CONF-800739--1

# SCLEROGLUCAN BIOPOLYMER PRODUCTION, PROPERTIES, AND ECONOMICS

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## ABSTRACT

Production and solution properties which may make scleroglucan polysaccharide economically advantageous for on-site production and use in tertiary oil recovery were investigated. Scleroglucan, which is similar in viscosity and shear thinning to xanthan, can be produced in a 3-day batch or 12 h continuous fermentation. Yield is nearly 50% based on input glucose. Gross biopolymer-biomass separation may be effected using microscreening, a low energy process, followed by polish filtration. Polymer flux may be improved by hydrolysis with an endolaminarinase from *Rhizopus arrhizius* QM 1032. Simple feedstock requirements and low growth pH, together with the difficulty of resuspending dried polymer, may encourage field biopolymer fermentation and use of purified culture broth.

## KEYWORDS

Biopolymer; *Sclerotium rolfsii*; polysaccharide; waterflooding; tertiary oil recovery; macromolecule separations; endolaminarinase.

## INTRODUCTION

Scleroglucan, a soluble fungal polysaccharide, has a structure composed of a laminarin backbone, $\beta$1,3-glucosylglucose, with single glucose unit sidebranches bonded $\beta$6,1 at intervals along the main chain. Water soluble polymers such as xanthan gum and hydrolyzed acrylamides are currently used in drilling and enhanced oil recovery applications. We are investigating some of the production and solution properties which may make scleroglucan economically advantageous for on-site production and use.

## LITERATURE REVIEW

Early structural research on scleroglucan was performed at the Pioneering Research Division of the U.S. Army Quartermaster Corps Research and Engineering Center (Reese and Mandels 1959), as a part of research on enzymatic determination of polysaccharide structure. Pillsbury developed scleroglucan polymers commercially based on early work by Halleck (1967). Halleck's patent, which covered structure of scleroglucan as determined by enzymatic hydrolysis using *Sporotrichum dimorphosporum* QM 806 culture filtrate and scleroglucan synthesis from a variety of carbohydrates by fungal species of the genera *Sclerotium*, *Helotium*, and *Stromatinia*, effectively protected Pillsbury's interest in the material's composition, method of production, and use. At that time, food related uses were a major consideration. Additionally, scleroglucan appeared to be suitable for use as a food-grade thickening agent. At that time, a patent filed by Williams (1968) indicates that there was some interest in use of scleroglucan as a thickening agent for water and surfactant floods, and for drilling muds. Interest, particularly for high temperature and salinity applications, is continuing (Akstinat 1980).

## DISCLAIMER

## LABORATORY SYNTHESIS

Figs. 1a and 1b show polymer production and biomass during a 5 day *Sclerotium rolfsii* ATCC 15206 fermentation. The medium containing, per L, 3 g $NaNO_3$, 1 g $KH_2PO_4$, 0.5 g $MgSO_4$, 0.5 g KCl, 0.1 g DOW AF (antifoam), 0.1 g DOW P-2500 (antifoam), 30 g glucose, and 1 g Ambrex 1003 yeast extract was sterilized and incubated at 28 C in a 14 L Chemapec GF0014 fermenter. Air was sparged at 1 vol air/vol broth/min and the fermenter agitated at 300 rpm during the fermentation. Analytical methods were as reported earlier (Griffith and Compere 1978). Polymer production is highest during early log-phase growth, with a rapid decline thereafter. Continuous culture is feasible, with a turnover rate of 2 vol broth/vol/day.

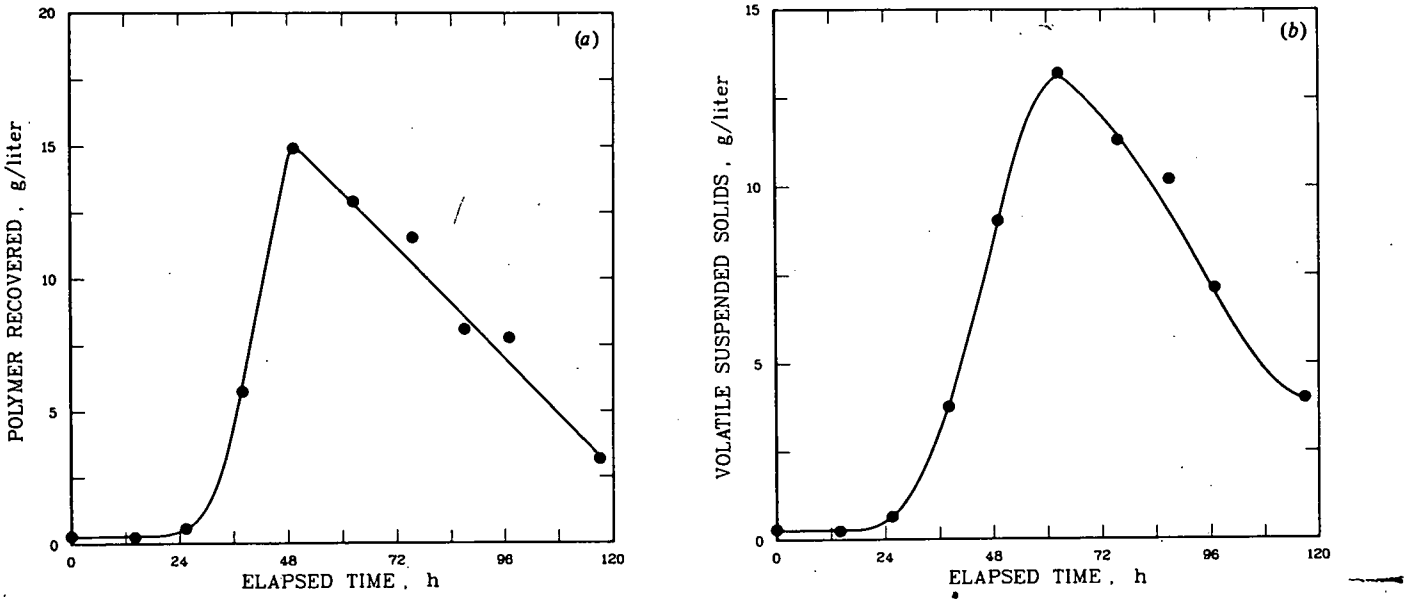


Fig. 1. (a) Polymer production by *Sclerotium rolfsii* ATCC 15206. (b) Culture biomass during polymer production.

As shown in Fig. 2a, culture viscosity increases after the peak in polymer production. This is probably due to the increasingly filamentous nature of the fungus. As shown earlier by Ferguson and Westover (1969), Westover and Ferguson (1969), and in Fig. 2b, culture viscosity is increased substantially by neutralization, heating, and blending.

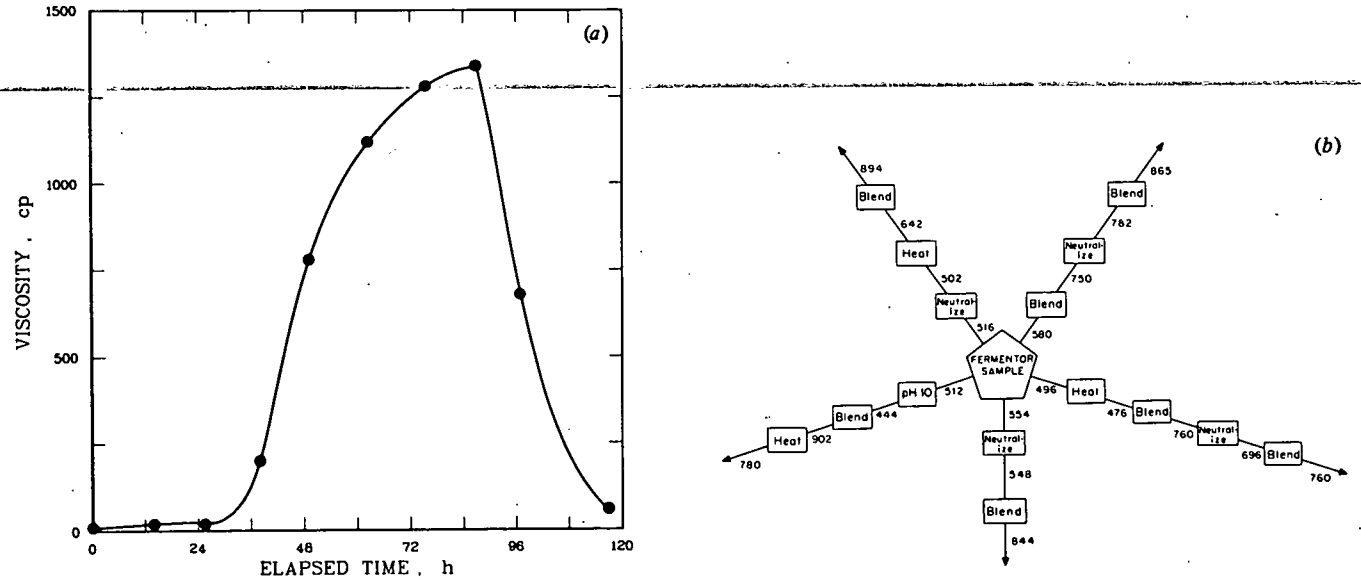


Fig. 2. (a) Broth viscosity during polymer production measured at 25° C. (b) Changes in broth viscosity with treatment. Treatment: blend - 60 s at low speed, Waring blender; heat - 90° C for 30 min; neutralize - to pH 6.5 to 7.0. Viscosities in centipoise, measured at 60 rpm, Brookfield LVT, spindle 3, 25° C.

## SOLUTION PROPERTIES

Fig. 3a shows variation in viscosity with concentration, at 25 C, of commercial xanthan (Xanflood) and scleroglucan in both distilled water and 0.05 *M* NaCl. Scleroglucan shows less viscosity change with addition of NaCl than does xanthan. At concentrations of <1 g/L, in distilled water, Xanflood is a more effective thickener than scleroglucan; otherwise, scleroglucan is slightly more effective. As might be expected from the structure, xanthan evinces cation bridging with concomitant changes in viscosity and shear response; scleroglucan, without carboxyl groups, does not. Scleroglucan has a slightly lower relative decrease in viscosity with increasing temperature than does Xanflood, as shown in Fig. 3b.

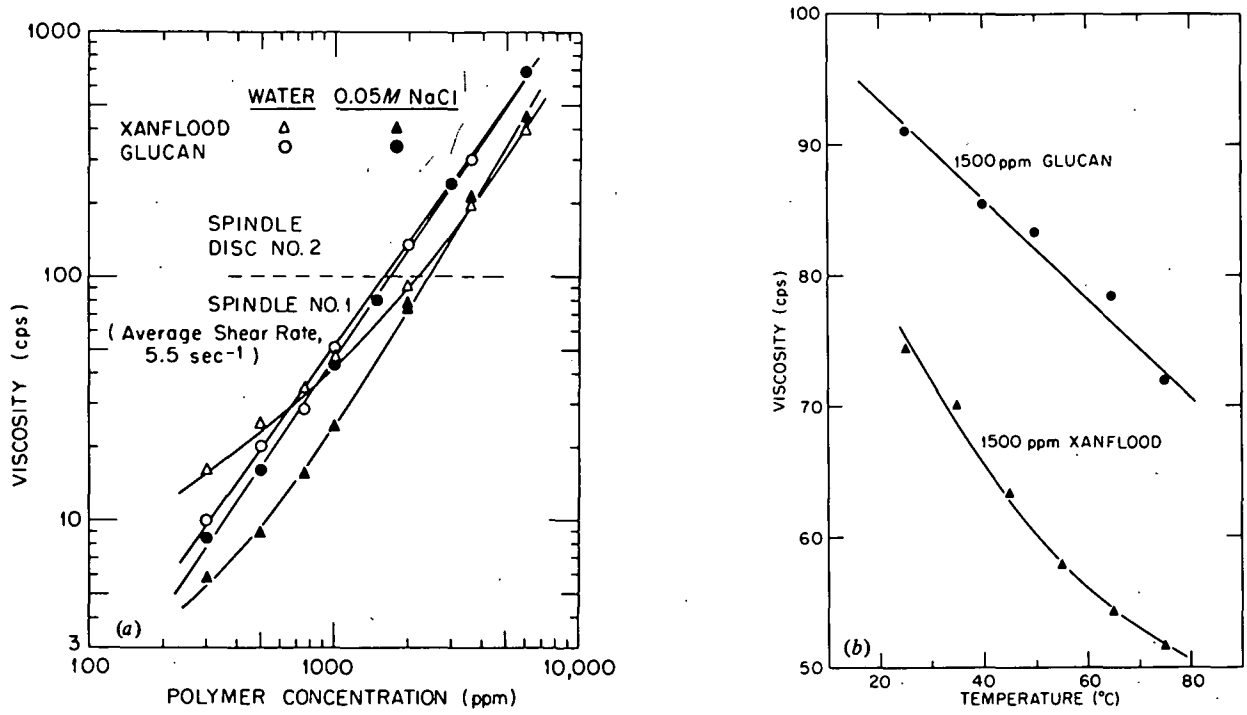


Fig. 3. (a) Viscosities of solutions of Xanflood and scleroglucan, measured at 25° C, Brookfield LVT, spindles 1 and 2. (b) Changes in viscosities of 1500 ppm solutions of Xanflood and scleroglucan with temperature.

## PURIFICATION

Industrial scleroglucan preparation includes neutralization, filtration, and alcohol precipitation (Rogers 1973). These steps are followed by polymer drying and grinding. At the use site, the polymer is resuspended under high shear, and possibly filtered prior to use. Where a liquid product could be shipped, the alcohol precipitation and solids handling steps could be omitted; however, shipping a product at a concentration of <1 %w/vol could be impractical. Diatomaceous earth (DE) filtration is currently used for scleroglucan purification. Disadvantages of DE filtration include partial removal of polymer along with biomass, production of a large mass of material difficult to dispose, and adulteration of biomass which could find byproduct use.

Tangential-flow methods, in which filtrate is removed normal to the fluid flow, appeared to be potential replacements for DE filtration. These methods, which include microscreening, axial filtration, and cross-flow filtration, produce a concentrated solids stream and a relatively clean effluent stream.

The use of a conventional microscreen was also investigated by Cravens and colleagues (1979) to separate *Sclerotium rolfsii* biomass from culture broth. Removal efficiencies obtained with 6 $\mu$m polyester media are about 80%, from Fig. 4a. No significant polymer rejection occurred at the low ($5 \times 10^3$ Pa) pressure used. Polish filtration of the microscreen effluent using the axial filter and the Gelman cross-flow cartridge with 1.2 $\mu$m Acropor type AN media improved the fluxes obtained in plugging tests run on the filtrates as shown in Fig. 4b. Energy and capital costs for three types of biopolymer purification are shown in Table 1.

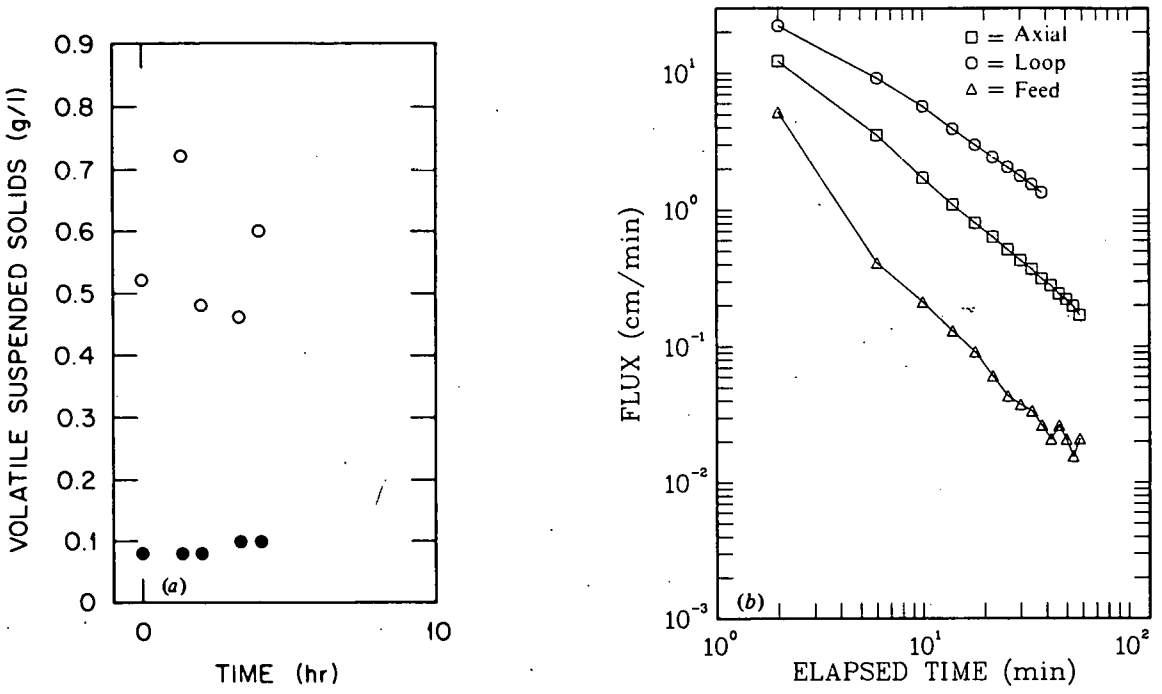


Fig. 4. (a) Volatile suspended solids removal by 6$\mu$m microscreen. (o) influent and (●) effluent. (b) Polish filtration of microscreen effluent. (△) microscreen effluent, (o) microscreen effluent polished using Gelman Instruments cross flow filter cartridge with 1.2 $\mu$m Acropor AN media, and (□) axial filter with 1.2 $\mu$m Acropor AN media mounted on rotor.

TABLE I Cost and energy of alternative processes for a 1,000 kg/day ($264 \times 10^3$ L/day) pilot installation.

| Process | Cost, $10^3$ $ | Power, hp |
|---|---|---|
| DE filter | 155 | 180 |
| Centrifuge[1] | 107 | 40 |
| Microscreen[2] | 80 | 8 |

[1]Maximum power demands up to 60 hp. [2]Polishing step required.

The filtration work also extended to cover axial filtration and circulating cross flow loop filtration, as described by Kraus (1974). Although this work was performed on *Sclerotium glucanicum* NRRL 3006 (Griffith, Tanny, and Compere 1979), the results are applicable to other *Sclerotium* strains. Permeate rates obtained using the axial filter to separate diluted culture broth from biomass are presented in Fig 5a. When a 125 $\mu$m screen was used, permeate rates were essentially constant as shown in Fig. 5a. No significant polymer was rejected. As measured by volatile suspended solids determinations, about 90% of the biomass was removed. With the axial-filter rotor covered with 5 $\mu$m Acropor Type AN media, the flux declined, and some polymer was rejected.

The fluxes obtained when the axial filter and the Gelman cross-flow cartridge were used to polish-filter the screened filtrate from the axial filter are shown in Fig. 5b. For comparison results obtained with a 142 mm flat-plate filter were included. Permeate rates were higher than those of a flat plate filter. With the exception of the flat-plate filter and the highest pressure cross-flow filtration run, little polymer rejection occurred.

## ENZYME DEGRADATION

Controlled enzyme degradation of scleroglucan has been used for two purposes: elucidation of polysaccharide structure and improvement of solution properties. Since its demonstration by Reese and Mandels (1959), the exolaminarinase of *Sporotrichum dimorphosporum* QM 806 has been used for controlled hydrolysis of polysaccharides with laminarin backbones. Halleck (1967) used this technique to demonstrate the scleroglucan structure. Compere and co-workers

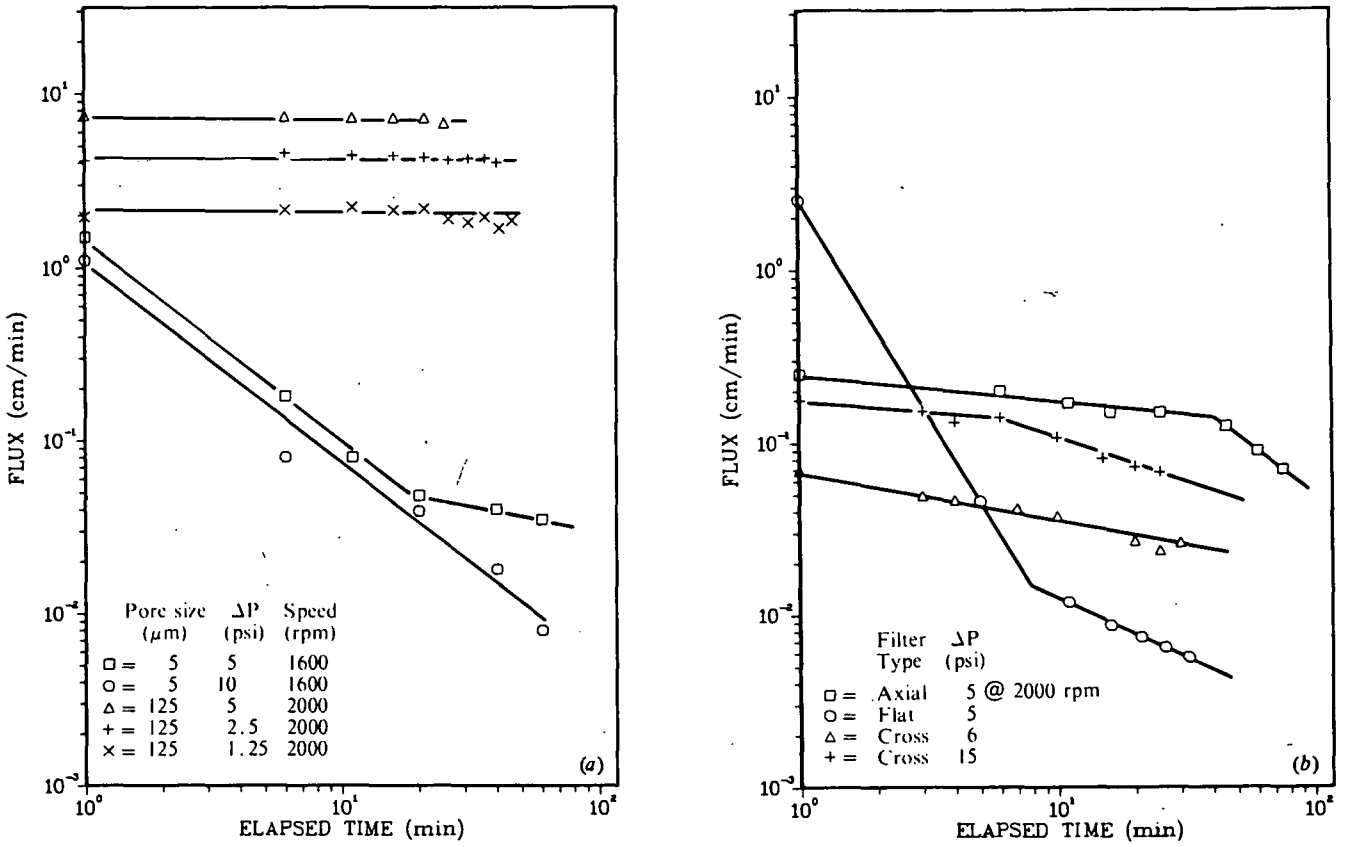


Fig. 5. (a) Permeate rates obtained with axial filter, no prescreening. (b) Permeate rates obtained with Acropor type AN 5μm membrane polishing axial filter effluent from 125 μm screen.

(1979) have investigated pH and temperature optima for scleroglucan and laminarin exohydrolysis. Endolaminarinases, however, by virtue of making mid-chain breaks, can be used to decrease the average size of polysaccharide chains. As shown in Fig. 6, this can provide for flux increases of nearly an order of magnitude through a porous body, in this case, a filter paper, without a major decrease in viscosity (Griffith, Compere, and Crenshaw 1980). This technique is also effective with xanthan.

## POTENTIAL USE CONSIDERATIONS

We have been concerned primarily with use of scleroglucan in enhanced oil recovery. The major consideration for this use is the dependable provision of high viscosity biopolymer which has maximum flux through the very small, often around 1μm diameter, reservoir pores. Even field pilot equipment is large (typical fermenter capacity for a small pilot experiment could be around 100 $m^3$), with a full size field operation 10 to 100 times larger. Alternatively, around $10^3$ kg of dried biopolymer per day would be required for a field pilot test.

On a large scale, it is probable that on or near-site synthesis will be used for two reasons: alcohol makeup for polymer precipitation is often the single largest production cost and polymer precipitation causes the formation of aggregates difficult to dissolve or resuspend. Another major consideration is biomass removal and disposal. Use of biomass as an animal feedstock would be desirable, and might well be feasible with fungal biopolymers, such as scleroglucan (Pillsbury 1963 to 1967). However, use of diatomaceous earth as a filtration medium and as body feed could add substantially to the cost of field synthesis, both in material expense and in disposal cost. Use of a fermentation operated continuously across an extended injection period could also decrease costs by decreasing capital equipment requirements: a continuous scleroglucan fermentation might be only 1/6 of the corresponding batch fermentation volume (Baldwin and co-workers 1979). The low fermentation pH, often 1.5 to 3, of many *Sclerotium* strains, together with the simple medium, makes it relatively easy to perform long-term continuous fermentations without contamination.

In summary, it appears that scleroglucan biopolymer could, under appropriate conditions, be suitable for use in tertiary oil recovery.

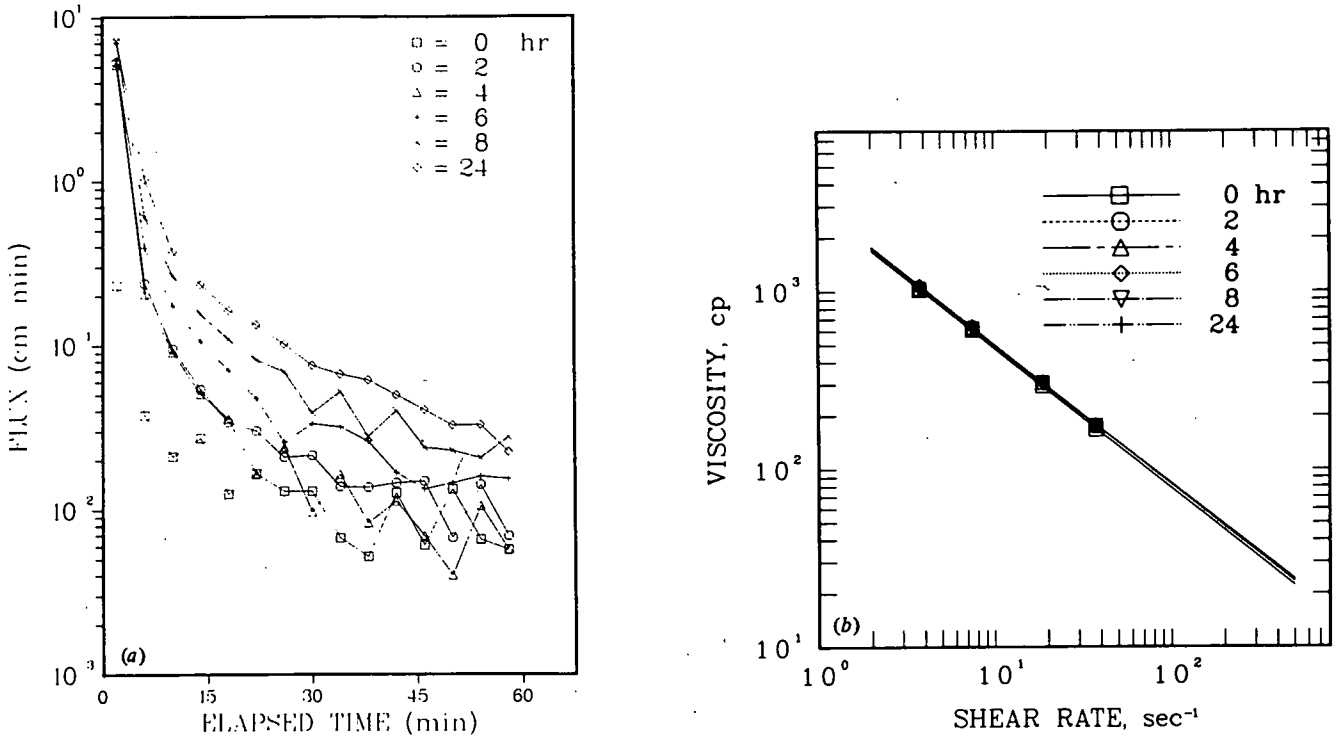


Fig. 6. (a) Flux of 0.5 g/L scleroglucan solution treated with *Rhizopus arrhizius* QM 1032 endolaminarinase. (b) Changes in viscosities of treated solutions measured at 25° C.

## ACKNOWLEDGEMENTS

Biopolymer separations work was supported by Division of Chemical Sciences, Basic Energy Sciences, and all other research by Division of Fossil Fuel Extraction, U.S. DOE, under contract W-7405-eng-26 with Union Carbide Corp.

## REFERENCES

Akstinat, M. H. (1980). Polymers for enhanced oil recovery in reservoirs of extremely high salinities and high temperatures. *Soc. Pet. Engr. J.* (USA) paper 8977.

Baldwin, W. H. and co-workers (1979). *Chemicals for enhanced oil recovery*. U.S. DOE report BETC/W26-4.

Compere, A. L. and co-workers (1979). *Chemicals for enhanced oil recovery*. U.S. DOE report BETC/W26-8.

Cravens, J. B. and co-workers (1980). Innovative enhanced oil recovery separations techniques. *Proceedings, Conference on Energy Optimization of Water and Wastewater Mangement*, New Orleans, La.

Ferguson, R. B., and J. D. Westover (1969). *Aqueous suspension of fungal polysaccharide subjected to shearing forces*. U.S. patent 3,436,311.

Griffith, W. L., and A. L. Compere (1978). Production of a high viscosity glucan by *Sclerotium rolfsii* ATCC 15206. *Dev. Ind. Microbiol.* (USA), 19, 609-617.

Griffith, W. L., G. B. Tanny, and A. L. Compere (1979). Evaluation of tangential filtration methods for the recovery of scleroglucans. *Dev. Ind. Microbiol.* (USA), 20, 743-750.

Griffith, W. L., A. L. Compere, and J. M. Crenshaw (1980). Modification of biopolymer solution properties by endoglucanohydrolases. *Dev. Ind. Microbiol.* (USA) 451-460.

Kraus, K. A. (1974). Cross flow filtration and axial filtration. *Proceedings 29th Purdue Industrial Waste Conference*, Lafayette, Indiana.

Hallack, F. E. (1967). *Polysaccharides and methods for production thereof*. U.S. patent 3,301,848.

Pillsbury (1963-1967). Unpublished data, food and animal feed uses of *Sclerotium* gum and cells.

Reese, E. T., and M. Mandels (1959). β-D-1,3 glucanases in fungi. *Can. J. Miocrobiol.* (Canada), 5, 173-185.

Rogers, N. E. (1973). Scleroglucan. In R. L. Whistler (Ed.), *Industrial Gums*. Academic Press, New York pp. 499-511.

Westover, J. D., and R. B. Ferguson (1969). *Process for preparing filterable aqueous polysaccharide solutions*. U.S. patent 3,436,346.

Williams, S. A. (1968). *Waterflood process employing thickened water*. U.S. patent 3,373,810.