Final Technical Progress Report

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Project Title: Optimization and Scale-Up of Fermentation Process for Production of Microbial Polysaccharide.

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This grant was awarded to provide for the scale-up of the process of production of a (1-3)- β -D-glucan which is produced by *Cellulomonas flavigena*. One of the goals was to provide sufficient amounts of the polysaccharide polymer to conduct a field test of its usefulness in subterranean permeability modification procedures of enhanced oil recovery.

Abbott Laboratories, Chemical & Agricultural Products Division, Custom Fermentations, North Chicago, IL was selected to provide the fermentation.

During September and October, 1994, fermentations and recoveries were done by Abbott Laboratories, to develop a process to provide at least 400 lbs of the glucan polymer for field testing. Shake flask runs and four fermentation runs were completed. A summary of the fourth fermentation run, conducted in a 40,000 liter fermentor, follows:

I. Fermentation conditions and yields

Corn syrup was used at a concentration of 25 gm/liter to provide the carbon and energy source for the growth of *Cellulomonas flavigena*. The culture was incubated for 72 hrs. It was then cooled to 20 C and prepared for harvest and extraction of the glucan. Table 1 shows the progress of the fermentation and the glucan produced by the bacteria.

Table 1. Growth of *Cellulomonas flavigena* in 40,000 Liter Fermentor Vessel

¹ Age	² OD	³ Glucose	⁴ PCV,%	⁵ NH ₃	⁶ Gel/15 ml	⁷ Glucan
0	0.19	27.7	-	530	-	-
12	1.73	26.3	0.7	391	-	0.12
24	8.64	21.3	3.5	10	-	0.65
36	16.6	16.5	5.5	2	3.7	2.48
48	28.4	11.8	5.4	2	5.3	4.6
60	29.1	6.8	5.3	2	6.1	6.72
72	26.5	4.4	5.5	2	6.6	9.11

¹ Hours incubation at 30C

² Optical density of culture

³ Residual corn syrup(as glucose) in medium

⁴ Packed cell volume (per cent of culture volume)

⁵ Ammonia nitrogen content

⁶ Grams hydrogel producible by 15 ml of culture

⁷ Concentration (gm dry weight) of glucan/liter of culture

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The optical densities reached, amount of glucose used, and glucan produced were what was hoped for on the basis of improvements from first three fermentations.

II. Recovery of (1→3)-β-D-Glucan - Production Scale

Figure 1 illustrates the steps involved in the extraction of the (1→3)-β-D-glucan from the bacteria and the partial purification of the extract. Thirty seven thousand and three hundred (37,300) liter of culture was processed. Table 2 provides the material balance for the procedures depicted in Fig. 1.

Table 2. Material Balance

Material	Kgs	g/l (1→3)-β-D-glucan	Kgs (1→3)-β-D-glucan
Broth	37,300	8.4	313
Cell-glucose slurry	5,700	56.8	324
Dilute extract	17,580	17.5	308
Cell slurry (waste)	3,800	8.8	33
Solution (unfiltered)	13,500	25.9	322
Solution (at load-out)	12,900	24.9	321

III. Field test of suitability of (1→3)-β-D-glucan for subterranean permeability modification.

On October 21, a shipment of 29,000 lbs of an alkaline extract containing more than 2% of the (1→3)-β-D-glucan was delivered from Abbott to a field site (Nelson Lease) in southeast Kansas. The conduct of the field test was supervised by the Tertiary Oil Recovery Project (TORP), University of Kansas. Almost all of the alkaline extract of the glucan was successfully injected into a test well. However, numerous unanticipated technical problems were encountered during the field test. Data are still being collected and analyzed, and final conclusions concerning the outcome of the test have not yet been determined.

The alkaline solution of the (1→3)-β-D-glucan produced by Abbott performed as expected, and gels of good quality were formed. Lab scale research on the use of the polymer in permeability modification is continuing, and we remain optimistic that polymer will have successful applications in enhanced oil recovery procedures.

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Figure 1. Recovery of β -Glucan: Production Scale