Task 1. Test Plan

Task has been completed.

Task 2. Culture Development

The anaerobic bacterium *Rhodospirillum rubrum* has been chosen for catalysis of the biological water gas shift reaction. Two bacteria, *Chlorobium thiosulfatophilum* and *Chlorobium phaeobacteroides*, are being evaluated as candidates for H2S conversion to elemental sulfur. Since these latter two organisms both grow and convert H2S in batch culture using standard basal medium, the choice of a suitable bacterium must be made in consideration of specific growth and uptake rates (see Task 3). Produced elemental sulfur stability against further oxidation to sulfate, and minimal use of H2 as a producing agent must also be considered.

Task 3. Mass Transfer/Kinetic Studies

The effects of temperature on the performance of *R. rubrum* were evaluated. It was found that the cell concentration was highest at
temperatures of 25 and 30°C, and that the specific uptake rate was highest at temperatures of 30, 32 and 34°C. No growth was observed at 37°C. Also, temperature did not affect the yield of H₂ from CO. Thus, R. rubrum may be used for biological rates gas shift at any temperature between 30 and 34°C, although growth is maximized at lower temperatures. Preliminary studies with C. thiosulfatophilum showed rapid utilization of H₂S from the gas and liquid phases with subsequent production of elemental sulfur. Elemental sulfur production interfered with cell concentrations measurements, although a technique has been developed to rectify this problem.

TEMPERATURE EFFECTS ON R. RUBRUM

The anaerobic photosynthetic bacterium Rhodospirillum rubrum has been chosen from among several members of the Rhodospirillaceae family to be the biocatalyst for the biological water gas shift reaction. R. rubrum requires light for growth on various organic carbon sources (although not CO) and catalyzes the reaction:

\[
\text{CO} + \text{H₂O} \rightarrow \text{CO₂} + \text{H₂} \quad (1)
\]

The yield of H₂ from CO and water by R. rubrum was found to be 0.87 mol/mol, which is 87 percent of the theoretical yield of Equation (1). Cell yields on acetate and ammonia were found to be 0.42 g/g and 13 g/g, respectively. Mass transfer and kinetic parameters have been obtained for R. rubrum in batch culture. In simple 150 mL serum stoppered bottles the mass transfer coefficient, \(K_{La}\), was calculated to be 9.7 - 14.4 h⁻¹ when utilizing an agitation rate of 150 rpm. The specific uptake rate of CO, \(q\), for R. rubrum was correlated by a Monod type equation modified for substrate inhibition:

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where $P_{CO}^G$ represents the gas phase partial pressure of CO. In examining Equation (2), it is seen that the maximum specific uptake rate, $q_m$ is 0.055 mol/g•hr and the Monod saturation constant, $K_p$, is 0.45 atm. The substrate inhibition constant, $W$, has a value of 0.106 atm, indicating that CO significantly inhibits CO uptake. In fact, the highest specific uptake rate found from Equation (2) is 0.011 mol/g•h, occurring at a partial pressure of 0.218 atm.

A Monod-type relationship was also obtained for light-limited growth on acetate as the carbon source. The specific growth rate, $\mu$, was modeled in terms of the incoming light intensity, $I_0$, by the equation:

$$\mu = \frac{0.052 I_0}{140 + I_0}$$  \hspace{1cm} (3)$$

In analyzing Equation (3), it is seen that the maximum specific growth rate, $\mu_m$, is 0.052H^{-1} and the Monod saturation constant, $K_p'$ is 140 lux. Thus, at a light intensity of 2660 lux, the specific growth rate is 95% of the maximum.

The models of Equations (2) and (3) were obtained at the published optimum temperature of 30°C. However, from observing the performance of other microorganisms as a function of temperature it was often found that the reported optimum was, in fact, not really an "optimum" temperature, but instead a "preferred" temperature of the two or three temperatures tested. Thus, the effects of temperature on growth and uptake by *R. rubrum* need to be
studied to establish true temperature optima in terms of maximizing the specific growth and uptake rates. Furthermore, since biological water gas shift may not be carried out under strictly isothermal conditions in industrial reactors, the effect of temperature on the performance of the bacterium needs to be well understood.

The purpose of this study is to present results on the effect of operating temperature on the performance of *R. rubrum*. The operating temperature was varied between 25 and 37°C in an effort to quantify the effects of temperature on the specific growth rate, the yield of H₂ from CO and specific uptake kinetics.

**MATERIALS AND METHODS**

The composition and preparation procedure, as well as the experimental and analytical procedures used, have been previously reported. Experiments were conducted at temperatures of 25, 30, 32, 34 and 37°C. The seed cultures for each of these experiments were grown at the temperatures of the proposed experiments except for 37°C. No growth was observed at 37°C. Each of the temperature studies included three experiments at various initial CO partial pressures ranging from 0.9 to 1.5 atm.

**RESULTS AND DISCUSSION**

Five temperatures were considered in the experimental study: 25, 30, 32, 34 and 37°C. As was mentioned previously, the reported "optimum" temperature was 30°C. Thus, temperatures both above and below the optimum were considered. The lowest temperature chosen was 25°C since it is well known that reaction rate generally increases with temperature, so that the specific rates of both growth and uptake would be expected to be quite low at temperatures below 25°C. The maximum temperature studied was 37°C since no
growth was obtained at this temperature. Small temperature increments between 30 and 37°C were used since it was expected that these small increases in temperature might significantly affect bacterial growth and CO uptake.

The effects of temperature on cell concentration in the exponential growth phase is shown in Figure 1, where the natural logarithm of the cell concentration is plotted as a function of time for four temperatures. Again, no growth was observed at 37°C. The experimental growth region was chosen for analysis in order to eliminate differences in inoculum and culture acclimation effects often present in the lag phase. In the exponential phase, the plot of ln X as a function of time should yield a straight line. As is noted in Figure 1, three experiments (corresponding to three initial partial pressures) were run at each temperature. In analyzing the data, it is seen that the highest cell concentrations (ln X) occurred at the lower temperatures of 25 and 30°C. Slightly depressed growth occurred at 32 and 34°C. If a single straight line is drawn through the truly exponential region of the data, the slope of the line is found to be 0.035 h⁻¹, which is equal to the specific growth rate, μ. Thus, any temperature between 25 and 34°C is suitable for the growth of R. rubrum.

Figure 2 shows the calculation of the yield of H₂ from CO, Yp/s, for the four temperatures. Previous results have shown a product yield of 0.87 mol/mol at 30°C, and the theoretical yield is 1.0 mol/mol from Equation (1). The slope of the line in Figure 2 is 0.96, which is 96% of theoretical. Also, there is seen to be no effect of temperature on the product yield. No explanation is offered for the increase in the yield in the temperature study compared with previous results at 30°C.
The general equation for specific uptake rate as a function of $p_{CO}^G$ considering substrate (CO) inhibition is:

$$q = \frac{q_m p_{CO}^G}{K_p + p_{CO}^G + \left(\frac{p_{CO}^G}{q_m}\right)^2}$$  \hspace{1cm} (4)

Equation (4) may be rearranged to yield an equation of quadratic form:

$$\frac{p_{CO}^G}{q} = K_p \frac{p_{CO}^G}{q_m} + \frac{p_{CO}^G}{q_m} + \frac{\left(\frac{p_{CO}^G}{q_m}\right)^2}{q_m}$$  \hspace{1cm} (5)

Thus, a plot of $\frac{p_{CO}^G}{q}$ as a function of $p_{CO}^G$ should yield a curve of quadratic form with an intercept of $K_p/q_m$, a slope of $1/q_m$ and a curvature of $\frac{1}{q_m^2}$.

A plot of $p_{CO}^G/q$ as a function of $p_{CO}^G$ is shown in Figure 3 for the four temperatures. As is noted, a single curve is essentially obtained for temperatures of 30, 32 and 34°C, indicating that the specific uptake rate is the same at these temperatures. (An exception to this observation is one set of data at 34°C which gives lower uptake rates and thus higher values of $p_{CO}^G/q$. Also, the rather steep curvature for one set of data at 30°C is due to the lag phase). At 25°C, it is seen that the specific uptake rate is lower for all three data sets, indicating that 25°C would be an inappropriate temperature for catalyzing the reaction of Equation (1).

Thus, the growth of R. rubrum may be satisfactorily carried out at 25 and 30°C, while uptake and thus conversion of CO best occurs at temperatures of either 30, 32 or 34°C. The reported optimum of 30°C is well suited for both growth and CO uptake. More importantly, small upward variations in temperature in industrial processes will not negatively affect CO uptake or H₂ production.
**H₂S CONVERSION TO ELEMENTAL SULFUR**

As was mentioned in Task 1, rate studies are being carried out in comparing *C. thiosulfatophilum* and *C. phaeobacteroides* for H₂S conversion to elemental sulfur. Both bacteria are photosynthetic and anaerobic, and both bacteria are able to grow on CO₂ in basal medium in converting H₂S to elemental sulfur. Also, both bacteria appear to utilize H₂ and elemental sulfur as sources of reducing power once the H₂S concentration has fallen to near zero.

Two problems have been encountered in developing rate expressions (specific uptake rate and specific growth rate) for the two bacteria:

1. difficulties in closing the sulfur material balance; and
2. difficulties in measuring cell concentrations in the presence of deposited elemental sulfur.

The sulfur material balance must account for a host of sulfur species, including H₂S (g), H₂S (l), HS⁻(l), S²⁻(l), SO₄²⁻(l) and S(s). In addition, it has been found that H₂S attacks the butyl rubber stoppers used in batch bottle experiments.

The purpose of this section of the report is to present results showing progress in closing the sulfur material balance, to present two methods for measuring cell concentration in the presence of sulfur and to present experimental data for the growth of *C. thiosulfatophilum* and subsequent utilization of H₂S. *C. thiosulfatophilum* is used in these initial results, but the results should be directly applicable to *C. phaeobacteroides*. 
MATERIALS AND METHODS

Equipment

All experiments were carried out in batch 150-mL glass serum bottles as previously described. The modified New Brunswick Model G25 shaker incubator was used for incubation at 30°C. Tungsten light was supplied in excess.

H₂S Balance Experiments in Water

As was mentioned previously, a sulfur balance on the batch system has been complicated due to problems of H₂S attack on butyl rubber stoppers. A host of techniques have been employed to counteract this problem. After several trials with different bottles and stoppers, it was finally decided to use thick butyl rubber stoppers since they were more effective against gas leaks when sampling. However, some small amount of reaction between H₂S and the rubber was noted.

Two experiments were carried out with 5 and 10 mL of gaseous H₂S in 75 mL of water in an effort to monitor the total sulfur concentration in the system. In this H₂S-water system, sulfur was present only in the liquid and gas phases. A sulfide probe may thus be used to monitor liquid phase sulfur (H₂S(1), S²⁻(1), HS⁻) as sulfide, and gas chromatography may be used to monitor the gas phase H₂S concentration. A plot of the total H₂S concentration in mmol as a function of time for two different H₂S-water systems is shown in Figure 4. As is noted, for a total H₂S concentration of 0.31 mmol, the total H₂S content varied by only 14 percent during the 37 h of study. Similarly, the total H₂S content for the 0.73 mmol experiment varied by only 16 percent in 45 h. Thus, a suitable batch system for containing H₂S throughout the duration of a typical H₂S consumption experiment has been developed. The addition of elemental sulfur and sulfide to the system should have no effect on the technique illustrated in Figure 4.
yielded straight lines. These calibration curves were constructed at a late stage in the fermentation when all of the elemental sulfur had been converted to sulfate. Thus, no interference of sulfur on the direct cell concentration measurements at 580 nm was observed.

**Sulfur Measurements**

The gas phase H$_2$S concentrations were measured by gas chromatography using a Hewlett-Packard (Kennett Square, PA) HP 5890 Series II gas chromatograph and an HP 7673 integrator equipped with a thermal conductivity detector. The column was a 1/8" x 6' teflon column packed with Chromosorb 107, 80/100 mesh (Altech Assoc., Inc. Deerfield, IL). The oven temperature was 80°C, and the detector and injector temperatures were kept at 175°C. The carrier gas was helium at a flow rate of 30 mL/min. Liquid phase sulfur concentrations (H$_2$S(l), HS$^-$, S$^{2-}$) were measured using a Corning Silver/Sulfide Electrode (Corning Glass Works, Medfield, MA) and an Orion Specific Ion meter (Orion Research, Inc., Cambridge, MA).

**RESULTS AND DISCUSSION**

Two preliminary fermentation profiles for *C. thiosulfatophilum* with 200 mmol of H$_2$S and 320 mmol of H$_2$S are shown, respectively, in Figures 7 and 8. Cell concentration measurements in the figures are by optical density measurements at 580 nm. As is shown in Figure 7, complete H$_2$S utilization from both the gas and liquid phases occurred in just over 30 h. The concentration reached approximately 160 mg/L, although a spike in cell concentration was observed due to elemental sulfur interference. The spike rapidly disappeared as the bacterium used elemental sulfur once the H$_2$S had been depleted from the liquid phase. Similar results are shown in Figure 8 where just over 50 h were required to completely utilize all of the 320 mmol
Medium and Operating Conditions

The medium for the studies with *C. thiosulfatophilum* contained per 100 mL: Pfennig mineral solution (2), 5 mL; Pfennig trace metals solution (3), 0.1 mL; B-vitamins solution (3), 0.5 mL; yeast extract (Difco), 0.05 g; and NaHCO₃, 0.4 g. Before sterilization, 75 mL of medium were added to each bottle under a N₂/CO₂ (80%/20%) atmosphere. The bottles were autoclaved at 15 psig for 20 minutes. Once sterile, the bottles were flushed water He/CO₂ (60%/40%) to remove any traces of O₂ and inoculated with 5 mL of seed culture. Gaseous H₂S was then added to the bottles in the desired amounts. The seed culture was grown for 72 h before being used for inoculation into the bottles.

Cell Concentration Measurements

As was mentioned previously, the presence of elemental sulfur crystals makes measurement of cell concentration by optical density difficult. Two methods have thus been used for measuring the cell concentration of *C. thiosulfatophilum*: direct o.d. measurement at 580 nm and o.d. measurement of the pigment chlorophyl at 670 nm. For the measurement of pigment, 1 mL of solution was first centrifuged at 15,000 rpm for 4 min. The supernatant water was then discarded. Methanol (1 mL) was then added to dissolve the chlorophyl present in the cells, followed by centrifugation for an additional 2 min. Methanol (1.5 mL) was then added to the new supernatant and the absorbance measured at 670 nm.

A calibration curve for cell concentration in mg/L as a function of optical density at 580 nm is shown in Figure 5 and a calibration for cell concentration based upon chlorophyl content in mg/L as a function of optical density at 670 nm is shown in Figure 6. As is noted, both calibrations
of \( H_2S \). Again, a spike in cell concentration was observed, which again disappeared as the elemental sulfur was used by the bacterium once all of the \( H_2S \) had disappeared from the medium. The maximum cell concentration in Figure 8 was approximately 260 mg/L.

Kinetic analysis of the data in Figures 7 and 8 will not be attempted due to the interference of elemental sulfur in all concentration measurements. Subsequent experiments are being conducted using the alternative "pigment" method for cell concentration estimations.

**Task 4. Bioreactor Studies**

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

**Task 5. Limiting Conditions/Scale-up**

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

**Task 6. Economic Evaluations**

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

\[ M. \text{ D. Ackerson} \]
Principal Investigator

\[ Edgar C. Clausen \]
Principal Investigator

\[ J. D. Gaddy \]
Principal Investigator
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Figure 1. Growth of *R. rubrum* in the Experimental Growth Phase as a Function of Temperature.
Figure 2. Calculation of the Yield of H₂ from CO by *R. rubrum* as a Function of Temperature.
Figure 3. Comparison of Specific Uptake Kinetics for *R. rubrum* as a Function of Temperature.
Figure 4. H₂S Balance in Gas and Liquid Phases for Two Different Initial H₂S Concentrations (System: H₂S, Water).
Figure 5. Cell Concentration Calibration Curve as a Function of Optical Density at 580 nm for *C. thiosulfatophilum*. 
Figure 6. Cell Concentration Calibration Curve (Based upon Chlorophyl Content) as a Function of Optical Density at 570 nm for *C. thiosulfatophilum*. 

\[ OD_{570} \]

\[ X_{ch} (\text{mg/L}) \]
Figure 7. Batch Cell Concentration and H₂S Profiles for *C. thiosulfatophilum* (200 mmol H₂S Initially).
Figure 8. Batch Cell Concentration and H$_2$S Profiles for *C. thiosulfatophilum* (320 mmol H$_2$S Initially).
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