Carbohydrate Polymers 80 (2010) 1037–1041

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Recovery and purification of the exopolysaccharide PS-EDIV from *Sphingomonas pituitosa* DSM 13101

Michael Andreas Bahl, Ellen Schultheis, Dietmar Christian Hempel, Bernd Nörtemann, Ezequiel Franco-Lara *

Institute of Biochemical Engineering, Technical University of Braunschweig, 38106 Braunschweig, Germany

ARTICLE INFO

Article history:
Received 15 October 2009
Received in revised form 7 January 2010
Accepted 11 January 2010
Available online 15 January 2010

Keywords:
Biopolymer
Down-stream processing
Sphingan
Polarization effect

ABSTRACT

The separation and purification of the exopolysaccharide PS-EDIV from the culture broth of *Sphingomonas pituitosa* DSM 13101 was studied. For this purpose a sequence of separation and concentration operations including centrifugation, ultrafiltration and precipitation were used. Cell removal by centrifugation was performed at four different temperatures, showing that at 40 °C clarification was best with a residual biomass concentration of 0.1 g L^{-1}. Subsequently ultrafiltration tests revealed that the cell debris containing polysaccharide solution did not cause any membrane fouling. The observed decrease of permeate flux at high PS-EDIV concentrations was rather attributed to a concentration polarization effect. The polymer solution could be concentrated up to 5 g L^{-1} whereby 90% of the initial polymer was recovered. In a last recovery step the polymer was precipitated using four different organic solvents: acetone, ethanol, propanol and isopropanol. Isopropanol gave the highest precipitation yield of 81% at a solvent ratio of 9:1 (v/v).

1. Introduction

Within the last 30 years microbial exopolysaccharides have found a wide range of applications in food, pharmaceutical and agricultural industry where they are mainly used as emulsion stabilizers, viscosity improvers, film formers and gelling agents (Podolsak & Tiu, 1996; van Kranenburg, Boels, Kleerebezem, & de Vos, 1999). Usually, these natural polymers were produced by submerged fermentation in stirred tank reactors. Numerous studies on the production of exopolysaccharides deal with the problems arising from limited bulk mixing and mass transfer in the highly viscous cultivation broths (Amanullah, Tuttiett, & Nienow, 1998; Funahashi, Maehara, Taguchi, & Yoshida, 1987; Herbst, Schumpe, & Deckwer, 1992). The corresponding down-stream processing of polysaccharides has also been reviewed by several authors (Godet, 1973; Pace & Righelato, 1980; Smith & Pace, 1982).

Generally, after cell inactivation by heat treatment, the biopolymer is directly recovered from the fermentation broth by addition of one to three volumes of alcohol. Since the acquired product also contains the producing cells or their fragments, its application is limited if a refined product is required or desired. In this case, the high viscosity of the broth has to be reduced by dilution to enable or facilitate cell removal by filtration or centrifugation (Johns & Noor, 1991). The resulting clarified polymer solution can be further concentrated by ultrafiltration to enhance the economy of the precipitation step. By these means the amount of alcohol necessary for the polymer recovery can be significantly reduced and low molecular impurities, e.g. salts, can be partially removed (Barker, Bhambra, Alsop, & Gibbs, 1987; Lo, Yang, & Min, 1997).

The present work was carried out to optimize the recovery and purification of the rhamsan-like capsular exopolysaccharide PS-EDIV produced by *Sphingomonas pituitosa*. PS-EDIV has a linear molecular structure with a partially branched repeating unit that consists of glucose, rhamnose, glucuronic acid and 2-deoxy-glucuronic acid and, due to the uronic acids, features a slight anionic character (Schultheis et al., 2008). The rheological behavior of the culture broth during exopolysaccharide production has been also characterized in detail (Schultheis et al., 2009). The culture broth exhibits a high viscosity which is strongly influenced by salt addition but weakly influenced by temperature or pH variations. Furthermore, the PS-EDIV containing broth presents a viscoelastic behavior, which is more gel-like than sol-like, and exhibits slight elastic properties. In the present work, a three step recovery of PS-EDIV from the culture broth was investigated consisting of a primary physical separation of the cells via centrifugation, a secondary concentration of the clarified polymer solution per ultrafiltration and finally the isolation and purification of the polysaccharide from the aqueous solution by means of precipitation.

* Corresponding author. Fax: +49 (0) 531/391 7652.
E-mail addresses: ibvt@tu-bs.de, e.franco-lara@tu-bs.de (E. Franco-Lara).

0144-8617/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.01.021

2. Materials and methods

2.1. PS-EDIV broth

Sphingomonas pituitosa DSM 13101 was cultivated in a medium containing (per liter) 50 g of sucrose, 3 g of $NaNO_3$, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.025 g of $FeCl_3$, 0.65 g of K_2HPO_4 and 0.28 g of KH_2PO_4. Cultivations were carried out in a 7 L Applikon bioreactor (Applikon, AC Schiedam, The Netherlands) with a working volume of 5 L, equipped with three Intermig stirrers. The batch process set-points were: agitation rate 600 min^{-1}, aeration rate 0.5 L L^{-1} min^{-1}, pH 7, 30 °C. The process was completed after 48 h of cultivation time. The growth medium (4.5 L) in the reactor was inoculated with 500 mL of an exponentially growing preculture.

The residual biomass in the diluted broth was determined by means of a correlation between the optical density (OD_{640}) and the cell dry weight. Viscosity was measured at 20 °C using a concentric viscosimeter (CS10, BOHLIN instruments, UK) at a constant shear rate of 1 s^{-1}.

2.2. Centrifugation experiments

Centrifugation was performed with diluted culture broth (1:10) in a laboratory centrifuge (Biofuge, Heraeus, Germany) at 20,000*g* to remove the *S. pituitosa* cells. The resulting polymer concentration was 0.8 g L^{-1}. The temperature during centrifugation was controlled at 4, 20 and 40 °C, respectively, and the operation time varied from 5 to 60 min. Prior to centrifugation, all samples were tempered according to the centrifugation temperature (4, 20 and 40 °C). Since high temperatures are assumed to play a major role in the primary recovery step, a further sample was first heated to 70 °C and directly centrifuged at the maximal device operation temperature of 40 °C.

2.3. Crossflow ultrafiltration

For the ultrafiltration experiments, a tubular polyethersulfone membrane hollow fiber ultrafiltration cartridge (Daicen FB02-CC-FUSS082, Germany) with a length of 364 mm was used. The inner diameter of each hollow fiber was 0.8 mm, and the total membrane area (molecular cutoff size 55 kDa) was 0.26 m^2.

A rotary piston pump (Pureflo 55 Series, United Kingdom) was used to drive the polymer solution (20 L in total) from a feed tank through the hollow fiber module at a fixed speed of 500 min^{-1}. The pressure at the inlet and the outlet of the module was monitored by two digital pressure gauges (Greisinger GMSD10BR, Germany). The trans-membrane pressure (tmp) was regulated at 0.5 bar with a valve at the outlet side (after the pressure gauge). The system was operated in batch mode by returning the retentate (non steady-state experiments) or both retentate and permeate (steady-state experiments) to the feed tank.

Concentrated polymer solutions were prepared by centrifugation of a diluted culture broth (1:10 with water) for 30 min at 40 °C. The polymer-containing supernatant was collected, and the polysaccharide was precipitated with 3 volumes of isopropanol. After drying the precipitate to constant weight at 60 °C, it was used for the preparation of the polysaccharide solutions at distinct concentrations (0.5, 1 and 2 g L^{-1}).

2.4. Precipitation experiments

The studies on the isolation of the exopolysaccharide from the aqueous solution were carried out using several organic solvents: acetone, ethanol, propanol, isopropanol. The polysaccharide used in the precipitation experiments was purified as follows: Culture broth was diluted 1:10 with water and centrifuged for 30 min at 20,000*g* and 40 °C. The polysaccharide-containing supernatant was collected in 10 kDa dialysis tubings (Serva Servapor, Germany) and dialyzed against 5 L of distilled water for 3 days to remove all low molecular compounds (salts, residual sugar, etc.). Dialyzed polymer was precipitated with 3 volumes of isopropanol (HPLC-

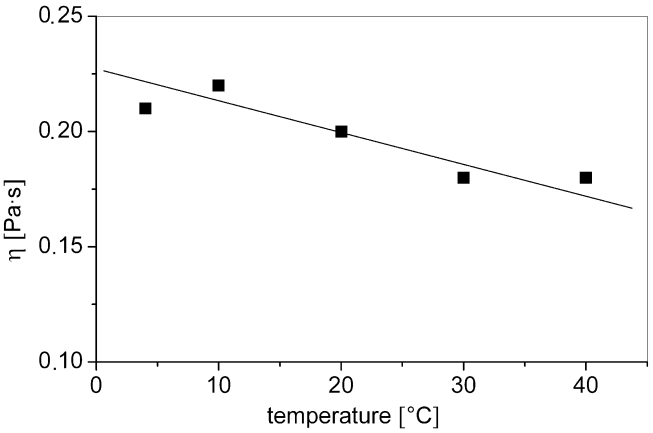

Fig. 1. Temperature dependence of solution viscosity ($C_{PS\text{-}EDIV}$ = 0.8 L^{-1}; γ = 1 s^{-1}).

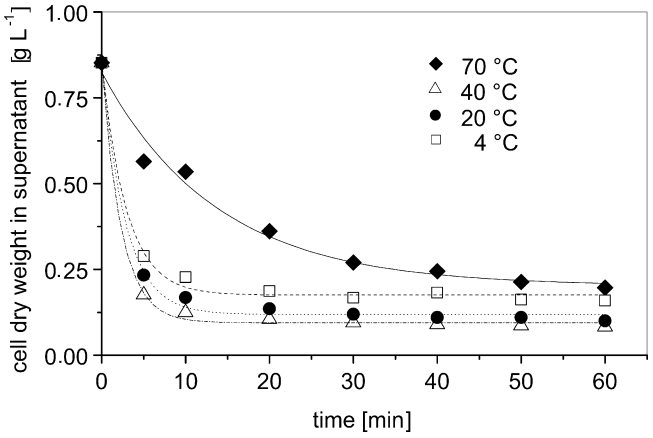

Fig. 2. Influence of centrifugation time and operation temperature on the cell separation.

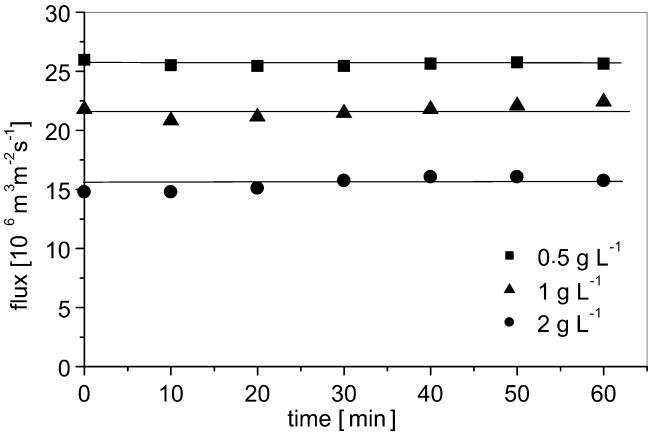

Fig. 3. Steady-state performance of ultrafiltration experiments at different PS-EDIV concentrations.

grade) and lyophilized. The lyophilized polymer was used to prepare the respective solutions (1 g L^{-1}) for the precipitation experiments. The organic solvents were added to the polysaccharide solution, mixed and allow precipitating and held for 24 h at 4 °C. For the gravimetric determination of the precipitation yield, the precipitates were centrifuged for 10 min at 20,000*g* and 4 °C and the pellets obtained were dried in an oven at 60 °C until they reached a constant weight.

3. Results and discussion

3.1. Cell separation by centrifugation

Fig. 1 depicts the apparent viscosity of a diluted culture broth (0.8 g L^{-1} PS-EDIV), which is strongly temperature dependent. The viscosity decreases with increasing operating temperatures for centrifugation. The separation of cells from the diluted broth

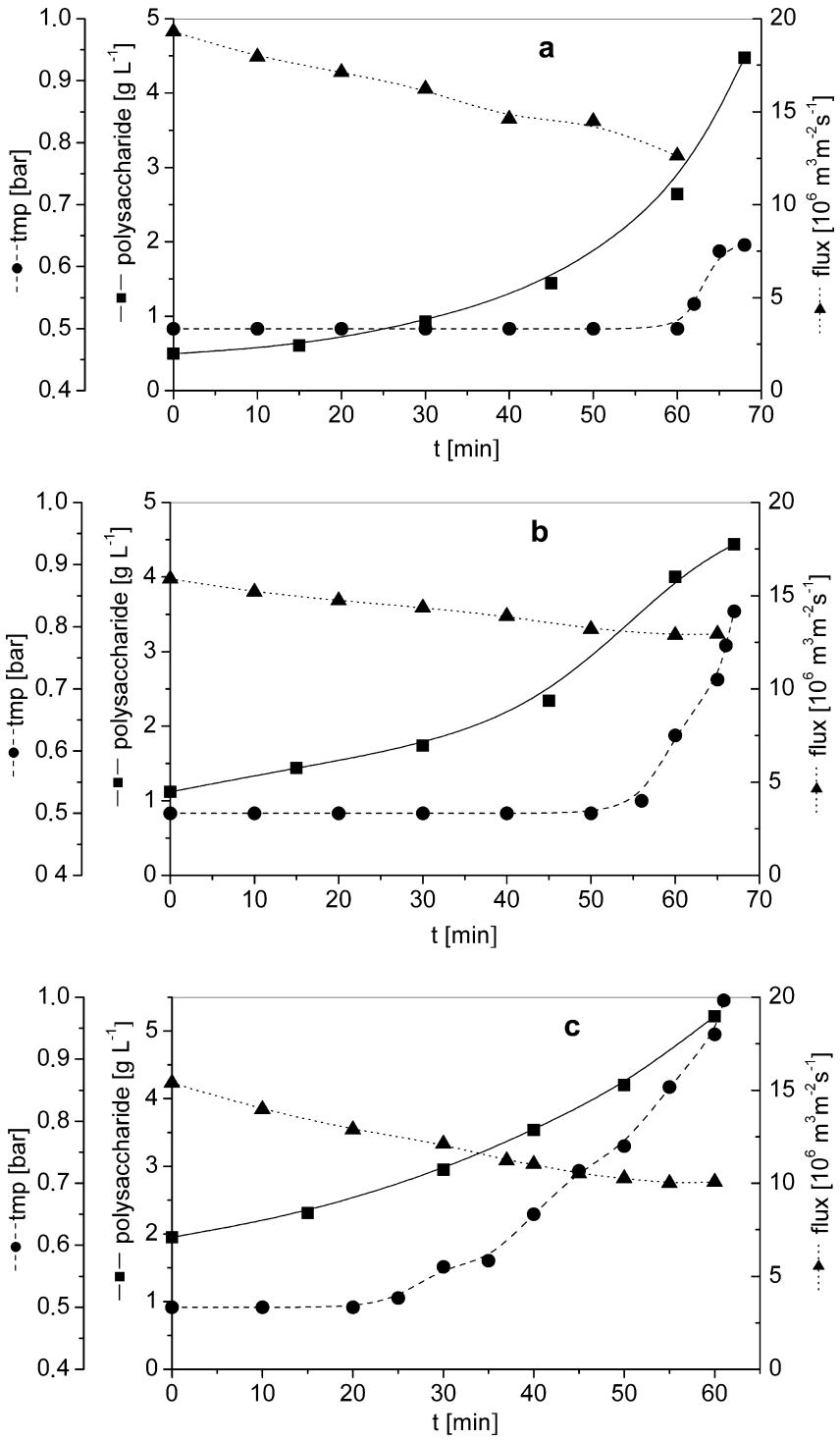

Fig. 4. Non steady-state performance of ultracentrifugation experiments as function of different initial PS-EDIV concentrations. (a) $C_{PS\text{-}EDIV}$ = 0.5 g L^{-1}, (b) $C_{PS\text{-}EDIV}$ = 1 g L^{-1} and (c) $C_{PS\text{-}EDIV}$ = 2 g L^{-1}. Working with PS-EDIV concentrations below 3 g L^{-1} the trans-membrane pressure (tmp) was kept fairly constant at the set-point of 0.5 bar.

was faster at 40 °C compared to lower temperatures, clearly indicating that low viscosities enhance the removal of cells.

At 40 °C about 79% of the cells could be removed after 5 min of operation compared to 72% and 66% at 20 and 4 °C, respectively (Fig. 2). After 30 min of centrifugation at 40 °C, the residual biomass in the supernatant was 0.1 g L^{-1}, corresponding to a clarification degree of 90%. Longer centrifugation times had almost no effect on the residual cell concentration. Actually, a total cell separation is not realizable, since according to the law of Stokes in a centrifugal field cell debris would settled down much slower compared to whole cells due to their smaller dimensions and could even be completely stabilized because of the yield stress of the surrounding polysaccharide solution. This could also explain the low separation efficiency at a temperature of 70 °C, where most of the cells were found to be subjected to rapid lysis. Pre-treatment methods like the addition of chaothrophic salts or a pH-shift, as suggested by Pollock (2002), did not increase the separation process performance (data not shown).

3.2. Concentration of PS-EDIV by ultrafiltration

It was previously reported that cell-containing xanthan and gellan solutions could significantly limit membrane operations by extensive fouling (Lo et al., 1997; Manna, Gambhir, & Ghosh, 1996). Because the recovered PS-EDIV solutions still contained about 0.1 g L^{-1} of cells or cell fragments, ultrafiltration tests were performed to concentrate the PS-EDIV solution, to further separation of the cell debris and, simultaneously, to quantify possible membrane fouling due to these materials. Usually membrane fouling can be identified by a decrease of flux according to a first-order mechanism (Cheryan, 1986; Patel, Mehaia, & Cheryan, 1987). As shown in Fig. 3, the flux did not significantly change over a period of 70 min in several steady-state filtration experiments with constant polymer concentration. This indicates the absence of an irreversible gel layer on the membrane. In contrast to the experiments of Lo et al. (1997) and Manna et al. (1996), the biomass concentration in the polymer solutions used was about 40-fold lower, which could explain the constant performance over more than one hour of operation time.

As a consequence, fouling could be excluded and, for this reason, the decrease of permeate flux with increasing polymer concentration was rather attributed to a concentration polarization effect. In contrast to fouling, concentration polarization acts as an additional hydrodynamic resistance caused by a (reversible) build-up of rejected feed components at the membrane surface. This results from the convective flow of solutes towards the membrane under the applied trans-membrane pressure gradient.

Non steady-state crossflow ultrafiltration was also carried out with three different initial concentrations of 0.5 g L^{-1} (Fig. 4a), 1 g L^{-1} (Fig. 4b) and 2 g L^{-1} (Fig. 4c). The results of these ultrafiltration experiments revealed a decline in permeate flux with increasing polymer concentration, which was in accordance with the results of the steady-state experiments. However, for all experiments it was not possible to maintain the trans-membrane pressure at a constant level (0.5 bar) throughout the whole experimental run. In general, the trans-membrane pressure started to increase at polymer concentrations between 2.5 and 3 g L^{-1} and could not be kept constant because the permeate valve reached its maximal capacity. At the end of operation a final concentration of 4.5–5 g L^{-1} PS-EDIV was achieved. The process was finished because of the low volume of the concentrated solution, which hampered the further pumping through the filtration cartridge and module. However the results showed that about 90% of the initial polymer could be recovered and purified by this means.

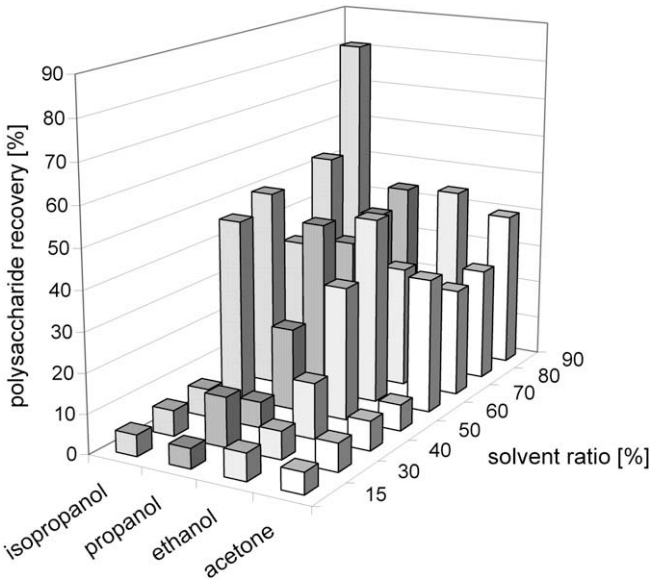

Fig. 5. Effect of solvent type and ratio on the PS-EDIV precipitation yield.

3.3. Recovery of PS-EDIV by precipitation

Experiments with regard to the precipitation of PS-EDIV by organic solvents were carried out with three alcohols and acetone. As shown in Fig. 5, the polymer recovery did not depend on the type of solvent used at solvent concentrations below 40% obtaining average values of about 7%. At higher solvent contents the yield increased to a maximum of 40% (acetone) and 81% (isopropanol), respectively. Furthermore, for each organic compound used at solvent ratios around 70% the precipitate concentration decreased by 5–15% compared to the maximum values determined at solvent ratios of 60% and 90%, respectively.

The limited precipitation of the polymer at solvent concentrations below 40% is in good accordance with the results obtained by Flahive, Foufopoulos, and Etzel (1994), who investigated the precipitation of the anionic polysaccharide xanthan using the same organic solvents. They observed that precipitation began within a narrow range of 30–40% alcohol concentration. Regarding PS-EDIV, a minor decrease of polymer yield occurred at solvent concentrations about 70%. We suggest that, in this range, the anionic polymer is subjected to a structural transition, slightly increasing its solubility in the alcohol–water mixture. This assumption is based in the tendency that sphingan polysaccharides have to arrange into double helices structures in liquid solutions (Chandrasekaran, Puigjaner, Joyce, & Arnott, 1988). For this reason, and probably due to a transition within the helical structure, the hydrophilic carboxyl group might be, at least partially, covered, while the more hydrophobic deoxysugar rhamnose might be exposed to a greater extend to the surrounding solvent. When the alcohol concentration is further increased, the hydrophobic surrounding finally forces the polymer to precipitate.

Comparing the absolute precipitation efficiency, the solvents can be ranked as follows:isopropanol > propanol ≈ ethanol > acetone.

4. Conclusions

The separation efficiency of *S. pituitosa* cells from diluted fermentation broths by centrifugation is temperature dependent with an optimal value at 40 °C. Even when operation at higher temperatures reduces the broth viscosity further centrifugation at higher

temperatures did not enhance the separation process. Pre-treatment at 70 °C and additional centrifugation even caused cell lysis and led to the release of intracellular compounds, which significantly boosted the turbidity of the solution. Moreover, even at the optimal centrifugation temperature (40 °C) a complete cell separation could not be accomplished regardless of a consecutive crossflow ultrafiltration step. However, during crossflow ultrafiltration the cell debris concentration did not lead to any membrane fouling thereby minimizing the amount of organic solvent required for the PS-EDIV precipitation. Precipitation experiments clearly showed that despite the use of the best suited solvent at solvent ratios of 90% not all PS-EDIV polymer could be recovered from the pure solution by means of precipitation. It is likely, that the precipitation efficiency could be increased by the addition of mono- or divalent cations, however under the drawback of obtaining salt-containing precipitates.

References

Amanullah, A., Tuttiett, B., & Nienow, A. W. (1998). Agitator speed and dissolved oxygen effects in xanthan fermentations. *Biotechnology and Bioengineering, 57*, 198–219.

Barker, P. E., Bhambra, K. S., Alsop, R. M., & Gibbs, R. (1987). Fractionation of dextran using ethanol. *Chemical Engineering Research and Design, 65*, 390–395.

Chandrasekaran, R., Puigjaner, L. C., Joyce, K. L., & Arnott, S. (1988). Cation interaction in gellan: An X-ray study of potassium salt. *Carbohydrate Research, 181*, 23–40.

Cheryan, M. (1986). Modelling of UF processes. *Ultrafiltration handbook* (vol. 4, pp. 73–229). Technomic publishing Comp. Inc..

Flahive, J. J., III, Foufopoulos, A., & Etzel, M. R. (1994). Alcohol precipitation of xanthan gum from pure solutions and fermentative broths. *Separation Science Technology, 29*, 1673–1687.

Funahashi, H., Maehara, M., Taguchi, H., & Yoshida, T. (1987). Effects of agitation by flat-bladed turbine impeller on microbial production of xanthan gum. *Journal of Chemical Engineering of Japan, 20*, 16–22.

Godet, P. (1973). Fermentation of polysaccharide gums. *Process Biochemistry, 8*, 33–34.

Herbst, H., Schumpe, A., & Deckwer, W. D. (1992). Xanthan production in stirred tank fermentors: Oxygen transfer and scale-up. *Chemical Engineering and Technology, 15*, 425–434.

Johns, M. R., & Noor, E. (1991). Recovery and purification of polysaccharides from microbial broth. *Austrian Journal of Biotechnology, 5*, 73–77.

Lo, Y. M., Yang, S.-T., & Min, D. B. (1997). Ultrafiltration of xanthan gum fermentation broth: Process and economic analyses. *Journal of Food Engineering, 31*, 219–236.

Manna, B., Gambhir, A., & Ghosh, P. (1996). Production and rheological characteristics of the microbial polysaccharide gellan. *Letters in Applied Microbiology, 23*, 141–145.

Pace, G. W., & Righelato, R. C. (1980). Production of extracellular microbial polysaccharides. *Advances in Biochemical Engineering, 15*, 41–70.

Patel, P. N., Mehaia, M. A., & Cheryan, M. (1987). Cross-flow membrane filtration of yeast suspensions. *Journal of Biotechnology, 5*, 1–16.

Podolsak, A. K., & Tiu, C. (1996). Rheological properties and some applications for rhamsan and xanthan gum solutions. *Polymer International, 40*, 155–167.

Pollock, T. J. (2002). Sphingan group of exopolysaccharides (EPS). In A. Steinbuechel (Ed.), *Biopolymers* (pp. 239–258). Weinheim: Wiley-VCH.

Schultheis, E., Dreger, M. A., Muñoz-Villegas, J. M., Escalante, J. I., Franco-Lara, E., & Nörtemann, B. (2009). Rheological characterization of the exopolysaccharide PS-EDIV from *Sphingomonas pituitosa* DSM 13101. *Biochemical Engineering Journal, 47*(1–3), 116–121.

Schultheis, E., Dreger, M. A., Nimtz, M., Wray, V., Hempel, D. C., & Nörtemann, B. (2008). Structural characterization of the exopolysaccharide PS-EDIV from *Sphingomonas pituitosa* strain DSM 13101. *Applied Microbiology and Biotechnology, 78*, 1017–1024.

Smith, I. H., & Pace, G. W. (1982). Recovery of polysaccharides. *Journal of Chemical Technology and Biotechnology, 32*, 119–129.

van Kranenburg, R., Boels, I. C., Kleerebezem, M., & de Vos, W. M. (1999). Genetics and engineering of microbial exopolysaccharides for food: Approaches for the production of existing and novel polysaccharides. *Current Opinion in Biotechnology, 10*, 498–504.