

THE MOLECULAR WEIGHT OF XANTHAN POLYSACCHARIDE PRODUCED UNDER OXYGEN LIMITATION

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SUMMARY

The molecular weight distributions of xanthan polysaccharide were determined by size-exclusion chromatography during batch fermentations in a bubble column. Xanthan with lower weight-mean molecular weight M_w^ was formed after growth had ceased. Under oxygen limitation, M_w^* decreased linearly with the specific oxygen uptake rate resulting in lower viscosity yield of the product.*

INTRODUCTION

Xanthan gum, the exocellular heteropolysaccharide produced by *Xanthomonas campestris*, has manifold applications as a thickening agent in the food, cosmetic, and pharmaceutical industries and for enhanced oil recovery by polymer flooding. The desired properties, i.e. high viscosity and extremely pseudoplastic flow behaviour, deteriorate the mass transfer, mixing, and heat transfer characteristics of the fermentor during xanthan production.

The product accumulation during batch cultivation may finally cause depletion of dissolved oxygen, particularly, at production scale (100 m³) with rather limited specific power input. Under oxygen limitation, the specific xanthan production rate of the bacteria becomes a linear function of the oxygen transfer rate (Peters et al. 1988, Pons et al. 1989). There is little information on the effect of fermentation conditions on the product quality. Recently, Peters et al. (1989) employed the size exclusion chromatography (Herbst et al., 1988) to study the molecular weights of xanthans obtained at various stirring speeds in a stirred tank fermentor. The lower the stirring speed, the earlier oxygen limitation was encountered and the lower was the weight-mean molecular weight M_w of the final product. This trend is of great practical importance since the viscosity yield is strongly affected by the molecular weight. For example, Herbst et al. (1990) showed that, at the gum concentration of 1 g/L, the specific zero-shear viscosity varies with M_w to the power of 4.5.

The objective of this work is to systematically investigate and quantify the effect of oxygen limitation on the molecular weight of the polysaccharide produced by *Xanthomonas campestris*. A bubble column fermentor is used because of its excellent mixing characteristics, specifically, the absence of dead zones (Suh et al. 1988, Pons et al. 1989).

EXPERIMENTAL METHODS

Xanthomonas campestris NRRL B-1459 S4L-II was cultivated in a 50-L bubble column fermentor with a diameter of 0.15 m. Aeration rates from 1.3 vvm to 9.6 vvm were applied, which corresponds to superficial gas velocities u_G of 0.051 to 0.361 m/s. The flow rate was kept constant in each fermentation. A defined synthetic medium was used with glucose (50 g/L) as the C-source and ammonium chloride (1.5 to 2 g/L) as the N-source. In one experiment ($u_G = 0.165$ m/s) additional glucose was fed in order to build-up higher gum concentration. The temperature was 28 °C and the pH was controlled at 7.0.

The flow behavior was investigated with a concentric cylinder viscometer (Contraves 115) at shear rates of about 10 to 1000 s⁻¹. The cell dry weight X and the xanthan concentration P were determined gravimetrically. Experimental details were described elsewhere (Peters et al., 1989).

The xanthan molecular weight distribution was determined by size exclusion chromatography on TSK PW 5000/6000 columns using a conventional HPLC system with a differential refractometer detector. For calibration of the molecular weight as a function of the elution volume, xanthan standards were used with molecular weight distributions characterized by low angle laser light scattering. Details were described by Herbst et al. (1988).

RESULTS AND DISCUSSION

Exponential growth started without any lag phase; the specific growth rate was 0.077 h⁻¹ independent of the aeration rate. The growth ceased due to exhaustion of ammonium. Oxygen transfer limitation started at about the end of the growth phase in the case of the lowest gas velocity and was avoided throughout in the case of the highest gas velocity. The specific xanthan productivity of the bacteria decreased under oxygen limitation as discussed by Peters et al. (1988, 1989).

The apparent viscosities μ of the pseudoplastic fermentation broths were well described as a function of the shear rate $\dot{\gamma}$ by the Ostwald - de Waele relation:

$$\mu = k \cdot \dot{\gamma}^{n-1} \quad (1)$$

Thomson and Ollis (1980) suggested that the evolution of the fluid consistency factor k and the flow behavior index n in a xanthan batch fermentation is related to the product concentration P as follows:

$$k = a \cdot P^b \quad (2)$$

$$n = c \cdot \ln k + d \quad (3)$$

with constant values of a , b , c , and d . Pons et al. (1989) used such relations, fitted to a dilution-series of commercial xanthan gum, when modelling the time-course of batch fermentations. This approach is not justified as to our results (Fig. 1). At constant gum concentration, higher consistency factors (and somewhat lower flow behavior indices) were obtained at higher aeration rates. Moreover, non-linearities were observed even in the absence of oxygen transfer limitation. These trends are considered to result from variation of the molecular weight.

In Fig. 2, two molecular weight distributions determined during the fermentation at the lowest gas velocity are compared: the one at the end of the growth phase and one in the final stage of the entirely oxygen-limited non-growth

phase. The shoulder at the higher molecular weight side was typical for the end of the growth phase. The same observation was already reported by Herbst et al. (1988). They considered varying degree of molecular association as an alternative explanation. However, we exclude this possibility based on the finding that neither the application of high shear (Ultra-Turrax stirrer) nor drying and redissolving had any effect on the distribution curves.

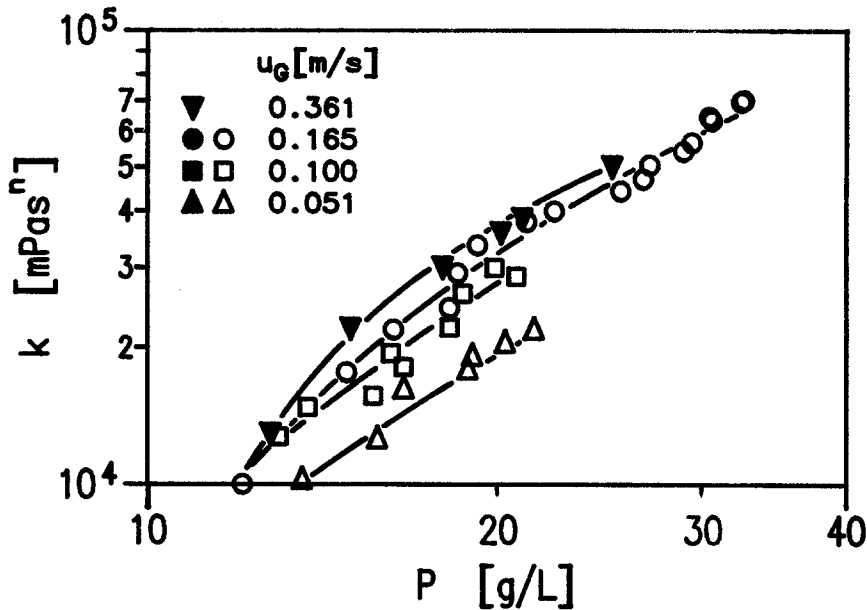


Fig. 1 Evolution of the fluid consistency factor k during batch fermentations (open symbols indicate oxygen transfer limitation).

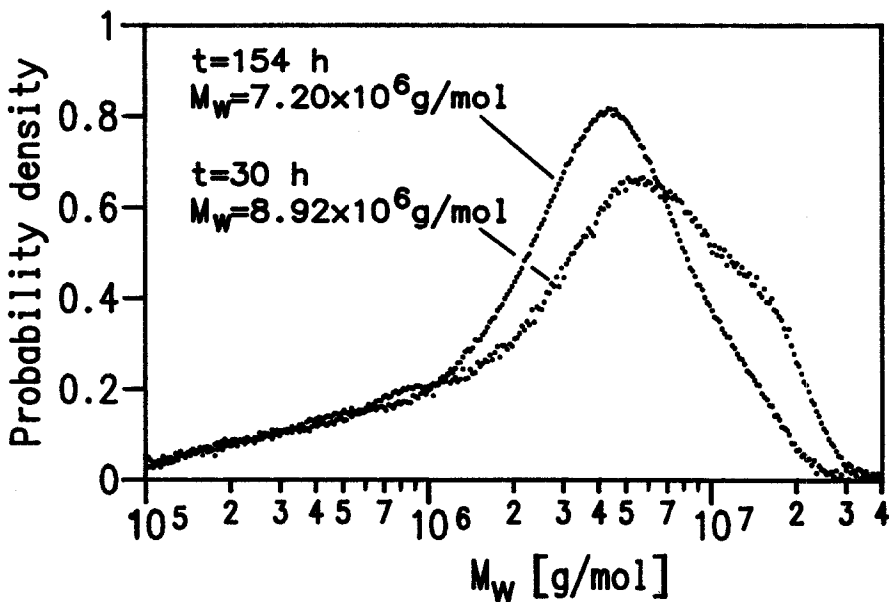


Fig. 2 Molecular weight distributions at the end of the growth phase and after oxygen-limitation in the non-growth phase ($u_G = 0.051$ m/s).

The evaluated time courses of the weight-mean molecular weight M_w at various gas flow rates are shown in Fig.3. The initial M_w was non-uniform but it increased rapidly to similarly high values ($8.8 \cdot 10^6$ to $9.4 \cdot 10^6$ g/mol) at the end of the growth phase (30 h). At this time the xanthan concentration was only $6.7 (\pm 0.3)$ g/L in all runs. In the non-growth phase, the mean molecular weight decreased at a rate strongly dependent on the gas velocity. Since most of the product accumulated during this phase, the degree of oxygen limitation determined the quality of the final product.

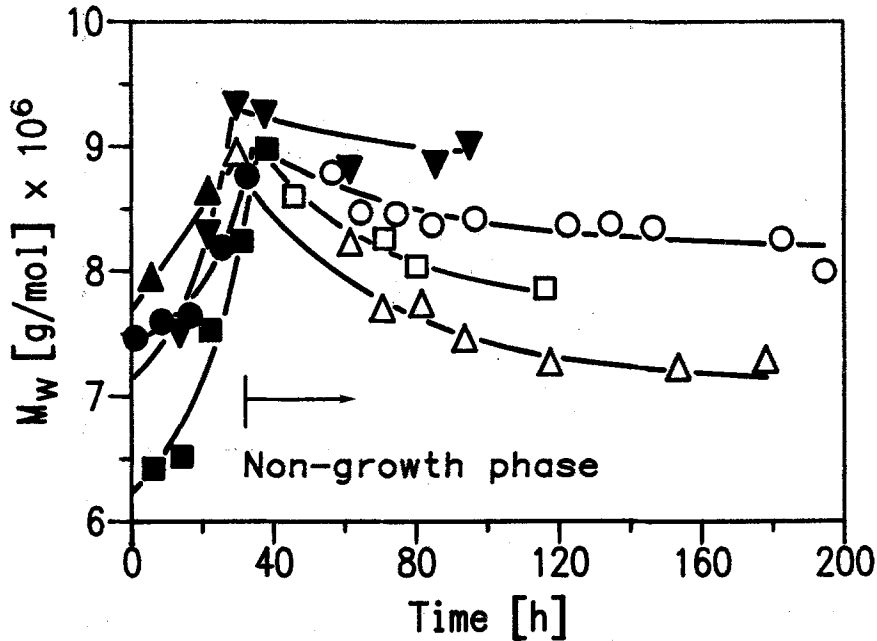


Fig. 3 Time-course of the weight-mean molecular weight M_w (open symbols indicate oxygen transfer limitation, cf. Fig. 1).

The weight-mean molecular weight M_w^* of the xanthan produced during a time interval (t_1, t_2) was calculated from the respective xanthan concentrations (P_1, P_2) and molecular weights (M_{w1}, M_{w2}) as follows:

$$M_w^* = (P_2 \cdot M_{w2} - P_1 \cdot M_{w1}) / (P_2 - P_1). \quad (4)$$

The variation of the initial molecular weight is probably caused by different degrees of oxygen limitation in the shake flask cultures used for inoculum cultivation. The application of Eq. (4) to the time-course of M_w in the exponential growth phase suggests the formation of xanthan with the molecular weight of $10.7 \cdot 10^6$ g/mol independent of the aeration rate. This value agrees with the M_w^* determined by Peters (1990) at the same growth rate during continuous cultivation in a chemostat. He reported a linear increase of the M_w^* with the growth rate. Correspondingly smaller molecules were formed in our experiments after growth had ceased. Without oxygen limitation ($u_G = 0.361$ m/s) the mean M_w^* of the non-growth product was $8.7 \cdot 10^6$ g/mol. At the lower aeration rates much smaller M_w^* was produced after the onset of oxygen limitation.

In order to quantify the effect of oxygen limitation, the oxygen transfer rate (OTR) has to be known. The change of the oxygen mole fraction in the gas was

sufficiently high for accurate measurement only at low gas velocity. Then the volumetric mass transfer coefficients $k_L a'$ (referred to unit volume of liquid) evaluated from the gas phase balance agreed well with the results of dynamic measurements in synthetic xanthan solutions (Suh et al., 1988, 1990). The volumetric mass transfer coefficients (81 data points) reported by Suh et al. (1990) for xanthan solutions in the same bubble column as used in this study are represented with 5.8 % mean error by the following empirical relation:

$$k_L a' = 8.95 \mu_{\text{eff}}^{-0.79} u_G^{0.48} \quad (5)$$

[h⁻¹] [Pa s] [m/s]

where the effective viscosity μ_{eff} is calculated from Eq. (1) introducing the effective shear rate $\dot{\gamma}_{\text{eff}}$ suggested by Schumpe and Deckwer (1987):

$$\dot{\gamma}_{\text{eff}} = 2800 u_G \quad (6)$$

[s⁻¹] [m/s]

The oxygen transfer rate could be estimated as the product of $k_L a'$ (Eq. 5) and the driving force measured with an oxygen electrode at half the dispersion height and corrected for the effects of the mean hydrostatic head and the salting-out by the glucose and dissolved salts (Schumpe et al., 1982):

$$\text{OTR} = k_L a' (c_L^* - c_L) \quad (7)$$

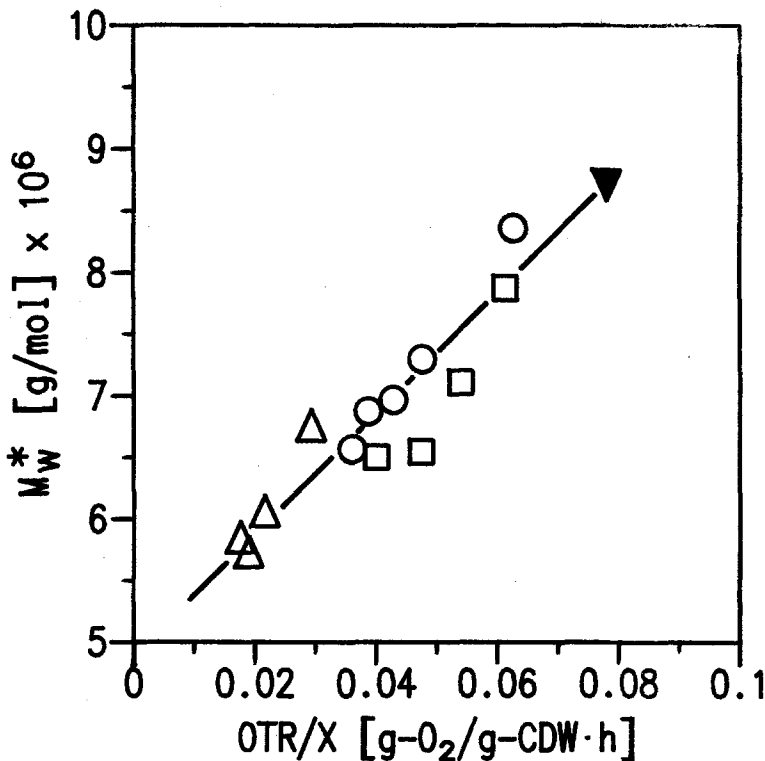


Fig. 4 Influence of the transfer-limited specific oxygen uptake rate in the stationary phase on the weight-mean molecular weight of the xanthan being formed (symbols as in Fig.1).

The oxygen transfer rates calculated for the fermentation without oxygen limitation agreed very well with the oxygen demand Q_{O_2} as to Peters (1990):

$$\begin{array}{rcl} Q_{O_2} & = & 0.59 \cdot (dX/dt) + 0.078 \cdot X \\ [g/h] & & [g/L \cdot h] \quad [g/L] \end{array} \quad (8)$$

In Fig. 4 the instantaneously produced M_w^* (Eq. 4) in the non-growth phase is plotted as a function of the specific oxygen transfer rate (OTR/X). In the case of oxygen limitation ($OTR/X < Q_{O_2}/X = 0.078 \text{ g-O}_2/\text{g-CDW} \cdot \text{h}$), the weight-average molecular weight becomes a linear function of the specific oxygen uptake rate:

$$\begin{array}{rcl} M_w^* & = & 4.9 \cdot 10^6 + 4.9 \cdot 10^7 \cdot OTR / X. \\ [g/mol] & & [g-O_2/g-CDW \cdot h] \end{array} \quad (9)$$

Equation (9) is considered to describe the effect of oxygen limitation on xanthan molecular weight independent of the reactor type. Poor mixing with local oxygen limitation in stagnant zones may be expected to result in local non-uniformity of the weight-mean molecular weight M_w^* being formed and thus lead to wider distributions (e.g., higher polydispersity index).

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