The Viscostat: Productstat Method of Feed-Rate Control in Continuous Fermentations

R. W. SILMAN and E. B. BAGLEY, *Northern Regional Research Center, Federal Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604*

Summary

Xanthan biopolymer has been produced in a single-stage continuous fermentation with *Xanthomonas campestris* NRRL B-1459, using a viscostat control method instead of the conventional chemostat method. A Bendix Ultraviscoson* sensed the fermentor viscosity, and the recorder-controller actuated the feed medium pump in an on-off control mode. Since all continuous fermentations eventually become contaminated or suffer culture variation, this work served also to demonstrate the effectiveness of the viscostat control. Neither the presence of a mold contaminant with specific growth rates lower than that of *X. campestris*, nor the presence of a bacterial contaminant of specific growth rate greater than *X. campestris*, affected the maintenance of constant viscosity in this control system.

INTRODUCTION

This laboratory has a long history of study on production, characterization, and use of microbial polysaccharides and, in particular, work on *Xanthomonas campestris* NRRL B-1459 has led to industrial uses in many processes and products. An overall description of the xanthan work at NRRC was reviewed by Jeanes et al.[1] This work included some studies of nitrogen-limited chemostat-type continuous cultures.[2,3] Other types of continuous cultures are the turbidostat and the productstat, which measure cell concentration and some product of the culture.

Productstats have been reported by Pechurkin et al.,[4] Lelieveld,[5] Wardley-Smith and White,[6] and Watson.[7] Pechurkin et al. studied a pH stat using *Candida tropicalis* on a paraffin substrate and monitored pH. They recognized that there was more than one mech-

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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anism of acid production. Lelieveld ran a pH stat for yogurt manufacture, which involves cultures of two bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Cultures were selected so that *L. bulgaricus* growth depended on *S. thermophilus* by using a strain of *S. thermophilus* that had a lower specific growth rate than the *L. bulgaricus* strain. Using *Photobacterium phosphoreum*, Wardley-Smith and White operated a luminostat. In this case, their feed control was based on the combined effect of luminescence and cell turbidity because neither provided steady state alone. Watson varied temperature at constant CO_2 production rate and studied resulting changes in specific growth rate and cell concentration with a CO_2-stat, using a culture of *Saccharomyces cerevisiae*. We decided to culture *X. campestris*, using a viscostat wherein viscosity in the fermentor is measured and a controlled setpoint activates the feed flow to maintain the viscosity at a constant value. The addition of feed dilutes the product in the fermentor and also provides further nutrient for making more cells and more product.

EXPERIMENTAL

Inoculum

Cultures of *X. campestris* NRRL B-1459 were maintained on YM agar slants (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, and 2.0% agar).[8] Transfers were made every two weeks with incubation of the slant for one day at 28°C followed by 13 days at 4°C. The inoculum buildup scheme from slant to fermentor inoculum is diagrammed in the Appendix.

Fermentation

The fermentation was conducted in the system described previously.[2] The fermentor was an 8 liter glass and stainless-steel tank of conventional configuration constructed in the Center's shops. Agitation was provided by one, six-bladed, disk turbine impeller mounted on a centered vertical shaft rotating at 1200 rpm. There were four evenly spaced baffles. Sterile air (1 v/v/m) was introduced through a single-hole sparger directly below the agitator. Fermentor temperature was controlled at 28 ± 1°C.

Medium composition is given in Table I. The medium was continuously sterilized in the pilot plant;[9] 20 liter Carboys were filled aseptically and medium was again transferred to an 8 liter feed bottle. About 3500 g medium were transferred to the fermentor,

TABLE I
Medium Composition[a]

Ingredient	Amount (g 1000 g)
Yeast extract	1
Malt extract	1
Peptone	1.7
Glucose	2.5
K_2HPO_4	5
$MgSO_4$	0.3
$CaCl_2$	0.5
GE60 antifoam	0.3

[a] pH is 7.3 before continuous sterilization at 138 C for 5 min.

inoculated, and allowed to incubate under batch conditions for 24 hr. At that time, viscostat operation began. Feed rates were calculated from weight change per time interval. Product was discharged from the fermentor via a dip tube set at the 3 liter level; air exhaust through the same line, when blocked by rising broth, removed the product.

The viscosity was monitored continuously by a Bendix Ultraviscoson viscometer. The sensing element is a vibrating reed probe connected to a transmitter that delivers a direct dial readout of viscosity and permits connection to a recorder-controller. The probe was calibrated outside the fermentor, disconnected, installed in the fermentor, and autoclaved in place in the fermentor. There was no change in calibration due to autoclaving after reconnection to the transmitter. The transmitter output was recorded on a Varian recorder; a microswitch, actuated by the pen pointer, controlled a New Brunswick 52 rpm peristaltic pump that served to deliver feed medium. The viscometer was calibrated with glycerol standards. This vibrating reed measures viscosity at very high shear rates but not at the low shear rates we have at 30 rpm when using a Brookfield LVT viscometer. The correlation between the Bendix and Brookfield viscosity is given in Figure 1. It shows that the correlation is useful above 200 cP measured by the Brookfield viscometer. The log-log plot conveniently linearizes the correlation.

Analytical

Analytical procedures remained the same as in previous work,[3] but supernatant total nitrogen was also run by the Kjeldahl

method.[10] Cell optical density (cell OD) was measured at 650 nm and is a net OD where the feed medium OD was the blank.

RESULTS

The fermentation was inoculated and allowed to run as a batch for 24 hr. At that time the viscosity was 2400 cP by Brookfield measurement, and the controller setpoint was adjusted to that corresponding value on the Varian recorder. The course of the run is shown in Figure 2. The run lasted nearly 16 days total. Unfortunately, the run was contaminated at about four days with a *Penicillium*-like mold. The contamination did not affect the control system but xanthan was produced at a decreased rate (either by reduced cell population or reduced specific production rate or both), which resulted in a decrease in dilution rate starting around 100 hr from 0.053/hr to 0.043/hr. The feed line plugged at about 236 hr with a resulting increase in viscosity above the control setpoint. A second, but bacterial, contamination occurred about then; from 0–236 hr the centrifuged cells were the normal bright yellow and after that time whitish cells also were seen, until by 378 hr they amounted to about half the pellet volume. The bacterial contaminant (viewed by microscope) obviously had a faster growth rate than *X. campestris*. This contamination eventually produced a more marked decrease in dilution rate than did the mold contaminant, taking from 236 to

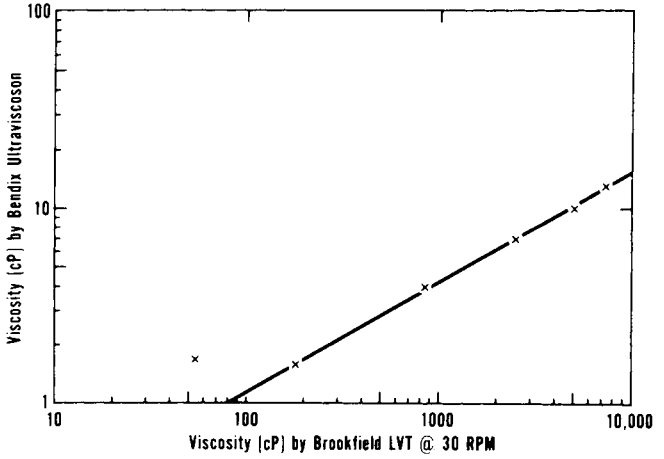

Fig. 1. Correlation of Bendix Ultraviscoson viscosity measurements with Brookfield LVT measurements at 30 rpm.

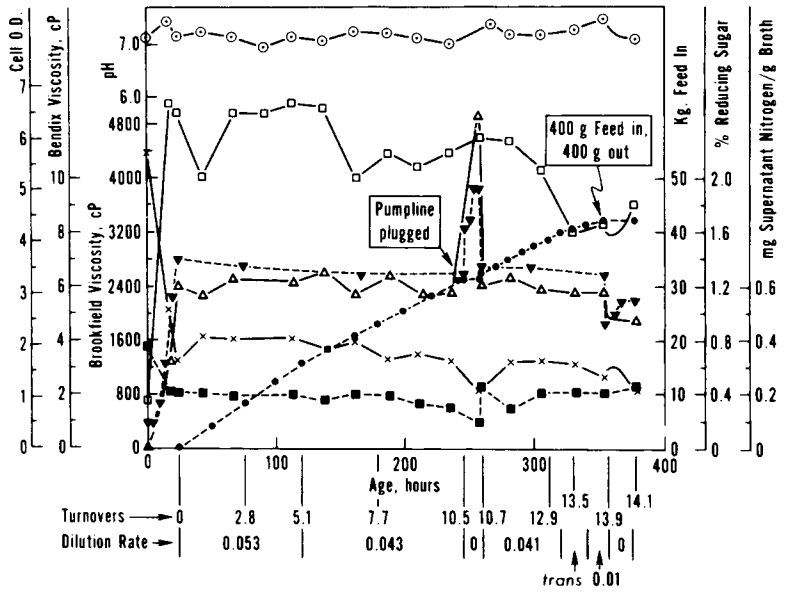

Fig. 2. Course of single-stage continuous xanthan fermentation under viscostat control of feed rate. (⊙) pH; (□) cell OD; (▼) Bendix; (△) Brookfield; (×) reducing sugar; (■) nitrogen; (●) cumulative feed medium fed to fermentor.

ca. 320 hr (ca. 2.5 replacement volumes or turnovers) to establish a sizable population.

DISCUSSION

The effectiveness of continuous viscosity control is particularly well illustrated in this fermentation in which a foreign microorganism was present. Such a "contaminated" system would normally not be reported, but contaminantion or culture variation will always occur eventually in a continuous fermentation process, unlike the batch process. In this work, two contaminants were present: a mold with a specific growth rate less than the *X. campestris*; a bacteria with a specific growth rate greater than *X. campestris*.

When the mold was present, xanthan production rate merely slowed down to a different steady state. In contrast, bacterial contamination caused production to slow to zero. The mold contaminant case illustrates again the point made by Lelieveld[5] that two organisms can be cultured simultaneously under production conditions and make a desirable product.

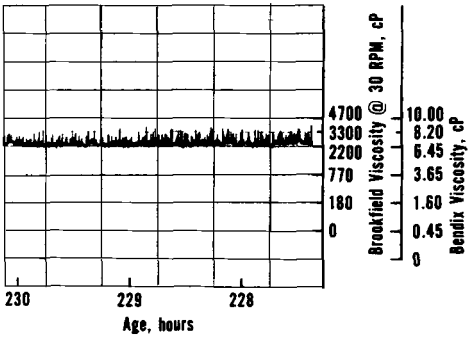

Fig. 3. Trace of viscosity recording at 2 in./hr at about 230 hr during xanthan continuous viscostat fermentation.

There are two other brands of viscometers available from Automation Products: (Dynatrol) Houston, Texas, and Nametre Co., Edison, N. J. They are both steam-sterilizable; i.e., they can be put in a steel tank through its wall and sterilized by live steam in the tank, but only the Bendix can be autoclaved in place in a glass fermentor. In Figure 3 is the recorder plot at 2 in./hr for a time period near 230 hr and it shows the "noise" associated with the viscosity reading. In addition, there seemed to be an irregular cycling of the bottom edge of the trace. The variation from 50 to 52.5 on the graph is 2200-2700 cP by the Brookfield. The sensitivity of the instrument at Brookfield viscosities above 1000 cP could be better. Also, there was drift of the Bendix Ultraviscoson transmitter as seen in Table II. The same standard viscosity solutions were used to calibrate the Bendix viscometer and to check the drift when the run was over. We feel that instrument improvement and modifications can be made that will increase the sensitivity and decrease the noise. Nevertheless, although this particular experiment was terminated because of contamination, the work serves to establish

TABLE II
Estimation of Drift of Viscosity Measurement with Bendix Ultraviscoson

	Original calibration (cP)	Final readings (cP)
Bendix	2.1	2.5
	8.5	9.6

the usefulness of continuous viscometric monitoring and control of certain fermentations.

APPENDIX: INOCULUM BUILDUP OF *Xanthomonas campestris* NRRL B-1459 FOR A 3 LITER FERMENTATION

One day old YM agar slant culture

↓

7 ml YM* in 18 × 150 mm test tube incubated 22 hr on rotary shaker (145 rpm, 2 in. eccentricity) at a 20° angle at 28°C

↓ ALL

35 ml YM* in 300 ml Erlenmeyer incubated 22 hr on rotary shaker (145 rpm, 2 in. eccentricity) at 28°C

↓ ALL

250 ml YM* in 1000 ml Erlenmeyer incubated 22 hr on rotary shaker (245 rpm, 2 in. eccentricity) at 28°C

↓ ALL

3500 g medium in 8-liter fermentor

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* YM medium without 2.0% agar.