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Detergents improve xanthan yield and polymer quality in cultures of *Xanthomonas campestris*

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The effect of the addition of detergents Tween 40, Tween 80, Chaps, and Triton X–100 on xanthan fermentation was evaluated in shake flasks. All assessed surfactants, when added after 24 h of culture, improved final xanthan gum concentration compared with the control which contained no detergent. Triton had the most important effect, producing 1.45–fold the gum produced in the absence of detergent. A time course of cultures developed in a 2-l fermentor revealed that the use of Triton led to higher oxygen concentrations during fermentation compared with the culture with no detergent. Observations under the microscope revealed that the bacterial cells were smaller than those seen in the absence of the detergent. Compared at the same xanthan concentration and ionic strength, the diluted broths of fermentations containing Triton were more viscous than those of a control fermentation. This suggested that the use of Triton affected factors determining the rheological quality of the polymer such as the molecular weight. The detergent, when added a posteriori *to xanthan broths, did not significantly affect the viscosity profiles. Since no severe foaming was observed as a result of detergent addition, the use of small amounts of this surfactant is a promising way for improving xanthan fermentation, thereby allowing higher xanthan titers and a high quality xanthan gum to be obtained.*

Keywords: Xanthan; detergents; *Xanthomonas campestris;* surfactants

Introduction

Xanthan gum is a well-known microbial polysaccharide with a tradition of more than 20 years in the hydrocolloid market. Xanthan gum production has been the subject of numerous publications[1,2] in the many aspects involved in its manufacture. Due to the highly competitive market of xanthan gum, more efficient processes are permanently needed. Xanthan fermentation has been improved by biological and engineering approaches. Recently, representative examples include the selection of improved *Xanthomonas* strains[3,4] and the manipulation of culture conditions such as the use of optimized culture media,[5,6] fed-batch culture,[7,8] and more effective mixing.[7,9,10]

In this work, an important improvement of xanthan fermentation has been achieved by conducting the culture in a medium containing a detergent. These type of surfactants are very useful in biology and biochemistry[11] and have been used in biotechnology for improving the yields of a number of enzymes produced by fermentation. Examples of this include lipases produced by a variety of microorganisms,[12–17] ligninases,[18,19] cellulases,[20,21] and amylases,[22,23] among the most important. The mechanisms by which detergents enhance extracellular enzyme production are not completely clear; however, increased cell membrane permeability, change in lipid metabolism, and stimulation of the release of enzymes are among the possible modes of action.[12–23]

This is the first report in which a detergent was successfully used for simultaneously improving the xanthan fermentation yield and the polymer rheological quality.

Materials and methods

Organism

Xanthomonas campestris IBT 148, a rifamycin-resistant strain[24] derived from the NRRL B–1459 strain was used in this work. The bacteria was kept on yeast-malt extract slopes at 4°C and trans-

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ferred every 30 days to fresh slopes. Inoculation started from a 24-hour-old slope cultured at 29°C.

Chemicals

All chemicals were reagent grade. The following detergents (Merck, Mexico City, Mexico) were used: Tween 40 (polyoxythylene sorbitan monopalmitate), Tween 80 (polyoxyethylene sorbitan monooleate), CHAPS (3–[(3–cholamidopropyl) dimethyilammoniol]–2–hydroxypropane–1–sulfonate), and Triton X–100 (nonaethylene glycol octylphenol ether).

Culture conditions

The composition of the inoculum and production medium has been previously described.[25] Experiments in shake flasks were conducted in 500-ml baffled Erlenmeyer flasks;[26] the detergent (0.1 or 0.5 g l^{-1}) was added aseptically at t= 24h of cultivation which lasted 54 h. Inocula were initiated by transferring 10 wire loops of a fresh slope to four 500-ml Erlenmeyer flasks containing 100 ml of yeast-malt medium which were cultured at 29°C for 24 h. Shake flasks were inoculated with 10 ml taken from this seed culture. In order to inoculate the 2 l bioreactor (containing 1.4 l of production medium), two seed Erlenmeyer flasks were used. The fermentor consisted of two identical glass vessels equipped with a set of two Rushton turbines (impeller to tank diameter = 0.68) which were coupled to a stirring device powered by a 1 Hp motor. Temperature was controlled by a water bath at 29 ± 2°C. The pH was controlled automatically by an Ingold (Wilmington, MA, USA) 2300 controller connected to a pH probe (Ingold) using 50% (v/v) ammonium hydroxide which was added by a peristaltic pump. Agitation speed was varied manually between 200–400 rpm as a function of the dissolved oxygen readings and for maintaining good mixing in the fermentor as determined from visual observations. Air flow rate was measured with a calibrated rotameter and remained at 0.5 vvm during the first 24 h of cultivation and at 1.0 vvm afterwards. The detergent (Triton) was added slowly (by droplets) to the fermentor with a syringe between hours 25 and 30 of the cultivation. Triton solution was prepared by suspending 0.15 g of the detergent in 2 ml of distilled water. This suspension was homogenized by vortex agitation and sterilized for 20 min at 1.4 kg cm^{-2} in an autoclave. A 50% (v/v) solution of silicon antifoam (AF, Dow Corning, Mexico City, Mexico) was used as needed in the fermentor (10–20 ml for the control experiment and 30–50 ml for the run containing the detergent).

Analytical and instrumental determinations

Bacterial biomass, xanthan concentration, and apparent viscosity were determined as previously described.[25] Ionic strength of the broths was measured with a conductimeter (The London Co., Denmark, Model CDM 2D). The ionic strength of diluted final broths was adjusted with NaCl. Microscopic observations were carried out under an optical (Nikon, Tokyo, Japan, Alphaphot 2542) microscope.

Results and discussion

Figure 1 shows the results of experiments conducted in shake flasks in order to evaluate the effects of different detergents at two concentrations (0.1 and 0.5 g l^{-1}) on the production of xanthan gum. All assessed detergents resulted in increased xanthan production when compared with the control experiment with no detergent. Experiments conducted with the highest (0.5 g l^{-1}) detergent concentration showed higher xanthan concentrations (1.03–1.2-fold) compared with the results obtained with the lowest detergent concentration (0.1 g l^{-1}); however, severe foaming was observed. Triton X–100 (either at 0.1 or 0.5 g l^{-1}) gave the best results, producing nearly 1.5-fold the xanthan produced in the control experiment. CHAPS improved xanthan production by 1.26–1.5-fold. Tween 80 and Tween 40 showed the mildest effect, improving xanthan production by 1.07–1.27-fold. Low final biomass concentrations (0.4–0.7 g l^{-1}) were observed when CHAPS and Triton X–100 were used whereas Tween 40 and Tween 80 turned out to have final biomass concentrations of 1.0–1.7 g l^{-1} which were similar to that of the control experiment with no detergent (1.3 g l^{-1}); however, it should be pointed out that the biomass in the shake flasks experiments is an end value and does not give information regarding the actual growth curve. Previous work[26,27] from our group has shown that, in baffled shake flasks with no detergent, a maximum amount of biomass (about 3 g l^{-1}) is obtained at 45 h of culture followed by a lysis period. Although biomass data is the result of different time courses of the cultures, no conclusions can be drawn regarding the effect of the detergent on the actual growth of the bacteria. Observations under the optical microscope of the culture containing Tween 40 (at 30 and 40 h of culture) evidenced that the bacterial cells were smaller in the experiment with the detergent.

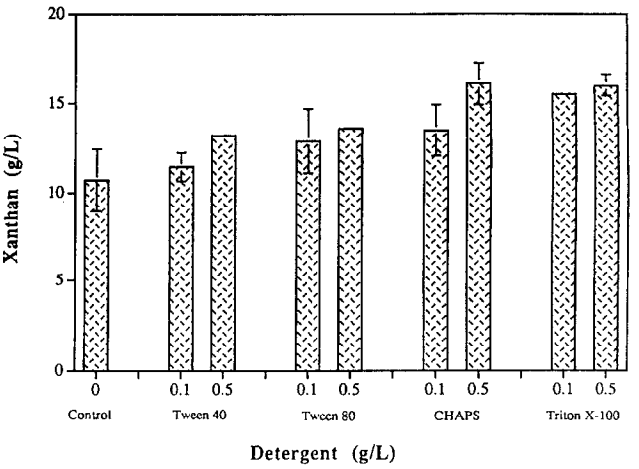

Figure 1 Effects of four detergents on xanthan flask fermentations. Data were determined after 54 h of culture. The detergent was added at t = 24 h of culture. The results are the average of three replicas. (When no error bar is shown, the standard error was less than 0.5%)

Since Triton X–100 gave the best results in terms of improved xanthan production in shake flasks, this detergent was evaluated under more controlled conditions in a 2-l bioreactor. Because no important effect was observed between the two detergent concentrations and the highest concentration tested (0.5 g l^{-1}) resulted in severe foaming, the experiments in the fermentor were conducted with 0.1 g l^{-1} of the detergent. *Figure 2* shows the evolution of xanthan and biomass of cultures conducted with and without Triton X–100. *Figure 3* shows a typical run in which dissolved oxygen was monitored during culture. In terms of biomass evolution (*Figure 2*), no differences were observed between the control (no detergent) and the culture containing 0.1 g l^{-1} of Triton X–100. Before the detergent was added, oxygen profiles of both cultures were very similar (*Figure 3*);

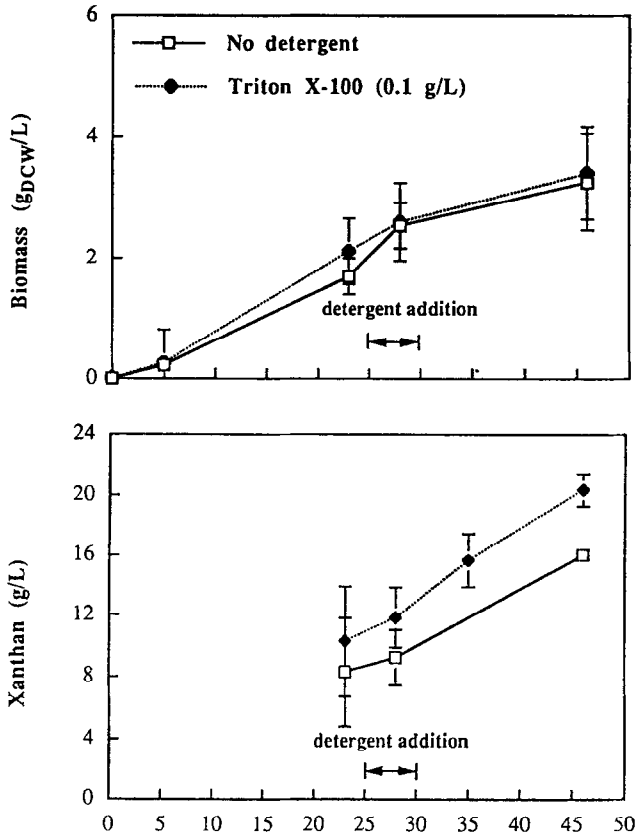

Figure 2 Evolution of xanthan fermentation in a 2-l bioreactor with and without a detergent present in the culture medium. Triton X–100 added between t = 25–30 h of cultivation. Data are from two independent runs

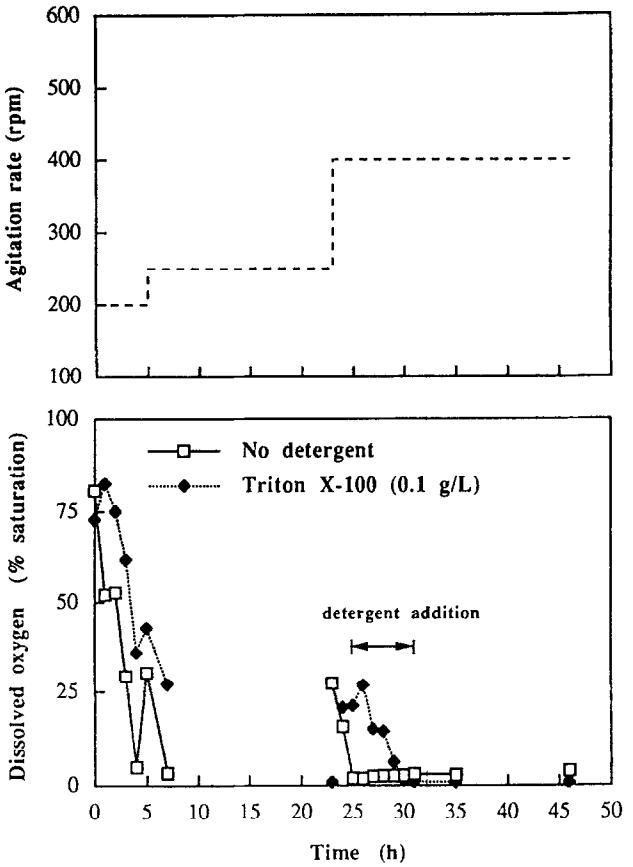

Figure 3 Oxygen and impeller speed profiles of a xanthan fermentation conducted in a 2-l bioreactor with and without a detergent present in the culture medium. Triton X–100 added between t = 25–30 h of cultivation

they showed a decrease in oxygen tension as the culture grew. At t = 24 h of cultivation (when the stirring speed was increased to 400 rpm) and before the detergent was added, oxygen tension was about 25% of saturation in both cultures. The control run experienced a drastic drop in oxygen tension, reaching unmeasurable values after t = 26 h of culture. In contrast, in the culture containing detergent, higher oxygen tensions were observed over a longer period of time. Unmeasurable levels were observed only after 35 h of cultivation. Observations under the microscope showed that the bacterial cells were smaller in the culture containing detergent compared with cells from the control run with no detergent.

Xanthan evolution was measured only after 24 h of cultivation to minimize sampling and because xanthan production during the first 24 h is not as important as afterwards. The culture containing the detergent produced considerably more xanthan than the control (*Figure 2*). When compared at the end of the culture, the run with Triton X–100 produced 4 g l^{-1} more than the control. This means that the use of a detergent like Triton X–100 gave an improvement of 1.24-fold in xanthan production. This improvement was lower than that observed in shake-flasks experiments. The reasons for the differences could be the better mixing achievable in the fermentor (resulting in improved mass transfer) and the fact that in the bioreactor, the pH was controlled.

Clearly, the detergent improved xanthan yield in shake flasks and a small bioreactor. The mechanism by which the detergent influences xanthan production is not clear; however, our data suggest that one mode of the action of the detergent is by altering oxygen transfer. As reviewed by Tsao and Lee[28] and Lee and Luk,[29] surfactants affect mass transfer either by changing the surface film resistance or the hydrodynamics. It has been shown[30] that the bubble-rise velocity is significantly reduced (in Newtonian and non-Newtonian fluids) in the presence of surface active materials due to existence of surface tension gradients at the bubble interface. Surfactants drastically change the rate of coalescence, thus making the bubble size more uniform.[31]

On the other hand, the fact that the cells were smaller in the presence of the detergent could have led to a higher oxygen uptake rate which is a parameter determining the xanthan specific production rate.[32–34] In addition, the action of the detergent could be by interacting with the bacterial cell membrane in a way which could enhance the polymerization process of the xanthan molecule (which occurs at the cell membrane)[35] and/or the turnover and release of the completed xanthan molecule at the cytoplasmic membrane. Evidently, further research is needed for the elucidation of the mechanisms of action of the detergents on xanthan biosynthesis.

In addition to promoting the increased production of xan-

than, the use of a detergent such as Triton X–100 resulted in a polymer of higher rheological quality. *Figure 4* is a rheogram (a log-log plot of the apparent viscosity as a function of the shear rate) of the behavior of solutions prepared at two different concentrations (by diluting the final broth and adjusting the ionic strength to 3.3 mMhos). At the two xanthan concentrations tested (10 and 15 g l^{-1}), the diluted broths (prepared from fermentation broth of the experiment described in *Figures 2* and *3*) showed higher viscosity in all values of shear rates measured. For instance, compared at 1 s^{-1}, the viscosity of the broth produced with Triton X–100 (containing 10 g l^{-1} of xanthan) was 3-fold higher than the value exhibited by the broth with no detergent. To rule out the possible effect of the detergent itself on the viscosity of the broth, 0.1 g l^{-1} of Triton X–100 was added to the broth produced in the control experiment and its viscosity profile was measured. The results (*Figure 4b*) clearly demonstrated that Triton itself did not affect broth viscosity in a significant manner and its effect on xanthan production results from influencing parameters of the fermentation. The fact that oxygen tension was higher for longer periods of time in the experiment with the detergent could explain why the xanthan produced under these conditions had a better quality; this is probably associated with characteristics such as a higher molecular weight. Oxygen concentration (under no limitations on oxygen),[36] and oxygen transfer rate (under oxygen-limited conditions)[37] importantly affect the average molecular weight of the xanthan. It can not be ruled out, however, that other characteristics determining the rheological quality (i.e., pyruvate or acetate content, quaternary structure, etc.) were not changed as well.

The obvious disadvantage of the use of detergents in this fermentation could be the generation of foam; nevertheless, in using 0.1 g l^{-1} of the detergent either in shake-flask experiments or the bioreactor, no serious problems with foam production were found. Although more foam was produced in the cultures containing the detergents, it was controllable. The reasons for this could lie in the low concentration of the detergent used (0.1 g l^{-1}) and in the fact that, due to the high viscosity, foam is not usually a problem in xanthan fermentation especially during the period when the polymer is predominantly produced (i.e., after 24 h of culture which is precisely when the detergent was added).

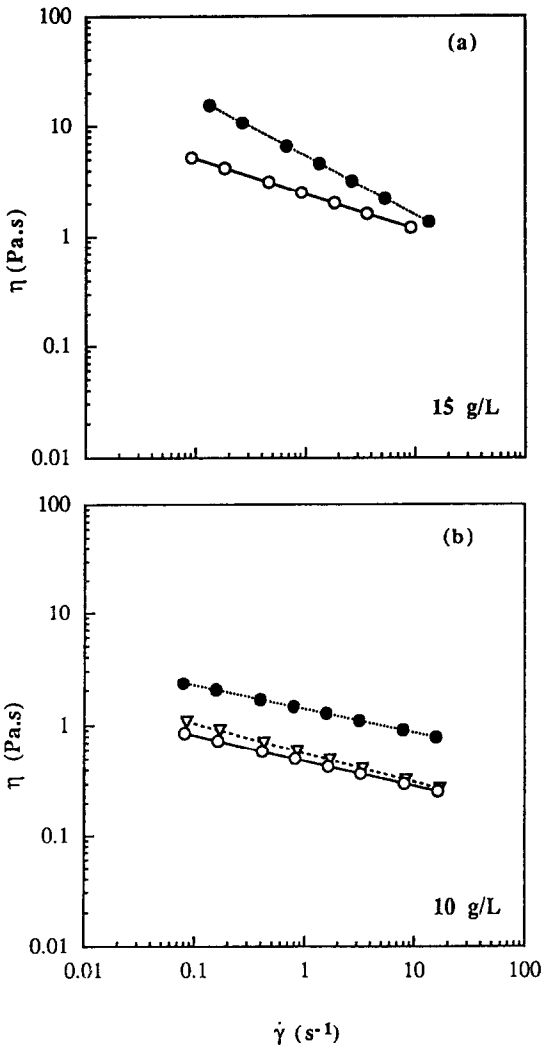

Figure 4 Rheograms of diluted xanthan broths arising from cultures with and without a detergent (Triton X–100, 0.1 g l^{-1}) present in the culture medium. Diluted broth from the culture with the detergent, ●; diluted broth from the broth without the detergent, ○; diluted final broth added with 0.1 g l^{-1} of Triton X–100, ▽. Ionic strength adjusted to 3 mMho with NaCl

Conclusions

Detergents improve xanthan production either in shake-flask (1.45-fold) or bioreactor (1.24-fold) cultures. At the low concentration used (0.1 g l^{-1}) and because of the time of addition (when viscosity is high), no severe foaming problems were observed. The detergent affects oxygen transfer which very likely is one of the reasons behind the improvement in xanthan production both quantitatively and qualitatively. Adding small amounts of a detergent such as Triton X–100 is a promising way of lowering fermentation costs in xanthan fermentation and allowing a polymer of higher quality to be synthesized.

Acknowledgments

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