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Growth Kinetics of *Thiobacillus thiooxidans* on the Surface of Elemental Sulfur

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The growth kinetics of *Thiobacillus thiooxidans* on elemental sulfur in batch cultures at 30°C and pH 1.5 was studied by measuring the time courses of the concentration of adsorbed cells on sulfur, the concentration of free cells suspended in liquid medium, and the amount of sulfur oxidized. As the elemental sulfur was oxidized to sulfate ions, the surface concentration of adsorbed cells per unit mass of sulfur approached a maximum value (maximum adsorption capacity of sulfur particles) whereas the concentration of free cells continued to increase with time. There was a close relationship between the concentrations of free and adsorbed cells during the microbial sulfur oxidation, and the two cell concentrations were well correlated by the Langmuir isotherm with adsorption equilibrium constant K_A and maximum adsorption capacity X_{Am} of 2.10×10^{-9} ml per cell and 4.57×10^{10} cells per g, respectively. The total concentration of free and adsorbed cells increased in parallel with the amount of sulfate formed. The total growth on elemental sulfur gave a characteristic growth curve in which a linear-growth phase followed the period of an initial exponential phase. The batch rate data collected under a wide variety of inoculum levels (about 10^5 to 10^8 cells per ml) were consistent with a kinetic model assuming that the growth rate of adsorbed bacteria is proportional to the product of the concentration, X_A, of adsorbed cells and the fraction, θ_V, of adsorption sites unoccupied by cells. The kinetic and stoichiometric parameters appearing in the model were estimated from the experimental data, and the specific growth rate, μ_A, and growth yield, Y_A, were 2.58 day^{-1} and 2.05×10^{11} cells per g, respectively. The proposed model and the parameter values allowed us to predict quantitatively the surface attachment of *T. thiooxidans* cells on elemental sulfur and the bacterial growth in both initial exponential and subsequent linear phases. The transition from exponential to linear growth was a result of two competing factors: an increase in the adsorbed-cell concentration, X_A, permitted a decrease in the unoccupied-site fraction, θ_V.

Thiobacillus thiooxidans is an acidophilic chemoautotrophic bacterium that uses elemental sulfur or reduced sulfur compounds as substrates at temperatures up to about 40°C and pH values as low as 0.5. This sulfur-oxidizing bacterium plays a major role in the cycling of sulfur in the biosphere. The ability of microorganisms to oxidize inorganic sulfur is applied in industry to the bioleaching of base and precious metals from various minerals and to the removal of pyritic sulfur (FeS_2) from coal (desulfurization). Although the mechanism of oxidation of inorganic sulfur compounds by *T. thiooxidans* has been extensively reviewed by Suzuki (10) and Ehrlich (3), the growth kinetics of *T. thiooxidans* on inorganic sulfur, especially elemental sulfur as a solid substance, has been poorly analyzed and modeled.

Previous work has indicated that the microbial oxidation of elemental sulfur by *T. thiooxidans* requires direct contact between the bacterial cells and sulfur (8, 11, 13). Takakuwa et al. (12) measured a batch growth curve of *T. thiooxidans* on elemental sulfur at an unadjusted medium pH and an inoculum level under no-forced-air conditions and showed that all the cells are attached to the sulfur surface during the early stages of growth, after which the number of free cells suspended in the liquid phase increases as the culture progresses. To gain a better understanding of the growth kinetics of cells adsorbed on elemental sulfur, it is necessary to determine the surface concentration of adsorbed cells during the course of growth. Moreover, collection and analysis of rate data concerning both bacterial growth and sulfur oxidation are important in obtaining the rate law for growth on solid substrates.

This paper describes the kinetics of growth of *T. thiooxidans* on elemental sulfur and subsequent oxidation to sulfuric acid in batch cultures. The batch growth and consequent sulfur oxidation were monitored by measuring the concentrations of free and adsorbed bacteria and the amount of sulfur oxidized as a function of time. Rate data collected at a wide variety of inoculum levels were analyzed to obtain the rate law and determine the kinetic and stoichiometric parameters for the microbial sulfur oxidation.

MATERIALS AND METHODS

Microorganism and medium. *T. thiooxidans* IFO 13724 was obtained from the culture collection of the Institute for Fermentation, Osaka, Japan. The basal salts medium was that described by Starkey (9). Starkey's medium contained the following components (in grams per liter of water): KH_2PO_4, 3.0; $(NH_4)_2SO_4$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.3; and $FeSO_4 \cdot 7H_2O$, 0.01. This liquid medium was supplemented with 30 ppb of molybdenum (12) and 1.0% (wt/vol) elemental sulfur (modified Starkey's medium). The substrate used was hydrophobic sulfur particles, and the dominant species was rhombic sulfur. The average diameter of the elemental sulfur particles was 29.9 μm, and the geometric standard deviation was 2.32. The sulfur particles added to the liquid medium had previously been washed by agitation in an aqueous solution of 1 M HCl for 30 min, the solution was removed, the particles were dispersed in 1 M NaOH solution, and the procedure was repeated twice more. The sulfur particles were then washed twice with distilled water and acetone; the washing with acetone was done in a short time (a few minutes) because elemental sulfur dissolves in acetone. Once again, the sulfur particles were dispersed in distilled water to remove the acetone and then dried at room temperature. The washing with HCl, NaOH, and acetone was required to remove inorganic nonsulfur elements and organic matter contaminating elemental sulfur.

T. thiooxidans was subcultured aerobically with gentle shaking in modified Starkey's medium. After 5 days of inoculation at 30°C, the suspension was passed through quantitative filter papers to remove sulfur particles and then centrifuged at 12,000 × *g* for 10 min to collect the cells. The collected cells were washed and

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resuspended in the liquid medium, and the cell suspension was immediately used as the inoculum in subsequent experimental runs.

Apparatus and procedure. The reactor used to perform the microbial oxidation experiments was an air-sparged vessel under agitation. It was charged with 3,000 ml of liquid medium and held at 30°C. A 5-ml sample of an active culture of *T. thiooxidans* was used as an inoculum. The reactor contents were mixed at 500 rpm by a paddle impeller with a 6-cm diameter and sparged continuously with air saturated with water vapor. It was initially established that air flow rates between 1,000 and 6,000 ml/min gave similar results with this reactor; thereafter, 3,000 ml/min was used.

A batch sulfur oxidation run was initiated by adding sulfur particles to the medium, and this was taken as zero time. The initial total cell concentration in the medium ranged from 3.58×10^5 to 1.59×10^8 cells per ml, and the initial sulfur/liquid loading ratio was 10 g of sulfur per liter of suspension. The medium pH was adjusted to an initial value of pH 1.5 by manual addition of KOH solution. In the present experimental study, the reactor was filled with the liquid medium and the hydrophobic sulfur particles were vigorously agitated with liquid medium by using the impeller. Consequently, the sulfur particles were in contact with the liquid medium and did not creep up the side of the reactor. In the presence of *T. thiooxidans*, furthermore, the adsorption of the bacteria onto the sulfur particles appeared to change the surface property of sulfur from hydrophobic to hydrophilic, because of the hydrophilic property of *Thiobacillus* cells (2).

A sample of 30 ml of sulfur-liquid mixture was periodically withdrawn from the bioreactor prior to analysis. The liquid samples were analyzed for inorganic sulfur compounds by ion chromatography (LC-6A chromatograph; Shimadzu). The number of cells in the liquid samples was determined by direct counts with a hemacytometer or by measurement of A_{660} in a spectrophotometer (UV-160A; Shimadzu) with cuvettes with a 1-cm light path. The relationship between the absorbance and the liquid phase cell concentration was linear up to at least 0.1 absorbance unit, so that the liquid samples were diluted with fresh medium to give an absorbance of 0.05 to 0.1 on the spectrophotometer. An absorbance of 0.1 was equivalent to 1.96×10^8 cells per ml.

In addition to the free-cell number, the number of cells adsorbed on elemental sulfur was measured by the following method. Samples for measuring the adsorbed-cell concentration were pipetted into duplicate bottles, one of which was for determination of the mass of elemental sulfur suspended in medium and the other was for counts of the total numbers of free and adsorbed cells. The residual sulfur in a bottle was filtered, washed with distilled water, dried, and weighed. The sulfur particles suspended in solution in another bottle were dissolved by adding 50% excess CS_2 solution, based on complete dissolution of sulfur particles (1.5 g of CS_2 was necessary to dissolve completely 1.0 g of sulfur at 30°C). Upon complete dissolution of the sulfur particles in the CS_2 solution, the cells adsorbed on the sulfur surface were all released into the liquid phase, as reported by Takakuwa et al. (12). The total number of cells in the liquid phase, which corresponds to the total number of free and adsorbed cells in the suspension, was determined by absorbance measurements. The CS_2 treatment had no effect on the absorbance measurements. The number of cells adsorbed on the sulfur particles was determined by subtracting the number of free cells in the liquid sample from the total cell number. The number of adsorbed cells per unit volume of the liquid phase was converted into the cell number per unit mass of sulfur by referring to the mass of elemental sulfur collected for the cell count.

The treatment of elemental sulfur with CS_2 solution was tested in preliminary runs: the concentration of cells adsorbed on sulfur was determined by direct counts from the difference of the concentrations of free cells in the liquid medium before and after the bacterial adsorption, whereas the concentration of adsorbed cells was also measured by the CS_2 method. The adsorbed-cell concentration determined by the CS_2 method was consistent with that based on the reduction in numbers of free cells in the liquid medium, within an error of 3%. This demonstrates that the use of CS_2 to measure the number of adsorbed cells is valid and reliable.

For cell viability measurements, *T. thiooxidans* cultures grown on elemental sulfur for 5 days were filtered with suction through quantitative filter papers to remove sulfur particles. The resulting cell suspensions were aerobically held at 30°C, and a 0.5-ml sample of the bacterial cell suspension was periodically taken for the assessment of viable cell numbers in the absence of elemental sulfur. The initial cell concentrations were $(5.8 \text{ to } 6.7) \times 10^8$ cells per ml. Since ATP was absent from nonviable cells, the viability of *T. thiooxidans* cells was monitored by measurements of cellular ATP content as a function of time. The ATP level was measured with an ATP analyzer (AF-100; TOA Electronics Ltd.) that measures luminescence produced by a reaction of ATP with luciferase and luciferin (14).

Aside from microbial oxidation runs, experiments were done to measure adsorption of *T. thiooxidans* onto elemental sulfur. An accurately weighed amount of sulfur particles (2.0 g) was mixed with 200 ml of liquid medium at pH 1.5 and 30°C in a flask. The initial concentration of free cells in the medium was varied from 2.20×10^8 to 1.84×10^9 cells per ml. The flask was shaken for 60 min. The number of free cells in the medium was determined by absorbance measurements. The number of cells adsorbed onto the sulfur surface was determined from the difference in the numbers of cells in the liquid phase before and after adsorption. The indirect measurement of the adsorbed-cell number on sulfur was consistent with direct measurement by the CS_2 method. In this experiment, the cell adsorption onto the flask wall was preliminarily tested in the absence of elemental sulfur: the suspensions with known concentration of free cells were poured into the flask, and then the liquid-phase concentration of free cells was measured as a function of time. The preliminary runs demonstrated that cell adsorption onto the flask wall was negligible. Thus, the above method of assessment of cell adsorption onto the surface of elemental sulfur was confirmed to be valid.

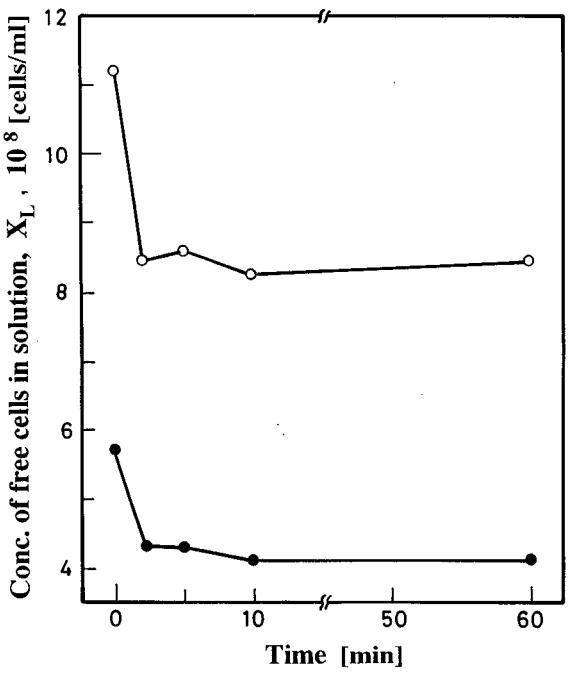

FIG. 1. Adsorption of *T. thiooxidans* onto elemental sulfur as a function of time at different initial concentrations of free cells.

RESULTS

Rate and equilibrium of bacterial adsorption. Since adsorption of *T. thiooxidans* onto elemental sulfur is considered to be a necessary part of the microbial oxidation process, the adsorption behavior was experimentally examined before the kinetics of growth and sulfur oxidation was addressed. Figure 1 shows the results of experiments in which the concentration of *T. thiooxidans* cells suspended in solution was measured as a function of time of contact between the cells and the sulfur particles. The free-cell concentration rapidly decreased with increasing contact time, implying that the free cells are extensively adsorbed from the liquid phase onto the sulfur surface. The number of adsorbed cells was dependent on the initial concentration of free cells. Moreover, the bacterial adsorption was a very fast process; equilibrium was attained within the first 10 min of exposure to sulfur particles.

Figure 2 shows equilibrium distribution of *T. thiooxidans* between the sulfur surface and the liquid medium. The concentration, X_A, of adsorbed cells per unit mass of elemental sulfur approached a limiting value as the concentration, X_L, of free cells in the liquid phase increased. The shape of this isotherm indicates that the equilibrium data can be modeled by the Langmuir equation:

$$X_A = (K_A X_{Am} X_L)/(1 + K_A X_L) \quad (1)$$

where X_{Am} is the maximum adsorption capacity per unit mass of elemental sulfur and K_A is the adsorption equilibrium constant.

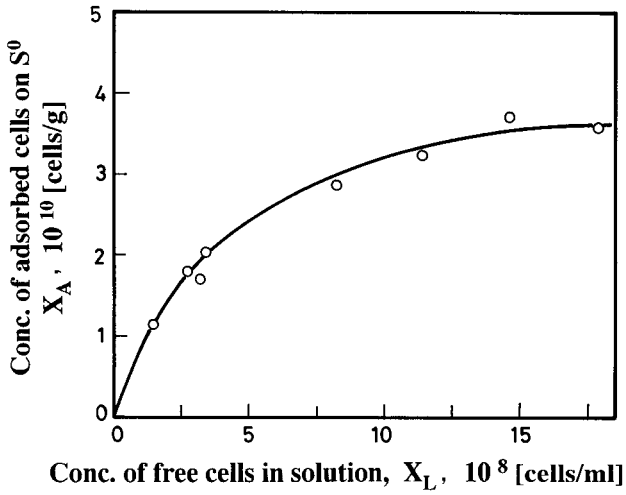

FIG. 2. Equilibrium adsorption isotherm for *T. thiooxidans* on elemental sulfur. The solid line represents the Langmuir isotherm.

For convenience in testing the experimental data, equation 1 was rearranged in linear form:

$$X_L/X_A = (X_L/X_{Am}) + 1/(X_{Am}K_A) \quad (2)$$

A plot of X_L/X_A versus X_L gave an excellent straight line (Fig. 3). A correlation coefficient for the fit of equation 2 to the data was 0.982, indicating that the equilibrium data shown in Fig. 2 conform to the Langmuir isotherm. From the slope and the intercept of the regression line fitted to the data, the two parameters in equation 2 were determined to be $X_{Am} = 4.57 \times 10^{10}$ cells per g of sulfur and $K_A = 2.10 \times 10^{-9}$ ml per cell. The Langmuir adsorption isotherm predicted from the estimated parameter values of X_{Am} and K_A was consistent with the equilibrium data (Fig. 2).

Cell growth on elemental sulfur. The air-sparged agitated reactor was used to avoid sulfur oxidation runs under gas transfer-limited conditions. To examine if growth of *T. thiooxidans* on elemental sulfur is limited by gas (O_2 or CO_2) transfer,

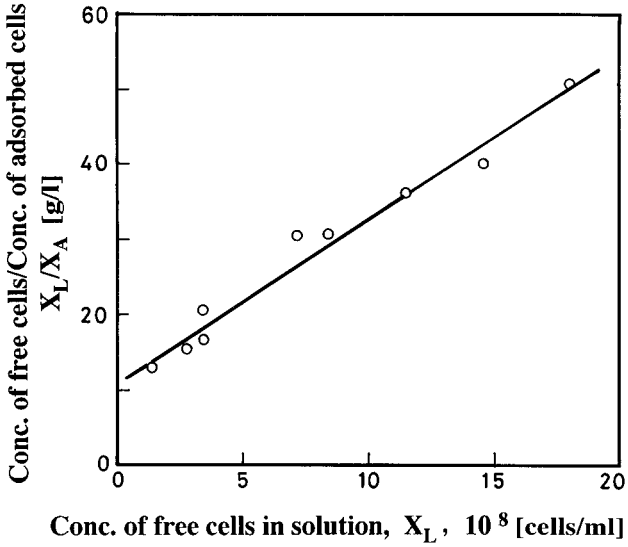

FIG. 3. Estimation of maximum adsorption capacity, X_{Am}, and adsorption equilibrium constant, K_A, in the Langmuir isotherm.

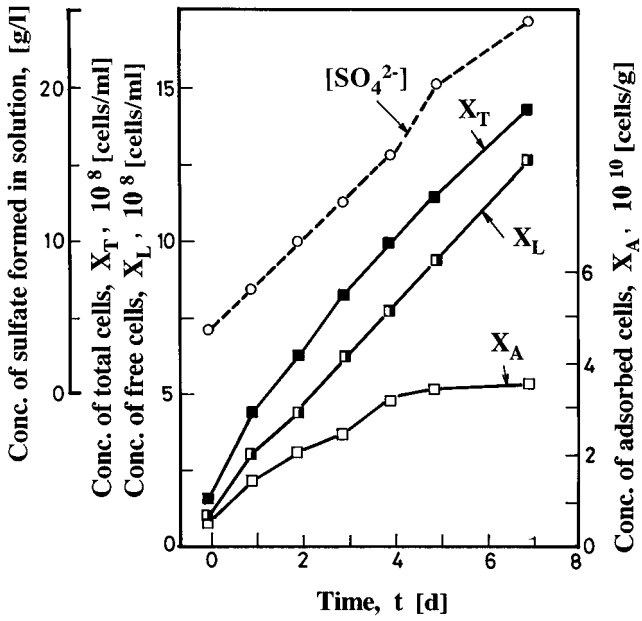

FIG. 4. Batch growth curve of *T. thiooxidans* on elemental sulfur at an initial total cell concentration of 1.59×10^8 cells per ml.

some microbial oxidation runs were carried out by varying the aeration rates at a constant liquid-phase stirring speed of 500 rpm. As the flow rate of air into the agitated reactor increased from 700 to 1,000 ml/min, the rates of growth and sulfur oxidation significantly increased, indicating that the microbial sulfur oxidation is limited by the gas transfer. However, increases in the air flow rates between 1,000 and 6,000 ml/min gave no increase in the growth and oxidation rates. Therefore, there was no gas transfer limitation when the air flow rate was higher than 1,000 ml/min; thereafter, the aeration rate was fixed at 3,000 ml/min for the reactor to remove the gas transfer limitation.

Figure 4 shows a batch growth curve for *T. thiooxidans* grown on elemental sulfur at an initial total cell concentration of 1.59×10^8 cells per ml, along with a time course of the liquid-phase concentration of sulfate ions. As *T. thiooxidans* cells grew on elemental sulfur, sulfate ions were accumulated in the liquid medium. To generate a mass balance with respect to sulfur, the mass loss of elemental sulfur was determined gravimetrically after microbial oxidation runs. The mass of adsorbed cells was negligible compared with the mass of sulfur. The amount of sulfate formed in the medium was equivalent to the mass loss of elemental sulfur, indicating that elemental sulfur was stoichiometrically oxidized to sulfate by *T. thiooxidans*:

$$S^0 + \frac{3}{2}O_2 + H_2O \rightarrow H_2SO_4$$

The amount of formed sulfate linearly increased with time, in parallel with the concentration, X_T, of total cells. The concentration, X_A, of adsorbed cells on sulfur increased significantly and then gradually approached a limiting value, i.e., the maximum adsorption capacity, X_{Am}, which was estimated as 4.57×10^{10} cells per g in the adsorption experiments. Even when the adsorbed-cell concentration remained almost unchanged, the concentration, X_L, of free cells in the liquid medium continued to increase with time. This significant increase in the free-cell concentration appeared to result from the release of bacteria which grew on the sulfur surface, because pre-

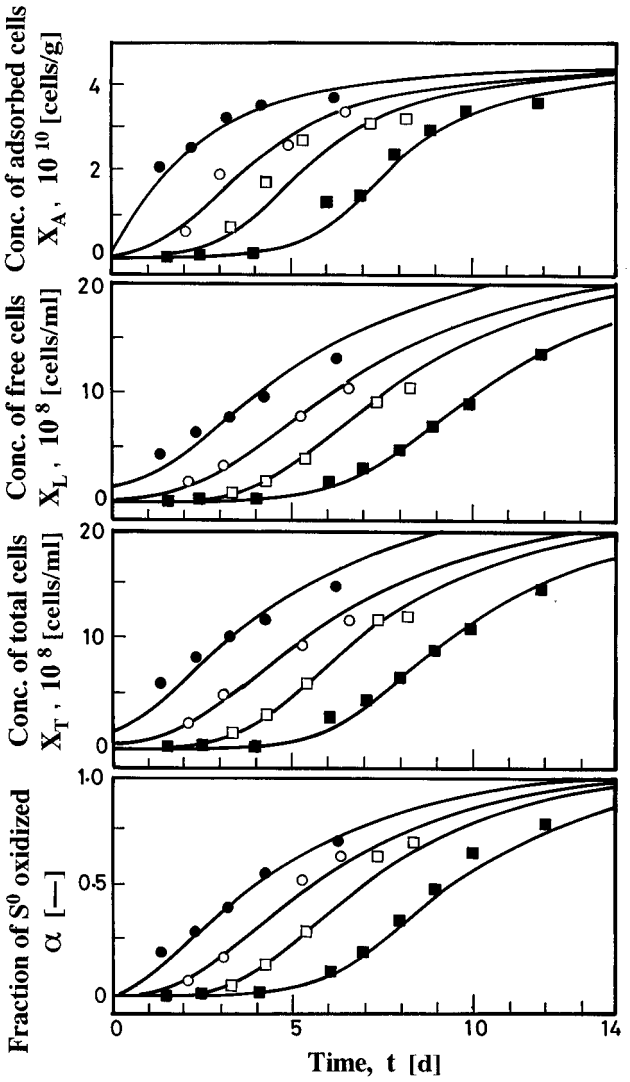

FIG. 5. Kinetics of batch growth and sulfur oxidation at different initial total cell concentrations, X_{T0}. Symbols: ■, $X_{T0} = 3.58 \times 10^5$ cells per ml; □, $X_{T0} = 3.96 \times 10^6$ cells per ml; ○, $X_{T0} = 3.14 \times 10^7$ cells per ml; ●, $X_{T0} = 1.59 \times 10^8$ cells per ml. Solid lines were calculated from the kinetic model described in the text.

vious work on the oxidation of sulfur by *T. thiooxidans* indicated that the bacteria are unable to grow in the liquid medium (12). The viability of free cells suspended in the liquid medium was monitored by measuring the cellular ATP content as a function of time. In the absence of elemental sulfur, the free *T. thiooxidans* cells in the medium were found to be about 70% viable after 7 days.

Figure 5 shows the effect of initial total cell concentration, X_{T0}, on both growth and sulfur oxidation. The fraction, α, of elemental sulfur oxidized was determined from the measured values of the aqueous sulfate concentration. At the lowest inoculum level of 3.58×10^5 cells per ml, a nearly linear increase of the oxidation fraction, α, with time was achieved after at least 4 days of incubation, and the time course of α approached an upper asymptote of 1.0. As the inoculum level increased from 3.58×10^5 to 1.59×10^8 cells per ml, nearly linear increases in the total cell number and the sulfur oxidation fraction tend to appear at earlier stages of incubation.

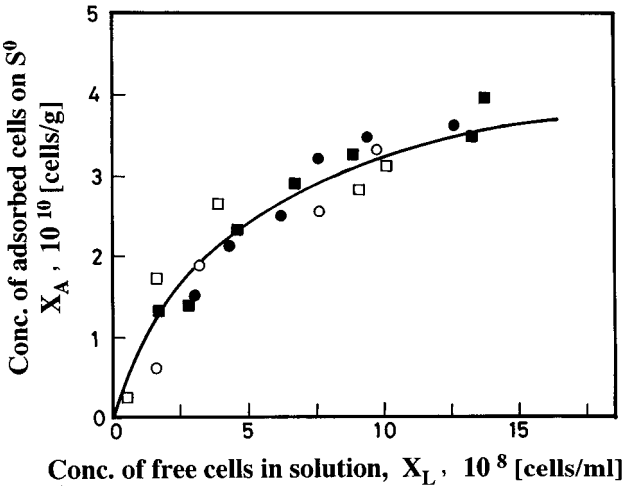

FIG. 6. Distribution of *T. thiooxidans* between sulfur surface and liquid medium during cell growth. Symbols are the same as in Fig. 5. The solid line was calculated from the Langmuir adsorption isotherm.

DISCUSSION

Free- and adsorbed-cell concentration during microbial sulfur oxidation. In modeling the growth of adsorbed bacteria on the solid surface, it is useful to derive information concerning the distribution of cells between the solid and liquid phases. The data for the concentrations of free and adsorbed cells during the growth of *T. thiooxidans* on elemental sulfur (Fig. 5) were plotted as X_A versus X_L in Fig. 6. The datum points had a form equivalent to that of a Langmuir-type equation, i.e., equation 1. To determine if the distribution data can be modeled by equation 1, the data for X_A and X_L in Fig. 6 were replotted in the form suggested by equation 2. The linearity of a plot of X_L/X_A versus X_L was checked, and the correlation coefficient was calculated as 0.948. The result indicates a close correlation between the free- and adsorbed-cell concentrations throughout the duration of the microbial sulfur oxidation.

In Fig. 6, the experimental data for the free- and adsorbed-cell concentrations, X_A and X_L, respectively, are compared with the Langmuir equation, which was calculated from equation 1 by using the X_{Am} and K_A values estimated in the above-mentioned adsorption equilibrium study. The solid curve representing the Langmuir isotherm provided a valid fit to the data. The good fit of the data by the equilibrium isotherm shows the distribution of *T. thiooxidans* cells to be at equilibrium even when significant growth occurs. The reason for this is that the adsorption of *T. thiooxidans* onto the sulfur particles proceeds on a much faster timescale than the cell growth: the bacterial adsorption attained equilibrium within 10 min, whereas the cell growth and sulfur oxidation lasted a few weeks. Consequently, it can be concluded that at all times during cell growth, the concentration of adsorbed cells on the sulfur surface is related to that of free cells in the liquid medium by the Langmuir adsorption isotherm. This quantitative relation is particularly important in kinetics analysis for the growth of adsorbed bacteria on the solid substrate.

Stoichiometry of cell growth and sulfur oxidation. A metabolic stoichiometry was established to describe cell growth and related substrate consumption. The growth yield, Y_A, is defined as the ratio of the total number of *T. thiooxidans* cells formed by growth to the mass of elemental sulfur oxidized:

$$Y_A = (X_T - X_{T0})V/(\alpha W_0) \quad (3)$$

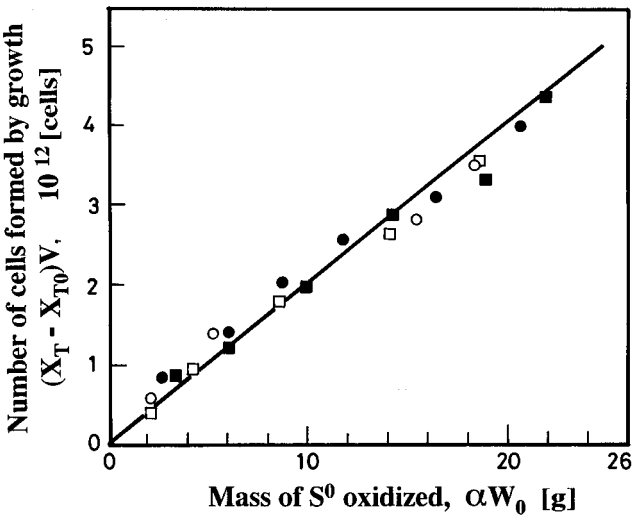

FIG. 7. Estimation of growth yield, Y_A, of *T. thiooxidans* on elemental sulfur. Symbols are the same as in Fig. 5.

where X_T is the concentration of total cells per unit volume of sulfur-liquid mixture, X_{T0} is the initial concentration of total cells, V is the volume of sulfur-liquid mixture, W_0 is the initial mass of sulfur particles, and α is the fraction of elemental sulfur oxidized.

The experimental data for the total cell growth and sulfur oxidation (Fig. 5) were plotted according to equation 3 to obtain the growth yield, Y_A. Figure 7 shows that a plot of $(X_T - X_{T0})V$ versus αW_0 resulted in a straight line passing through the origin, regardless of the inoculum level. A correlation coefficient for the fit of equation 3 to the data was 0.989. The growth yield, Y_A, on elemental sulfur was estimated as 2.05×10^{11} cells per g of sulfur oxidized from the slope of the regression line fitted to the data.

Growth kinetics. Mathematical models could be successfully used to describe the kinetics of direct microbial oxidation of pyrite (FeS_2) or sphalerite (ZnS) in bioleaching processes (1, 4–7). A similar approach was adopted to analyze and model the growth kinetics of *T. thiooxidans* on elemental sulfur.

For a well-mixed vessel, the concentration, X_T, of total cells can be expressed in terms of the surface concentration, X_A, of adsorbed cells per unit mass of sulfur and liquid-phase concentration, X_L, of free cells per unit volume of liquid:

$$X_T = X_A(W_0/V)(1 - \alpha)^{2/3} + (1 - \phi)X_L \quad (4)$$

where ϕ is the volume fraction of sulfur particles in the solid-liquid mixture. The ϕ value was taken as zero under the present experimental conditions in dilute suspensions. The term $(1 - \alpha)^{2/3}$ in equation 4 is used to represent a decrease in the surface area of sulfur particles as a result of dissolution.

According to the previous model (1, 4–7), the growth rate, R_A, of adsorbed cells on elemental sulfur is directly proportional to the product of the concentration, X_A, of adsorbed cells and the fraction, θ_V, of adsorption sites unoccupied by cells:

$$R_A = \mu_A X_A \theta_V (W_0/V)(1 - \alpha)^{2/3} \quad (5)$$

with

$$\theta_V = (X_{Am} - X_A)/X_{Am} \quad (6)$$

where μ_A is the specific growth rate of adsorbed cells and X_{Am} is the maximum adsorption capacity. This kinetic model differs significantly from Monod-type equations. Because *T. thiooxidans* cells multiply on the sulfur surface and no growth of free cells occurs in the liquid medium, the rate of increase in total cells can be written in terms of equation 5:

$$dX_T/dt = \mu_A X_A \theta_V (W_0/V)(1 - \alpha)^{2/3} \quad (7)$$

The oxidation rate of elemental sulfur can be related to the growth rate:

$$-dW/dt = W_0(d\alpha/dt) = (1/Y_A)V(dX_T/dt) \quad (8)$$

with

$$\alpha = (W_0 - W)/W_0 \quad (9)$$

where W is the mass of elemental sulfur at any time.

These model equations contain four key parameters. As described above, the stoichiometric parameter, Y_A, and the adsorption parameters, X_{Am} and K_A, were determined from the experiments. Thus, the remaining kinetic parameter, μ_A, remains to be estimated. Equations 7 and 8 were solved numerically by the Runge-Kutta method with the help of equations 1, 4, 6, and 9. The specific growth rate, μ_A, was estimated by fitting the numerical results to the experimental data by the method of nonlinear least squares. The sensitivity of the time courses of the total cell concentration, X_T, and the sulfur oxidation fraction, α, to the adjustable parameter μ_A was calculated for a change in μ_A. The total cell concentration, X_T, was strongly dependent on the parameter μ_A. After 4 days of growth, for example, the calculated value of X_T varied from 2.54×10^8 to 1.11×10^9 cells per ml as the μ_A value increased from 1.3 to 3.9 day^{-1}. The sensitivity was quite adequate for the accurate estimation of μ_A. Also, the sensitivity of α was adequate to enable the μ_A value to be accurately estimated.

The specific growth rate, μ_A, was estimated as 2.58 day^{-1} on the basis of the kinetic data collected in the four experimental runs under a wide range of initial total cell concentrations from 3.58×10^5 to 1.59×10^8 cells per ml. Figure 5 compares the experimental data with the model predictions by using the estimated parameter values of μ_A, Y_A, X_{Am}, and K_A. The model predictions were consistent with the experimental data concerning the time courses of the concentrations, X_A, X_L, and X_T, of adsorbed cells, free cells, and total cells, respectively, and of the fraction, α, of sulfur oxidized.

General discussion. The growth of *T. thiooxidans* cells adsorbed on the surface of sulfur particles exhibited sigmoidal growth curves on a linear scale (Fig. 5). The total growth data were graphed semilogarithmically (Fig. 8). The solid curves were calculated by using the above kinetic model, equations 1, 4, and 6 through 9, together with the estimated parameter values of μ_A, Y_A, X_{Am}, and K_A. During the early stages of incubation at the lowest inoculum level of $X_{T0} = 3.58 \times 10^5$ cells per ml ($t < 4$ days), the growth of adsorbed cells on sulfur was exponential. After the first 4 days, the cells could not multiply exponentially, and there was a nearly linear increase in the total cell concentration, X_T, as seen in the linear plot of X_T versus time (Fig. 5). The length of the exponential growth phase was dependent on the inoculum level, X_{T0}; especially at the highest inoculum level of $X_{T0} = 1.59 \times 10^8$ cells per m^3, the exponential phase practically disappeared. The adsorbed cells of *T. thiooxidans* utilized the majority of the initially added elemental sulfur in the linear growth phase, whereas only a very small percentage (about 1% or less) of the total sulfur was actually consumed in the exponential phase. To gain a good understanding of the kinetics of microbial sulfur oxidation, it is important to analyze and model the growth rates in the linear phase as well as the exponential phase. It should be noted that

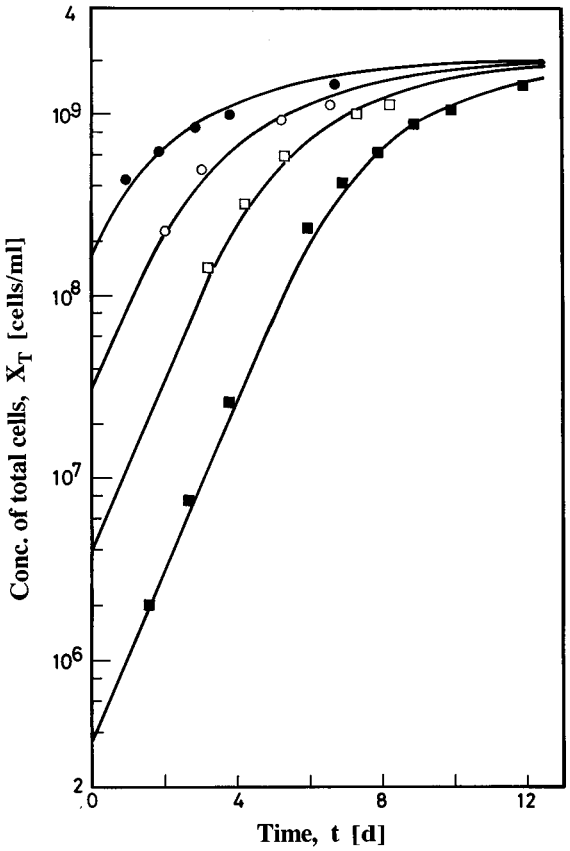

FIG. 8. Semilogarithmic plot of batch growth on elemental sulfur at different initial total cell concentrations. Symbols are the same as in Fig. 5. Solid lines were calculated from the kinetic model described in the text.

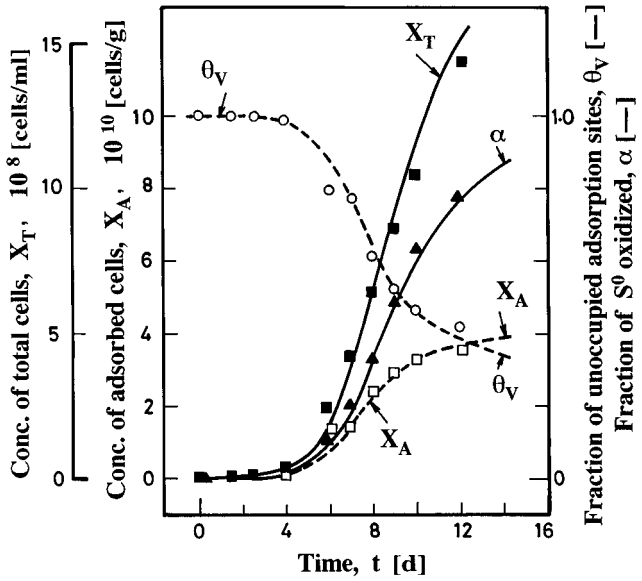

FIG. 9. Model simulations (lines) and experimental data (points) for batch growth of *T. thiooxidans* on elemental sulfur at an initial total cell concentration of 3.58×10^5 cells per ml.

the batch growth in both the initial exponential and subsequent linear phases can be quantitatively described by the rate law (equation 7), assuming that the growth rate of adsorbed bacteria is a function of the adsorbed-cell concentration, X_A, and the unoccupied-site fraction, θ_V.

Although bacteria generally have the capacity to grow exponentially, a number of factors often arrest the exponential increase in cell numbers without stopping growth. In the case of microbial sulfur oxidation, the deviation from exponential growth was not a consequence of limited O_2 and CO_2, as described above. Moreover, the estimated values of the specific growth rate, μ_A, and the growth yield, Y_A, were independent of the inoculum level in both exponential and linear phases, indicating that the rates of growth and oxidation were not limited by nutrient consumption in the basal salts medium or accumulation of toxic products. These results showed that *T. thiooxidans* grew with sulfur limitation. Therefore, the nearly linear growth observed in Fig. 4 and 5 is presumably caused by an increase in the sulfur surface covered by cells, that is, a decrease in the unoccupied-site fraction, θ_V. Figure 9 shows the changes with time in the adsorbed-cell concentration, X_A, and the unoccupied-site fraction, θ_V, when the initial inoculum level is 3.58×10^5 cells per m^3. During the exponential growth phase ($t < 4$ days), the adsorbed-cell concentration, X_A, was very low compared with the maximum adsorption capacity, X_{Am}; thus, the θ_V value remained essentially unchanged ($\theta_V \approx 1.0$). Consequently, the growth rate is apparently independent of θ_V, the growth being exponential. After the exponential phase ($t < 4$ days), the values of X_A and θ_V varied markedly with time. The transition from the exponential phase to the linear phase is a result of two competing effects which act in opposite directions: an increase in the adsorbed-cell concentration, X_A, always permits a decrease in the unoccupied-site fraction, θ_V, as was seen in equation 6. The sigmoidal dependence of the total cell concentration, X_T, and the oxidation fraction, α, exhibited by both model and experiment was evidence that the sulfur surface uncovered by *T. thiooxidans* cells has a significant effect on the cell growth on the solid substrate.

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