TRANSACTIONS, ILL. STATE ACAD. SCI.
Vol. 70, No. 1 (1977)

BIOPOLYMER FORMATION BY ACTIVATED SLUDGE IN THE PRESENCE OF METHANOL

Edwin N. Davis
Northern Regional Research Center, Agricultural
Research Service, U.S. Department of Agriculture,
Peoria, Illinois 61604

ABSTRACT

Activated sludge from four municipal waste disposal plants contained microorganisms which produced a viscous product when aerated with added methanol. Activated sludge from an industrial corn products firm made heteropolysaccharides only if acid was repeatedly neutralized. The influence of aeration, backseeding, and time of methanol feeding on viscosity and methanol utilization was examined in four 10-liter fermentations for 7 days. After methanol depletion, viscosity declined. Readily soluble, freeze-dried crude products were prepared. The crude product mixed with fertilizers or pesticides was shown, by other investigators, to have some promise as a slow-release agent.

INTRODUCTION

Previous work in our laboratory (Davis and Wallen, 1976) has demonstrated that mixed cultures of organisms in activated sludge (AS) from a waste treatment plant, when aerated with low concentrations of methanol, will produce a highly viscous crude product containing glycan polymer. During the course of a week-long fermentation, activity of biopolymer-producing organisms is enhanced by the presence of methanol. The addition of some of the final product from a recent methanol fermentation as backseed to fresh AS results in a more rapid fermentation with a more efficient conversion of methanol to polymer. Data previously reported were obtained mostly with AS from the waste treatment plant of the Greater Peoria (IL) Sanitary District. Fermentations were conducted in Fernbach flasks using 500 cc aliquants and rotary shaker aeration.

Results from the use of the AS from three additional smaller municipal plants and one agribusiness experimental waste treatment plant are reported here. Also reported are four fermentations in 10-liter quantities to study variables which are controllable only in such fermentors.

MATERIALS AND METHODS

Comparison of Activated Sludges

Approximately 3-liter samples of AS were taken from the municipal waste treatment plants in (1) Peoria, (2) Pekin, (3) East Peoria, and (4) Washington, Illinois, and (5) from the experimental waste processing unit of a corn starch-dextrose manufacturer. The Peoria plant processes about 30 million gallons per day, the others about 1 million or less. Within 3 hr of collection, 500 ml aliquants of these AS samples were placed in nonsterile 2800 ml Fernbach flasks and 5 ml methanol (ACS grade) was added (1% v/v). A duplicate series received 450 ml AS plus 50 ml of 5-day fermentation material from Peoria AS as backseed. The flasks were incubated at 25 C on a rotary shaker at 200 rpm. An additional 5 ml methanol was added after 1 day and 10 ml after 2 days for a total of 4% (v/v). Small samples were withdrawn each day for determination of pH and viscosity.

Ten-Liter Studies

Results obtained in laboratory-scale equipment cannot always be duplicated in commercial-size fermentations. Therefore, a series of 10-liter fermentations were run in identical 20-liter capacity fermentors using conditions found effective in flask fermentors. Three variables were tested: backseeding, timing of methanol additions, and degree of aeration. The backseed was 4-day fermentation material from flask fermentations. Antifoam was not required. Ten gallons of Peoria AS was collected at 8 a.m. and screened through double cheesecloth. Ten liters AS was put in one nonsterile fermentor, and 9.5 liters AS plus 500 ml 5-day backseed was used in each of three other fermentors. The protocol is given in the legend of Figure 1. Five-hundred ml aliquots were withdrawn into sterile Fernbach flasks as controls from two set fermentors. These flasks were incubated on a rotary shaker at 25 C with 2% additional methanol added after 2 days. Samples were taken daily, and pH and viscosity were determined immediately; small samples were frozen for later assay of residual methanol. About 4 liters of 7-day products from two fermentors were freeze-dried in trays in a Model 25-SRC-3 Virtis Sublimator.

Analyses

pH was determined by glass electrode. Viscosity (in centipoises) was determined with a Brookfield Viscometer (Model RVT, No. 4 spindel, 30 rpm at 25 C) by averaging several measurements in different locations in each sample. Residual methanol was determined by gas-liquid chromatography. Five or 10 microliters of thawed supernatant was injected in replicate. Volume percent concentration was calculated by comparing peak area to a standard.

RESULTS

Comparison of Activated Sludges

Table 1 shows that all of the municipal treatment plants sampled contain organisms capable of converting methanol to viscous product. All gave comparable responses to methanol-acclimated backseed and showed little variation in pH throughout the time cycle. Two of the AS samples without backseed had a longer lag period in initiating polymer formation and thus required more methanol. These two matched the others when reinforced with backseed.

Table 1. Methanol Fermentations by Activated Sludge from Different Treatment Plants

Plant	Days	Activated Sludge		Act. Sl. + 10% Backseed	
		pH	Viscosity (cp)	pH	Viscosity (cp)
Peoria	4	7.2	2996	7.3	7464
Pekin	4	6.9	3196	7.2	5324
East Peoria	4	7.0	1708	7.3	6212
Washington	4	7.3	1006	7.3	7452
Agri-business (corn starch)	3	4.9(6.9)*	0	6.3(7.2)*	2370
	4	5.3(6.7)*	1364	6.6(6.9)*	3904
	6	5.1	3354	6.7	2722

* Neutralized with NaOH

Previous attempts to form biopolymer with "clarifier underflow" (AS) from the waste treatment facilities of a corn processing plant resulted in failure. Five-day acidities were pH 4.5 or below. Backseeding with Peoria beer gave similar results, i.e., only slight viscosity and slightly higher final pH. This AS did become viscous when the accumulated acid was neutralized daily as shown in Table 1. Backseeding promoted rapid utilization of methanol as judged by viscosity at comparable times. The decrease in viscosity after 4 days may have coincided with depletion of the methanol, as indicated by subsequent experiment.

Ten-Liter Fermentations

Figure 1 shows curves of viscosity and methanol utilization over the 7-day fermentation period. For unknown reasons, AS without backseed (Fig. 1a) did not convert methanol to viscous polymer as efficiently in the 10-liter fermentation as in the shaken flask control. The aliquot in the flask gave viscosities expected from previous experience.

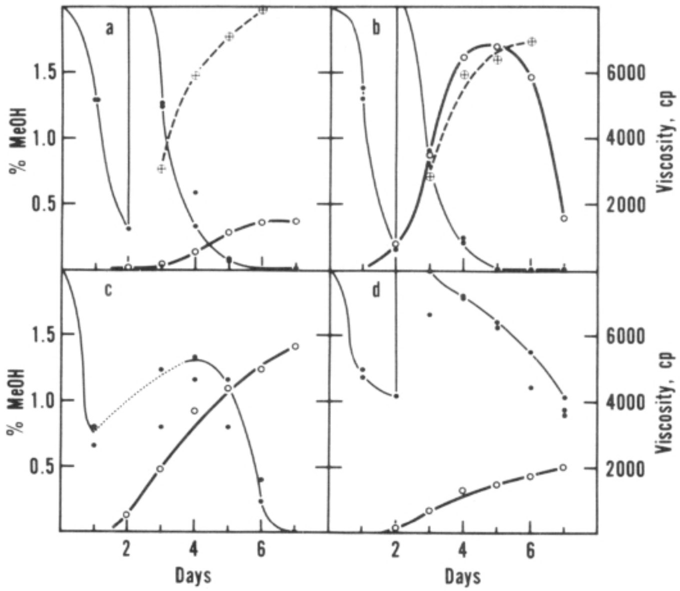

Figure 1. Ten Liter Fermentations with Peoria Activated Sludge (4 day backseed, 25°C, 200 RPM)

Ferm.	5%	% MeOH added			Aeration
	Backseed	at 0	1	2 days	lit./min.
a	none	2	0	2	15
b	yes	2	0	2	15
c	yes	1	3*		15
d	yes	2	0	2	2.5

* 0.05% each hour, 24 thru 84 hours

•—• % Methanol

○—○ Viscosity (cp) - Pilot Plant

⊕---⊕ Viscosity (cp) - Control Flasks

With backseed added (Fig. 1b), control flask and 10-liter fermentation viscosities paralleled each other. The methanol was utilized faster than without backseed and resulted in higher viscosity product. Biopolymer formation, as judged by increased viscosity, tapered off as methanol approached low concentration. A decrease in viscosity occurred after the exhaustion of methanol. The air to this fermentor was cut off after 6 days, but this did not retard further degradation of viscosity during the next 24 hr. No glucose was found by glucose oxidase test (TESTAPE) in degraded samples during this period.

Essentially continuous addition of methanol (Fig. 1c) prolonged its utilization over the 7-day period. Accumulated biosynthesis gave about the same maximum viscosity as in the fermentation with only two additions (Fig. 1b) of methanol. Again, the increase in viscosity began to taper off as the methanol approached low concentration.

With a critically low rate of aeration (Fig. 1d), methanol was not as rapidly metabolized. Only three-fourths of the 4% added methanol was utilized over the 7-day period with a very low rate of polymer synthesis.

Solids content of the high viscosity product was 1.75% (Fig. 1c) and 2.05% in the lowered viscosity product (Fig. 1b). When these freeze-dried products were resuspended to the original concentrations some months later, viscosities of smooth suspensions were 1795 cp and 608 cp as compared to 5646 cp and 1536 cp in the original liquid products.

DISCUSSION

No biopolymer has been isolated from the viscous product and no proof was found that methanol is necessarily the precursor of viscous products. However, it was previously reported that glucose comprised about three-fourths of the reducing sugars found in an incomplete acid hydrolysate of viscous product. Papergrams of this hydrolysate showed compounds with the R_f of glucose and of oligosaccharides. Also previously shown, viscosity increases only in the presence of methanol and not with other alcohols or sugar. It is a reasonable assumption that some of the organisms present in AS convert methanol to heteropolysaccharides as found by Ballerini and Parlouar (1974) and Finn et al. (1975, 1976) with other organisms.

The viscosity exhibited by the heteropolysaccharides in admixture with AS solids must be due to the composition of the biopolymer, the amount of polymer formed, the length of the polymer chains or the physical aggregation of the chains, or a combination of these. No glucose was found in samples taken during the period when viscosity was decreasing rapidly. If free glucose is released when viscosity drops, the organisms may have utilized it as fast as it formed. Or, chain shortening may have occurred by enzymatic breaking of internal bonds. If it is inconvenient to harvest at the time of maximum viscosity, the biopolymer may apparently be stabilized by addition of methanol to maintain a low concentration.

Automatic continuous addition of methanol might be convenient in large-scale operations and may give the most efficient utilization of substrate. However, improper feeding rates could result in continuous low levels of methanol concentration, and there is some evidence that such low methanol concentrations give low yields of polymer.

When a suspension of thick or diluted AS-methanol fermentation product is spread or sprayed on a surface, evaporation of water leaves a friable film which is not easily redispersed or displaced by water. This property has been utilized by others in our laboratory to prepare admixtures of fertilizers or pesticides in which the viscous product acts as a slow release agent (Roth, 1977).

Fermentative production of polysaccharides from methanol by the soil isolates Achromobacter sp. (Ballerini and Parlouar, 1974) and Methylamonas mucosa (Finn et al., 1975, 1976) have been reported. These organisms were grown in sterilized semi-synthetic media in pure culture. Activated sludge, in contrast, permits a nonsterile fermentation with a self-selected mixed culture as shown in a previous paper. The starting material is constantly available and requires only the addition of methanol and air. Open tanks, such as the aeration bays of waste treatment plants, should make satisfactory fermentors. The product is pumpable, and retention of a small fraction of the fermented product in the fermentor would be convenient as backseed for the next fermentation.

LITERATURE CITED

BALLERINI, D. and D. PARLOUAR. 1974. Microbiological Production of Polysaccharides. Fr. Demande 2,231,748 (Dec. 27, 1974); [C.A. 83(5): 41541 (1975)].

DAVIS, E. N. and L. L. WALLEN. 1976. Viscous Product from Activated Sludge by Methanol Fermentation. Appl. Environ. Microbiol. 32: 303-305.

FINN, ROBERT K., A. L. TANNAHILL, and J. E. LAPTEWICZ, JR. Production of Heteropolysaccharide by Fermentation of Methanol. U.S. 3,923,782, Dec. 2, 1975 [C.A. 84: 57403 (1976)]; U.S. 3,932,218, Jan. 13, 1976 [C.A. 84: 103,858 (1976)].

ROTH, WILLIAM B. Methanol Treated Activated Sludge as an Agricultural Chemical Carrier. U.S. 4,065,287, Dec. 27, 1977.