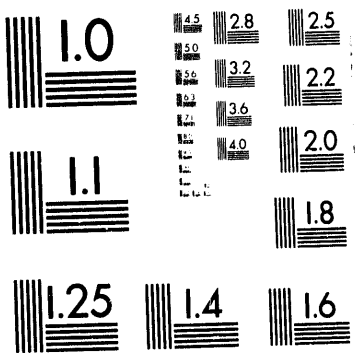
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6

1 of 1

PROJECT STATUS REPORT

October 1, 1992

Report No. 2-4
Reporting Period:
July 1, 1992
September 30, 1992

GRANT NUMBER AND TITLE

Biological Conversion of Synthesis Gas
U. S. Department of Energy No.: DE-FG21-90MC27225

GRANT PERIOD

September 4, 1990 - September 3, 1993

Task 1. Test Plan

Task has been completed.

Task 2. Culture Development

Task has been completed.

Task 3. Mass Transfer/Kinetics Studies

Task has been completed.

Task 4. Bioreactor Studies

A continuous bioreactor system has been used for the conversion of H_2S to elemental sulfur by *Chlorobium thiosulfatophilum*. Although system limitations prevented the optimization of the system, H_2S conversion to elemental sulfur was demonstrated in this CSTR system. Future experiments will be carried out in a CSTR system with continuous sulfur removal.

Technical Discussion

The anaerobic, phososynthetic bacterium *Chlorobium thiosulfatophilum* is able to convert H_2S and COS in synthesis gas to elemental sulfur. The bacterium grows on CO_2 as its carbon source at 30°C. In the absence of

sulfide, the formed elemental sulfur is converted to sulfate. Thus, bioreactor designs must incorporate sulfur removal as an integral part of the bioprocess.

In this initial study, *C. thiosulfatophilum* was used to convert H_2S to elemental sulfur in a continuous stirred tank reactor (CSTR) with continuous gas and liquid feed. Sulfur removal was not part of this initial system design, but will be an added feature in future work. The gas used in this study contained 2.52 percent H_2S, 10.00 percent CO_2, 14.99 percent CH_4 (as a tracer) and 72.49 percent He. The liquid flow rate to the 1380 mL reactor volume ranged from 10.8-23.6 mL/min and was a variable in the study. The initial gas flow rate was 11.6 standard mL/min, although it was also changed twice during the study. The temperature was maintained at 31°C and the agitation rate was held at 200 rpm in the Bioflo reactor.

Cell density was monitored by the chlorophyl method and gas composition was monitored by gas-solid chromatography. Light at 2200 lux was supplied using two 40W tungsten light bulbs on the outside of the glass reactor vessel. The light intensity was measured at the front of the reactor vessel, but was not uniform around the reactor.

Figures 1 and 2 show cell concentration and H_2S conversion measurements in the CSTR with *C. thiosulfatophilum*. Several changes were made during the course of the experiment as is noted on the two figures. Complete H_2S conversion was noted at a gas flow rate of 11 standard mL/min (2.09 hr gas retention time). The gas conversion fell at increased gas flow rates, mainly due, however, to temperature control problems.

Two problems were noted in operating the system. First, the use of external light in the temperature controlled room caused a temperature

increase inside the reactor, thus lowering the H_2S conversion and cell concentration. Secondly, a yellowish color was observed in the reactor after several hours of operation due to the accumulation of sulfur in the reactor. Both of these problems will be addressed in future studies.

A chiller will be installed in the cooling water line to help control the cooling water temperature. Light intensity will be controlled by installing a variable autotransformer with the lights.

Sulfur will be removed from the system by employing a separatory funnel and a recycle loop between the reactor and the separator. The cell culture with suspended sulfur will be pumped into the separatory funnel. Another pump will recirculate the cell culture back to the reactor. An overflow line on the funnel will take care of the flow imbalances between the pumps. Settled sulfur will be removed from the bottom of the funnel as needed.

Task 5. Limiting Conditions/Scale-up

As a first step in scaling up the bioprocesses for sulfur gas removal and shift conversion, the effects of cell concentration on density, viscosity and surface tension were determined. Logarithmic functions for density as a function of inverse absolute temperature were found for water, *R. rubrum* cells in medium and *C. thiosulfatophilum* cells in medium, although a different relationship was found for each of the three systems. A single logarithmic function was found for viscosity as a function of inverse absolute temperature for the three systems. No simple relationship was found for surface tension as a function of temperature.

Technical Discussion

Little is known about the effects of bacterial cells and medium on physical properties. In fact, various literature references show a variety of

effects on physical properties, including both decreases and increases with cell concentration. In order to accurately scale-up bioprocesses, information must be gathered on the effects of bacterial type, cell and medium concentration, and temperature on physical properties. Studies were thus carried out using both *Rhodospirillum rubrum* and *Chlorobium thiosulfatophilum* at various cell concentrations in measuring the effects of temperature on density, viscosity and surface tension.

R. rubrum and *C. thiosulfatophilum* were grown in continuous culture to various cell concentrations by varying the light intensity to the reactor. A New Brunswick Bioflo II-C fermenter was used as the CSTR in each bacterial study. The media for *R. rubrum* and *C. thiosulfatophilum* were as described previously. The operating conditions for the *R. rubrum* system were as follows:

Liquid Flow Rate:	7.08, 3.54 mL/hr
Gas Flow Rate:	3.95 Standard mL/min
Gas Composition:	9.95% CO_2, 20.04% H_2, 15.27% Ar (tracer), 54.75% CO
Reactor Temperature:	30°C

The operating conditions for the *C. thiosulfatophilum* system were as follows:

Liquid Flow Rate:	16.2 mL/hr
Gas Flow Rate:	8.4 mL/min
Gas Composition:	2.52% H_2S, 10.00% CO_2, 14.99% CH_4 (tracer), 72.49% He
Reactor Temperature:	31°C

Density (specific gravity) was measured using the ASTM standard test for specific gravity of creosote fractions and residues (D-369-84)[1], which is identical in substance to the Standard Method of Determination of the Specific Gravity of Distillation Fractions. Viscosity and surface tension were measured using the technique presented by Findlay[2]. An Ostwald viscometer was used for viscosity measurement and a 250 mm borosilicate glass capillary tube was used to measure surface tension.

The effects of cell concentration for both *R. rubrum* and *C. thiosulfatophilum* on density at 25°C is shown in Figure 3. The available cell concentration range for *R. rubrum* was 400-1800 mg/L, while the available cell concentration range for *C. thiosulfatophilum* was only 0-700 mg/L. *R. rubrum* obviously is able to grow to higher cell densities in the CSTR under similar conditions. This phenomenon does not necessarily mean that the global rate of conversion is greater for *R. rubrum*. As is noted in the figure, the density for the *R. rubrum* system was less than the density for *C. thiosulfatophilum*. The density for *R. rubrum* ranged from about 1.000 - 1.001 g/mL. The density for the *C. thiosulfatophilum* system ranged from 1.002-1.003 g/mL, increasing gently with cell concentration.

As is shown in Figure 4, the density of water, *R. rubrum* and *C. thiosulfatophilum* each decreased with increasing temperature. The density of *R. rubrum* was the highest (at both cell concentrations shown), followed by *C. thiosulfatophilum*, followed by water. The density of each of these systems may be fit by a logarithmic function with inverse absolute temperature (see Figure 5), using the equation:

$$\ln \rho = \ln \alpha + \frac{\beta}{T} \qquad (1)$$

Task 6. Economic Evaluations

No work was scheduled on this task during the reporting period.

M. D. Ackerson
Principal Investigator

Edgar C. Clausen
Principal Investigator

J. L. Gaddy
Principal Investigator

LITERATURE CITED

1. ASTM, "Standard Test Method for Specific Gravity of Creosote Fractions and Residue, D 369-84, 1989.

2. Findlay, A., *Practical Physical Chemistry*, 4th ed., Longmans Green Company, New York, pp. 70-81, 1923.

Table 1

Relating Density to Temperature for *R. rubrum* and *C. thiosulfatophilum*

$$\ln \rho = \ln \alpha + \beta/T$$

System	α	β
Water (literature)	0.9113	26.786
R. rubrum (950 mg/L)	0.9153	27.309
R. rubrum (1750 mg/L)	0.9582	13.395
C. thiosulfatophilum (525 mg/L)	0.9059	29.720

temperature range: 22-37°C

Table 2

Relating Viscosity to Temperature for *R. rubrum* and *C. thiosulfatophilum*

$$\ln \mu = \ln \gamma + \frac{\delta}{T}$$

System	γ	δ
Water (literature)	1.19 E-03	1971.73
Water (measured)	3.53 E-03	1647.15
R. rubrum (950 mg/L)	1.52 E-03	1907.56
R. rubrum (1750 mg/L)	2.82 E-03	1720.94
C. thiosulfatophilum (525 mg/L)	1.36 E-03	1936.38

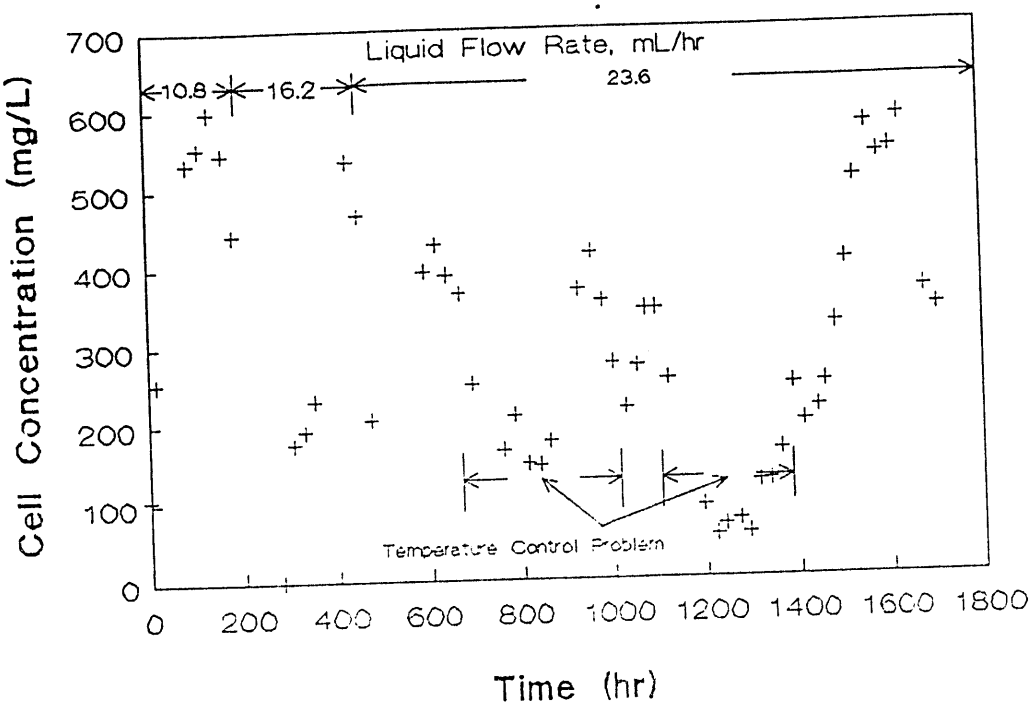

Figure 1. Cell Concentration Measurements for H_2S Conversion by *C. thiosulfatophilum* in the CSTR.

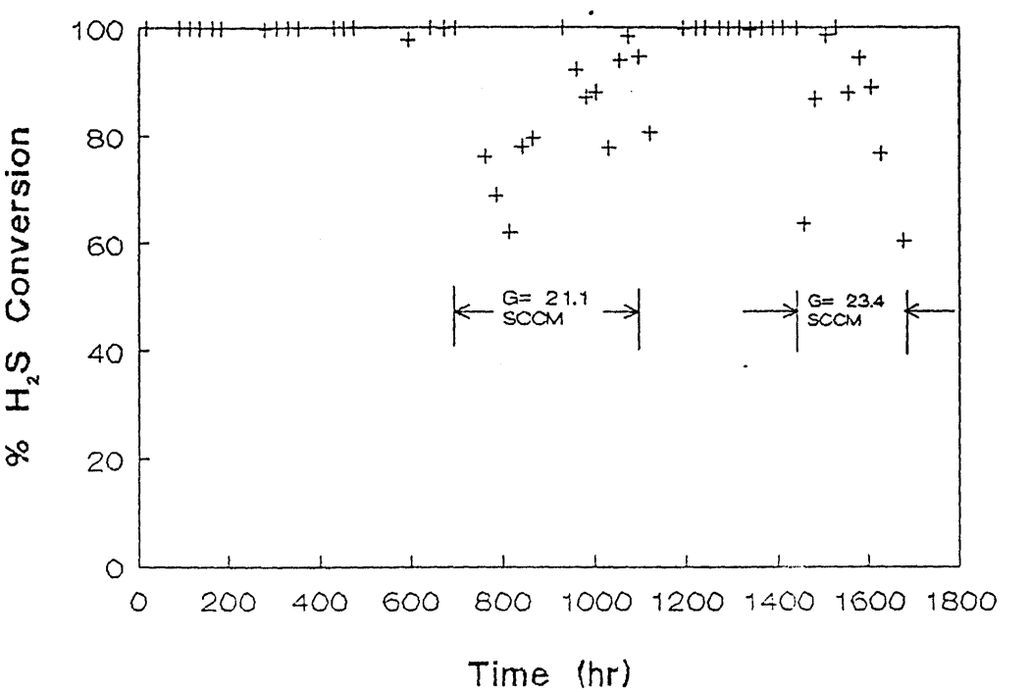

Figure 2. H_2S Conversion Measurements for H_2S Removal by *C. thiosulfatophilum* in the CSTR.

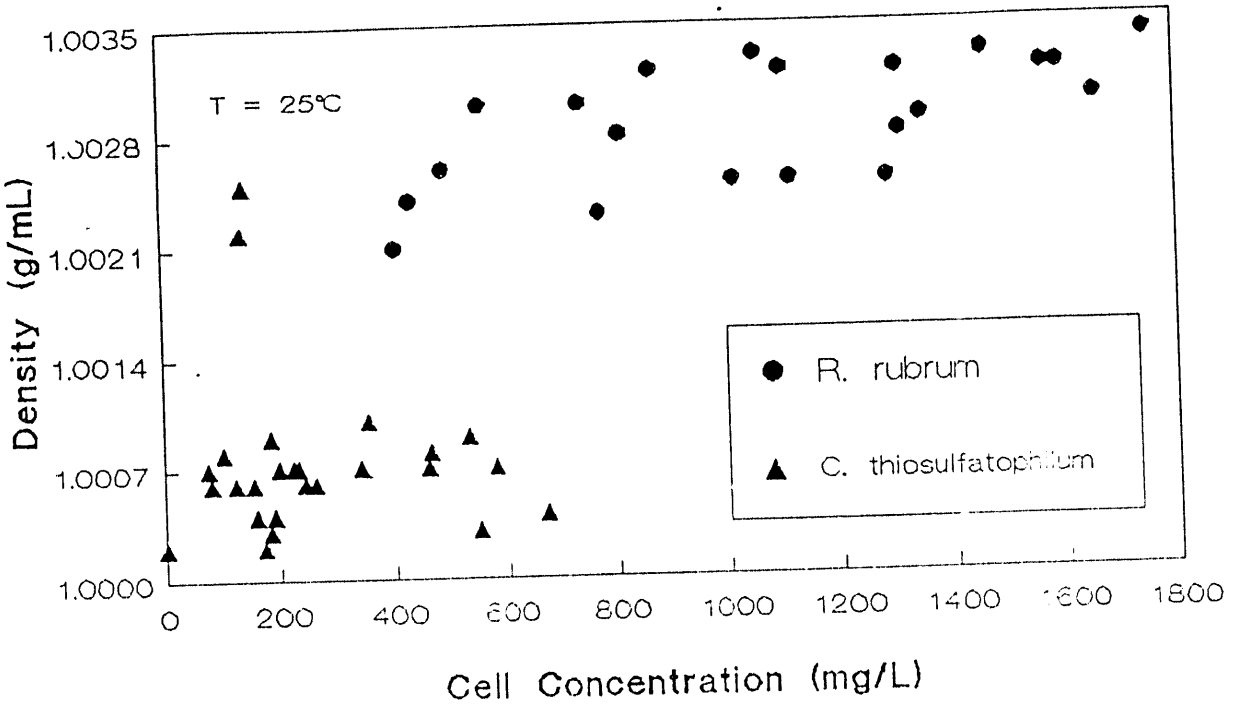

Figure 3. The Effect of Cell Concentration on Density for *R. rubrum* and *C. thiosulfatophilum*.

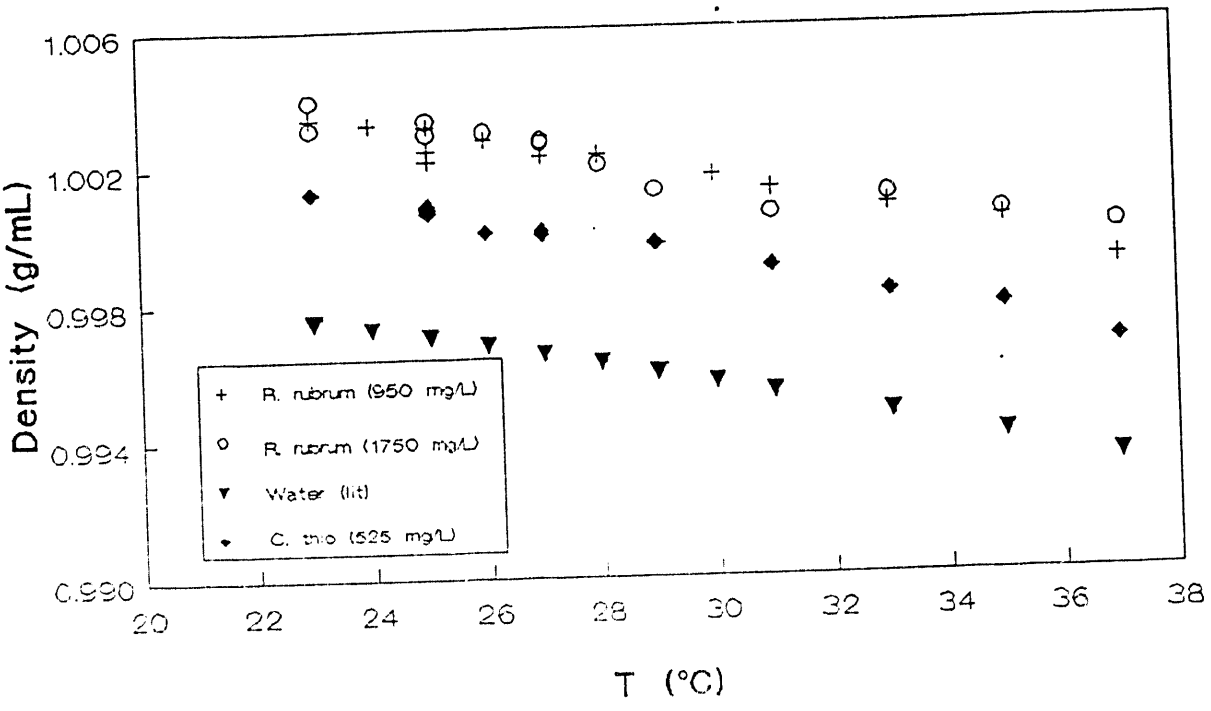

Figure 4. The Effect of Temperature on Density of Water, *R. rubrum* and *C. thiosulfatophilum*.

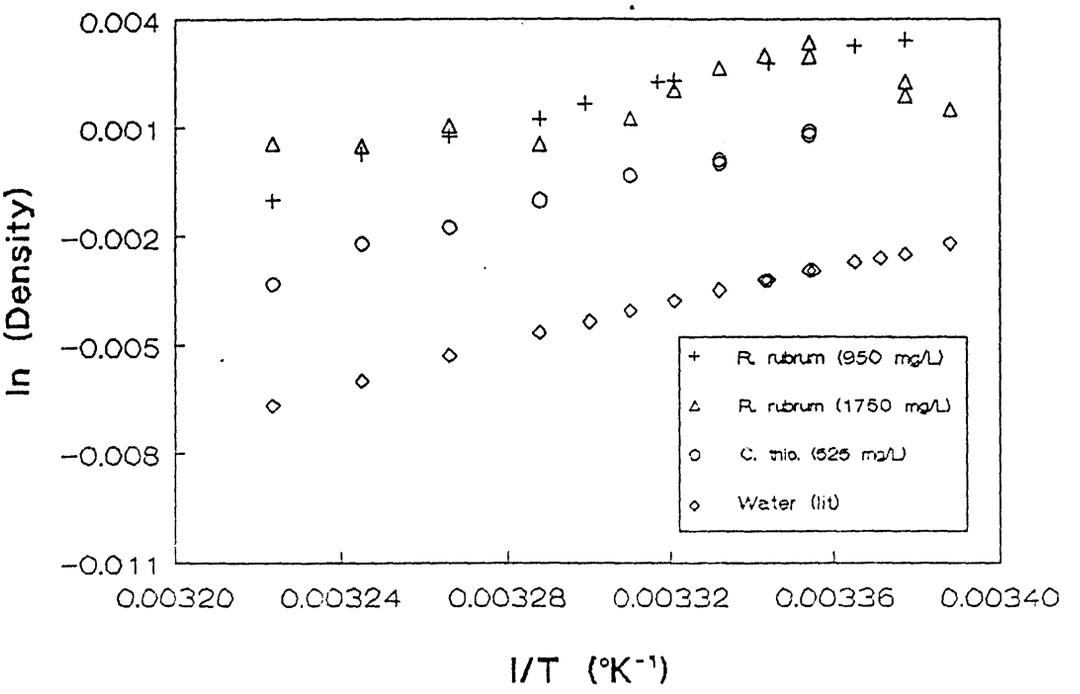

Figure 5. Test of Logarithmic Relationship Between Density and Inverse Absolute Temperature.

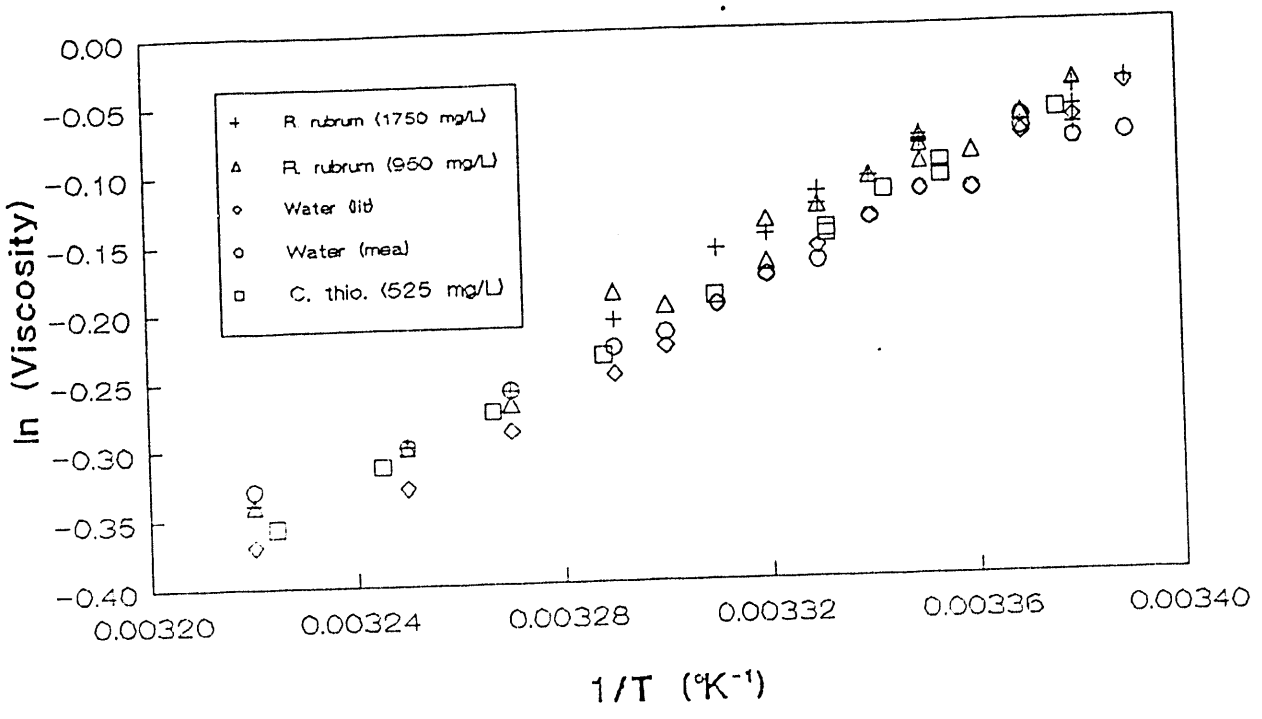

Figure 7. Test of Logarithmic Relationship Between Viscosity and Inverse Absolute Temperature.

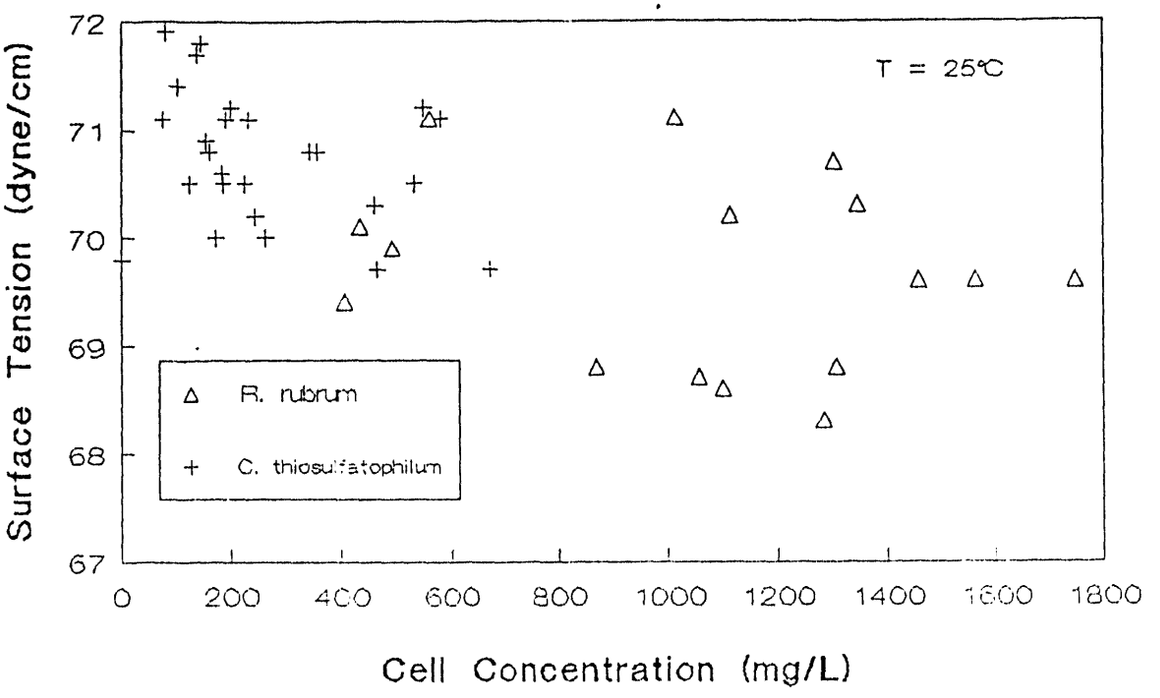

Figure 8. The Effect of Cell Concentration on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*.

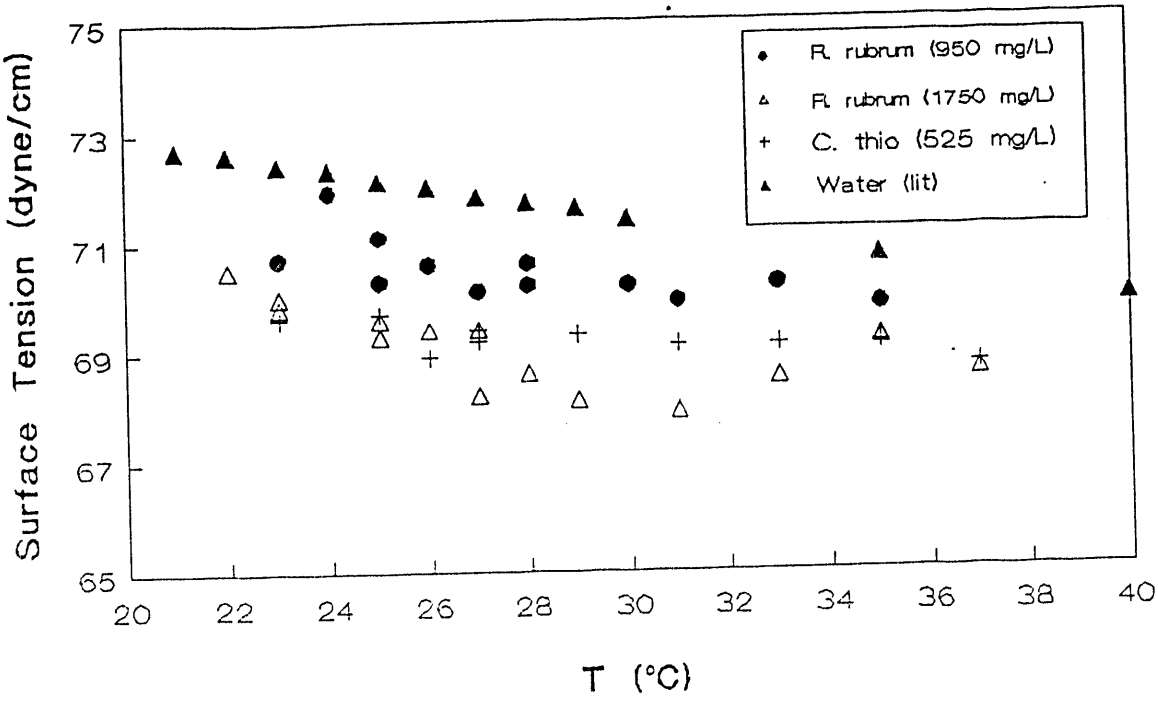

Figure 9. The Effect of Temperature on Surface Tension for *R. rubrum* and *C. thiosulfatophilum*

DATE
FILMED
4 / 4 / 94
END