is low (say ≤2.5 wt %) and the consistency factor $k$ is low as well and seemingly independent of the pyruvate content. At low ammonium chloride in the medium the pyruvate content increases (inversely proportional from about 3–7%) giving a higher level of $k$. Obviously the data used in the correlation of Peters et al. (10) are predominantly taken from this range as the xanthan products were usually obtained from cultivations with NH$_4$Cl concentrations of 2 g/L. The narrow transition range from low to high pyruvate as qualitatively shown in Figure 7 could be in agreement with the aggregational behavior as proposed for the polymer chains in solution. Further experimental data are yet needed to clarify the impact of NH$_4$Cl on the degree of pyruvilation and its effect on the rheological parameters. A metabolic flux analysis is under preparation to better understand the metabolic effect of NH$_4$Cl on product composition.

References and Notes


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