0273-2289/99/82/0175/$12.25

Xanthan Gum Production by *Xanthomonas campestris* w.t. Fermentation from Chestnut Extract

M. Liakopoulou-Kyriakides,*[,1] S. K. Psomas,[1] and D. A. Kyriakidis[2]

[1]Department of Chemical Engineering and [2]Faculty of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece, E-mail: markyr@vergina.eng.auth.gr

Received July 12, 1999; Revised November 2, 1999; Accepted November 10, 1999

Abstract

Xanthomonas campestris w.t. was used for production of xanthan gum in fermentations with chestnut flour for the first time. Fermentations were carried out with either chestnut flour or its soluble sugars (33.5%) and starch (53.6%), respectively, at 28°C and 200 rpm at initial pH 7.0 in flasks. The effect of agitation rate (at 200, 400, and 600 rpm) on xanthan gum production was also studied in a 2-L batch reactor. It was found that xanthan production reaches a maximum value of 3.3 g/100 mL at 600 rpm and 28°C at 45 h.

Index Entries: *Xanthomonas campestris*; fermentation; xanthan gum; chestnuts.

Introduction

Xanthomonas campestris is a Gram-negative bacterium that produces xanthan gum, a water-soluble extracellular polysaccharide. Xanthan is composed of pentasaccharide repeating units, containing D-glucose, D-mannose, D-glucuronic acid, acetal-linked pyruvic acid, and D-acetyl groups *(1)*. Xanthan gum, owing to its excellent rheological properties, has various applications, mainly in the food industry as a thickening, suspending, and stabilizing agent. It is also used as an emulsifier, lubricant, and thickening or mobility-control agent in oil recovery *(1–3)*.

Microbial production of xanthan gum from mannose and molasse has been reported *(4)*. In addition, a constructed strain of *X. campestris* has been found to produce xanthan gum from whey in large quantities *(5)*.

*Author to whom all correspondence and reprint requests should be addressed.

The construction of lactose-utilizing *X. campestris* either by conjugation or by transformation has already been reported *(6–8)*. Recently, the wild type of *X. campestris* has been found to produce xanthan gum from peach pulp *(9)*.

Chestnuts are seasonal fruits produced in many Mediterranean countries, e.g., Spain, Corsica, Italy, France, and Greece. They are used in the confectionery industry and directly as a food. Chestnut flour, in combination with regular flour, is used in some countries for the production of bread and other prepared foods. In this article, we report on xanthan gum production from the wild type of *X. campestris*, using, for the first time, chestnut flour as substrate. Fermentations were carried out with native chestnut flour, its isolated soluble sugars, and starch in flasks. Experiments were also conducted in a 2-L batch reactor at various agitation rates.

Materials and Methods

Bacterial Strain

The wild-type strain *X. campestris* ATCC 1395 was used.

Growth Medium

X. campestris was grown in Luria-Bertani (LB) broth, pH 6.8 *(10)*, plus 0.2% glucose (LBG). MacConkey agar was used for its maintenance.

Chestnut flour was a gift from the Agricultural Corporation of Corsica. It was used mainly as native chestnut flour, and in some experiments its soluble sugars (33.5% [w/w]) and starch (53.6% [w/w]), obtained as described in the next section, were used.

Separation of Soluble Sugars and Starch

One hundred grams of native chestnut flour in 400 mL of water was stirred at room temperature for 1 h. The suspension was centrifuged at 3000*g* for 10 min. The pellet was diluted with another 200 mL of water and stirred for 1 h at room temperature. The supernatant was removed by centrifugation. The pellet was dried in a desiccator over $CaCl_2$ for 24 h, and the two supernatants were lyophilized (at –20°C overnight) to give 33.5 g of solid material, hereafter called soluble sugars and the pellet starch.

Bacterial Growth

Single colonies of *X. campestris* were transferred into LB broth. The cultures were incubated at 28°C until they reached an optimal density (600 nm) of >0.8. Ten milliliters of this was transferred into 100 mL of the medium. The cultures were incubated at 28°C with shaking. Growth was estimated by measuring the optical density at 600 nm.

Fermentations

Fermentations in flasks were carried out at 28°C and 200 rpm at an initial pH of 7.0. Batch fermentations were carried out in a 2-L LSL Biolite

(St. Germain en Laye, France) fermentor with a working volume of 1 L. The operation variables were the same in all experiments described here and had been adjusted as in the case of flasks. Airflow was 0.6 L/min and experiments were conducted at three different agitation rates: 200, 400, and 600/min, respectively.

Determination of Xanthan Gum

Xanthan gum was determined as previously described *(11)*, and all the data are presented as the average of duplicate experiments.

Determination of Reducing Sugars

Ten-milliliter cultures were centrifuged at 5000*g* for 10 min, and the supernatant was used for the determination of reducing sugars. The dinitrosalicylic acid method of Miller *(12)* was used.

Total Sugars

Total sugars were determined by the chromatometric method of phenol-sulfuric acid *(13)*.

Proteins and Fats

Proteins were determined as reported previously *(14,15)* and fats according to the method of the American Association of Cereal Chemists *(16)*.

Pyruvate Content

Xanthan gum obtained from alcohol precipitation was hydrolyzed in 1 *N* HCl at 100°C for 3 h. Pyruvate content was then determined by high-performance liquid chromatography (HPLC) on a C8 column with 0.01 *N* orthophosphoric acid. The flow rate was 0.5 mL/min and an ultraviolet detector was set at 210 nm.

Results and Discussion

Previous work has demonstrated the production of xanthan gum by *X. campestris* fermentation using various substrates such as molasses, glucose, whey, and peach pulp *(4,5,9,17)*. *X. campestris* w.t. fermentation with chestnut flour is reported for the first time. Analysis of the chestnut flour used in these experiments showed the presence of 33.5% total soluble sugars, 53.6% starch, 0.66% proteins, and 3% fats.

Fermentation of *X. campestris* w.t. with chestnut flour at 28°C and 200 rpm showed that xanthan gum is produced in satisfactory yields. Xanthan production increased with an increase in concentration of chestnut flour in the medium, and reached a maximum value of 2 g/100 mL with 5% (w/v) chestnut flour (Fig. 1). The concentration of reducing sugars produced in the medium was also measured in this case. As shown in

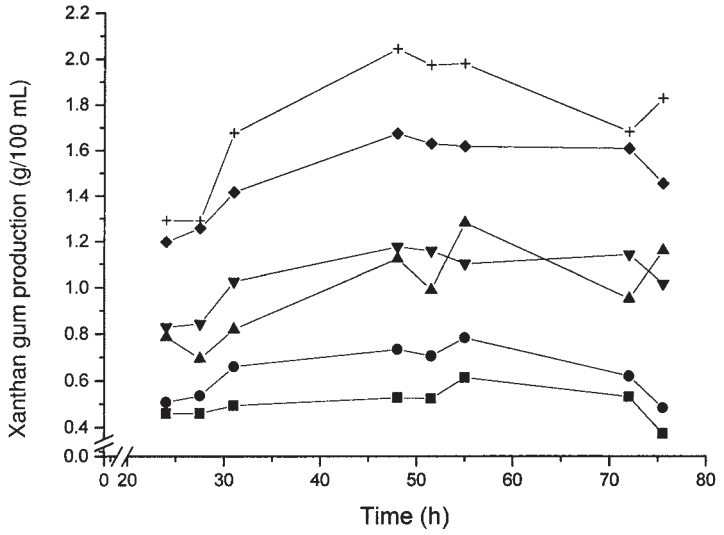

Fig. 1. Xanthan gum production during *X. campestris* w.t. fermentation with chestnut flour. ■, LBG; concentration of chestnut flour: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

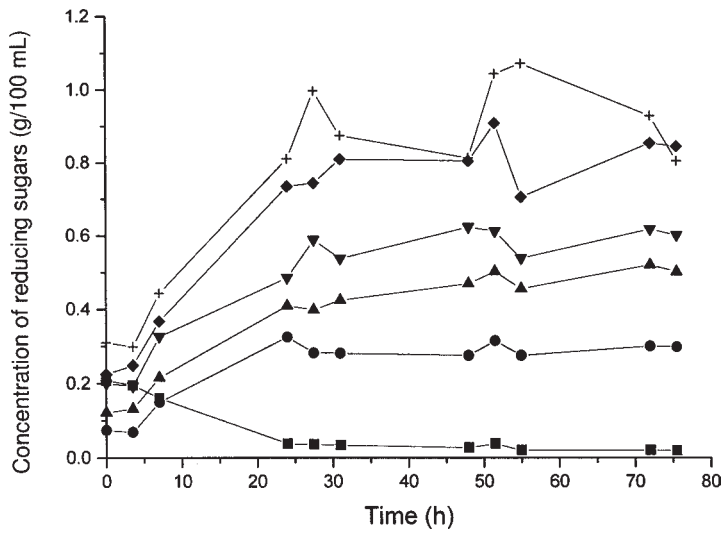

Fig. 2. Concentration of reducing sugars vs time during *X. campestris* w.t. fermentation with chestnut flour. ■, LBG; concentration of chestnut flour: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

Fig. 2, the production of reducing sugars increased upon the time at all concentrations of chestnut flour tested and reached a maximum value after 30 h.

In another series of experiments, total soluble sugars and starch were separated from the native chestnut flour, as described in Materials and Methods, and used separately for fermentations with *X. campestris*. It was

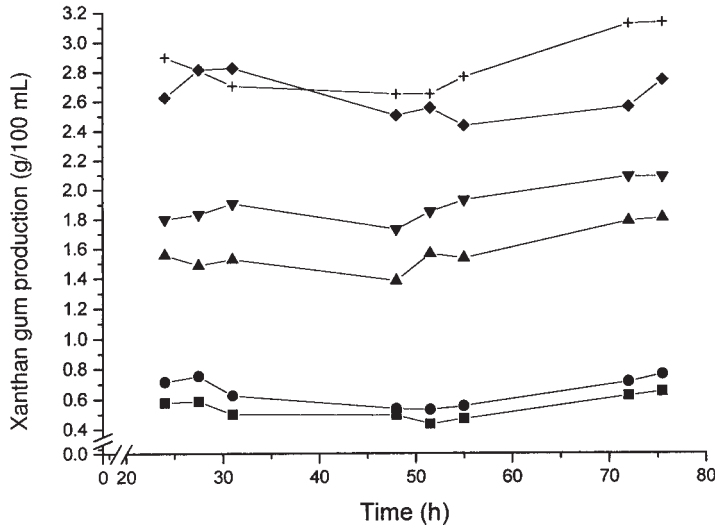

Fig. 3. Xanthan gum production during *X. campestris* w.t. fermentation of starch obtained from chestnut flour. ■, LBG; concentration of starch: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

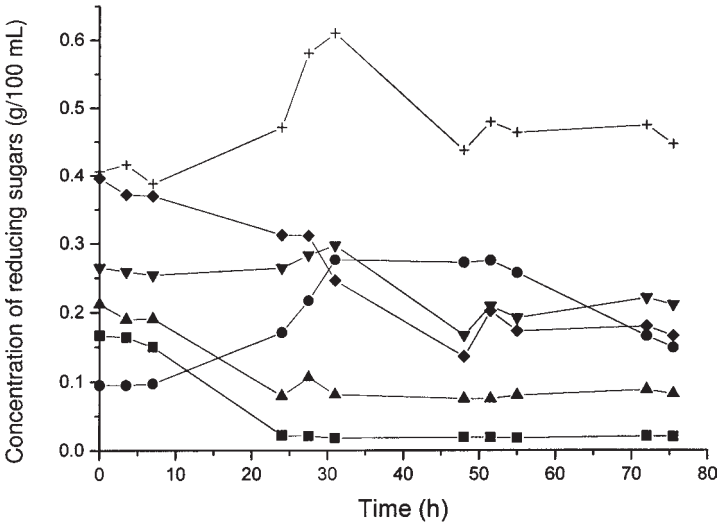

Fig. 4. Concentration of reducing sugars vs time during *X. campestris* w.t. fermentation of starch obtained from chestnut flour. ■, LBG; concentration of starch: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

found that in the case of starch, xanthan gum production at all concentrations tested began after 20–22 h with a plateau value until 50–55 h (Fig. 3). After that time a small increase in xanthan gum production was observed. At 5% (w/v) starch concentration the production of xanthan was 3 g/100 mL. Figure 4 shows the profile of reducing sugars during the fermentation of

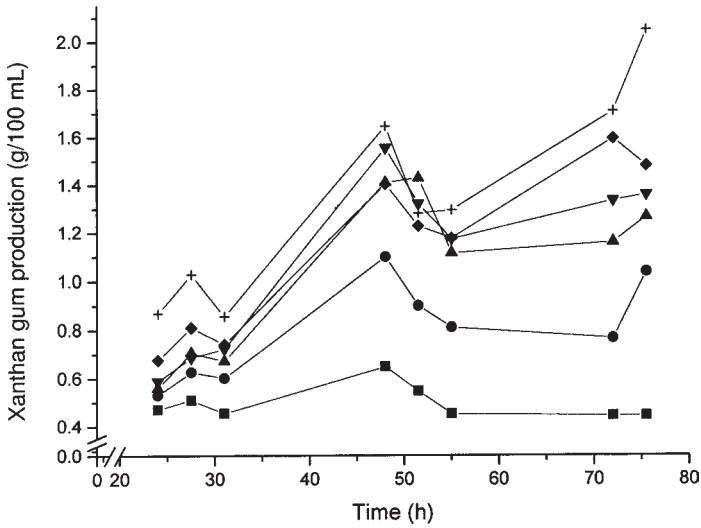

Fig. 5. Xanthan gum production during *X. campestris* w.t. fermentation of reducing sugars obtained from chestnut flour. ■, LBG; concentration of reducing sugars: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

X. campestris with the starch. The rate of decrease of reducing sugars is in good agreement with the rate of xanthan gum production. Note that *X. campestris* starts synthesizing xanthan gum only at the late log phase, which is usually 24 h of growth.

Similar experiments were performed with soluble sugars (obtained from chestnut flour), in which the majority were reducing sugars (about 98%). Xanthan gum production reached a maximum value of 2.5 g/100 mL at a concentration of 5% after 50 h of fermentation (Fig. 5). In the case of soluble sugars, as expected, the consumption of reducing sugars on time was significant and reached a minimum value at around 50 h (Fig. 6).

Figure 7 shows the effect of agitation rate on xanthan gum production, which was studied using a 2-L batch reactor. Xanthan gum reached a maximum concentration of 3.3 g/100 mL at 600 rpm at 45 h. Crude polysaccharide at this concentration formed a high-viscosity solution, and stirring was unsuccessful after 50 h. Xanthan gum production obtained at 200 rpm in flasks was higher than that in the reactor at the same rpm (Figs. 1 and 5), meaning that higher agitation rates (400 or 600) are necessary for sufficient stirring of the medium in the fermentor. Figure 8 shows the effect of agitation rate on the concentration of reducing sugars vs time.

It was reported earlier that in medium supplemented with lactose or glucose, the amount of xanthan gum production was 4.2 g/L *(18)*. In a lactose medium using a mutant strain of *X. campestris*, the production of xanthan gum was 14.0 g/L *(7)*, whereas in conjugant strain in 100% (w/v) whey, the production of xanthan gum was 14 g/L *(11)*. In a defined medium, in-flow cultures the amount of xanthan gum was 7.5 g/L *(19)*. In an indus-

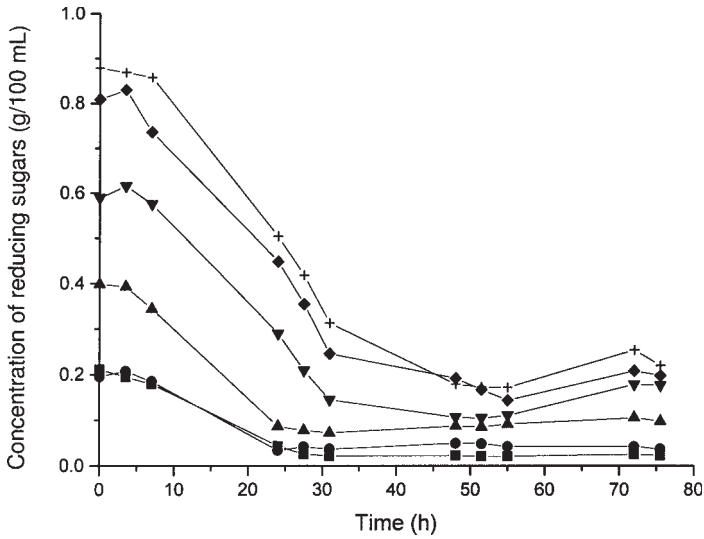

Fig. 6. Concentration of reducing sugars vs time during *X. campestris* w.t. fermentation of reducing sugars obtained from chestnut flour. ■, LBG; concentration of reducing sugars: ●, 1%; ▲, 2%; ▼, 3%; ◆, 4%; +, 5%.

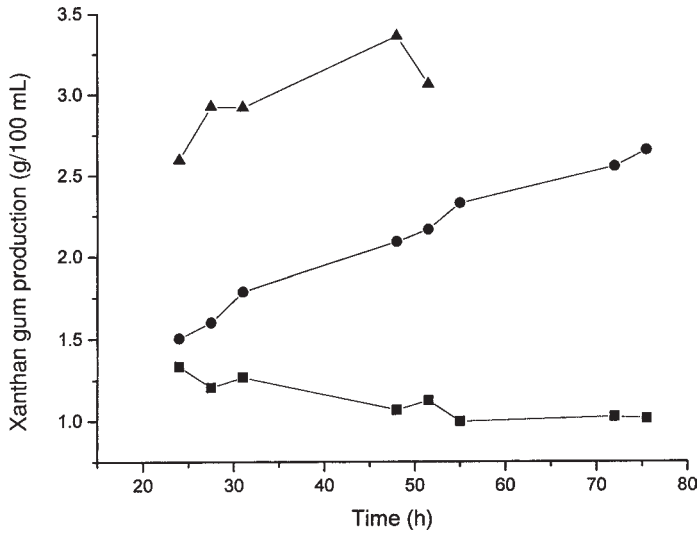

Fig. 7. Effect of agitation rate on xanthan gum production during *X. campestris* w.t. fermentation with chestnut flour at 5% concentration: ■, 200; ●, 400; ▲, 600 rpm.

trial medium (4% glucose, 4% sucrose, 10% molasses, or 2.8% sirodex A as a carbon source) the amount was 20 g/L *(4)*. More recently, using a peach pulp medium (10% [w/v]) the production of xanthan gum was 3 g/L *(9)*. Therefore, our results showed that chestnut flour gave a better yield and encouraged us to pursue our experiments to determine the possibility of using this substrate industrially.

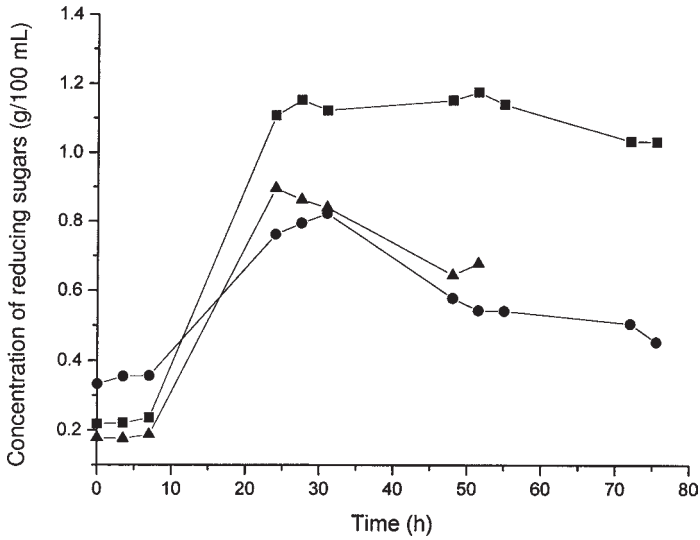

Fig. 8. Effect of agitation rate on the concentration of reducing sugars vs time during *X. campestris* w.t. fermentation with chestnut flour at 5% concentration: ■, 200; ●, 400; ▲, 600 rpm.

The extent of acetylation, and particularly pyruvylation, in xanthans can vary considerably, depending on fermentation conditions or starch variants *(20–22)*. The pyruvate content of the xanthan gum obtained from fermentation of chestnut flour under the experimental conditions described previously was determined by HPLC on a C8 column and was found to be 1.5%. The effect of various experimental parameters on the pyruvate content will be further studied, and the influence of its content on the properties of xanthan in solution have yet to be reported.

Although the pyruvate content of xanthan gum depends primarily on the appropriate choice of the strain and the medium, it has been reported that the degree of pyruvylation of the xanthan side chains decreases when the microbial oxygen demand is not met *(23)*.

Conclusion

The data show that chestnut flour can be used for production of xanthan gum by *X. campestris* w.t. fermentation in satisfactory yields. This finding also gives an alternative for the use of chestnuts, particularly in Greece, where the bulk of the chestnuts are not collected by humans and are accidentally eaten by animals. In addition, our results showed that the polysaccharide concentration in the stirred-tank fermentor increased with an increase in the agitation rate with a maximum value at 600 rpm.

Acknowledgment

We are indebted to R. Papi for assistance with the initial experiment.

References

1. Moorhouse, R., Walkinshaw, D., and Arnott, S. (1977), in *Extracellular Microbial Polysaccharides*, ACS Symposium Series, American Chemical Society, Washington, DC, pp. 91–102.
2. Andrew, T. R. (1997), in *Extracellular Microbial Polysaccharides*, ACS Symposium Series, Sanford, P. A. and Laskin, A., eds., American Chemical Society, Washington, DC, pp. 231–241.
3. Jeans, A. (1974), *J. Polymer Sci. Part C, Polymer Symp.* **45,** 209–227.
4. De Vuyst, L. and Vermeire A. (1994), *Appl. Microbiol. Biotechnol.* **42,** 187–191.
5. Papoutsopoulou, S. V., Ekateriniadou, L. V., and Kyriakidis, D. A. (1994), *Biotechnol. Lett.* **16,** 1235–1240.
6. Fu, J. F., Chang, R. Y., and Tseng, Y. H. (1992), *Appl. Microbiol. Biotechnol.* **37,** 225–229.
7. Konicek, J. and Konickova-Radockova, M. (1992), *Folia Microbiol.* **37,** 102–104.
8. Thorne, L., Tansey, L., and Pollock, T. J. (1998), *J. Ind. Microbiol.* **5,** 321–328.
9. Papi, R. M., Ekateriniadou, L. V., Beletsiotis, E., Typas, M. A., and Kyriakidis, D. A. (1999), *Biotechnol. Lett.* **21,** 39–43.
10. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989), *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
11. Ekateriniadou, L. V., Papoutsopoulou, S. V., and Kyriakidis, D. A. (1994), *Biotechnol. Lett.* **16,** 517–522.
12. Miller, G. L. (1959), *Anal. Chem.* **31,** 426–428.
13. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956), *Anal. Chem.* **28,** 350–356.
14. Bearden, J. C. (1978), *Biochim. Biophys. Acta* **533,** 525–529.
15. Bradford, M. M. (1976), *Ann. Biochem.* **72,** 248–254.
16. American Association of Cereal Chemists (1983), *Approved Methods of the American Association of Cereal Chemists*, 8th ed., AACC, St. Paul, MN.
17. Liakopoulou-Kyriakides, M., Tzanakakis, E. S., Kyparissidis, C., Ekateriniadou, L. V., and Kyriakidis, D. A. (1997), *Chem. Eng. Technol.* **20,** 354–360.
18. Fu, J. F. and Tseng, Y. H. (1990), *Appl. Environ. Microbiol.* **56,** 919–923.
19. Carlos Roseiro, J., Nick Emery, A., Simoes, P., Estevao, F., and Amaral-Collaco, M. T. (1993), *Appl. Microbiol. Biotechnol.* **38,** 709–713.
20. Cadmus, M. C., Knutson, C. A., Lagoda, A. A., Pittsley, J. E., and Burton, K. A. (1978), *Biotechnol. Bioeng.* **20,** 1003–1014.
21. Davidson, I. W. (1978), *FEMS Microbiol. Lett.* **3,** 347–349.
22. Sutherland, I. W. (1981), *Carbohydr. Polym.* **1,** 107–115.
23. Peters, H.-U., Suh, I.-S., Schumpe, A., and Deckwer, W.-D. (1993), *Biotechnol. Lett.* **15,** 565, 566.