EVALUATION OF GROWTH RATE OF *ACIDITHIOBACILLUS FERROOXIDANS* OXIDIZING ELEMENTAL SULFUR

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HODNOTENIE RASTOVEJ RÝCHLOSTI BAKTÉRIÍ *ACIDITHIOBACILLUS FERROOXIDANS* OXIDUJÚCICH ELEMENTÁRNU SÍRU

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Abstrakt

Bakteriálna oxidácia elementárnej síry predstavuje heterogénnu reakciu na rozhraní tuhá fáza- kvapalina, pričom dochádza ku konverzii hydrofóbnej síry S^0 až na formu S^{6+} a k následnému vylúhovaniu rozpustnej oxidovanej síry do roztoku (rovnica 1).

$$2S + 3O_2 + 2H_2O \xrightarrow{A.\ ferrooxidans} 2H_2SO_4 \qquad (1)$$

Chemická oxidácia síry molekulárnym kyslíkom je extrémne pomalá. Elementárna síra predstavuje stabilný produkt pri oxidačnom lúhovaní kovových sulfidov a často býva príčinou zníženia kinetiky lúhovania v dôsledku pasivácie reakčného povrchu [1]. Bakteriálna oxidácia síry a redukovaných sírnych zlúčenín hrá významnú úlohu v geochemických procesoch a spolu s oxidáciou pyritu sú dominantnými kyselinotvornými reakciami v procese tvorby kyslých banských vôd.. Predkladaná práca popisuje priebeh oxidácie elementárnej síry katalyzovanej baktériami *Acidithiobacillus ferrooxidans* vo vsádzkovom reaktore v aeróbnych podmienkach. Kinetika bakteriálneho rastu a oxidácie síry bola hodnotená z analýzy plynnej a kvapalnej fázy v priebehu kultivácie. Parametre rastu baktérií boli stanovené z rýchlosti spotreby oxidu uhličitého zo vzduchu ako jediného zdroja uhlíka (autotrófny rast). Oxidačná aktivita bola meraná z rýchlosti spotreby kyslíka ako konečného akceptora elektrónov oxidácie síry, z koncentrácie síranov v roztoku a hodnoty pH. Citlivá analýza plynov umožnila stanoviť aktuálnu rastovú rýchlosť bakteriálnej kultúry, aktuálne množstvo biomasy a následný výpočet špecifických rýchlostí v priebehu kultivácie (Obr. 1). Bakteriálna kultúra vykazovala maximálnu špecifickú rastovú rýchlosť (μ) 0,043 h^{-1}, (ktorá zodpovedá dobe zdvojenia 16 hod) v exponenciálnej fáze rastu (Obr. 3). Intenzita miešania a aerácie reaktora významne ovplyvňovala kinetiku bakteriálneho rastu a oxidácie síry v tejto fáze. Zvýšenie rýchlosti prietoku vzduchu zapríčinilo prekvapujúci pokles špecifickej rastovej rýchlosti kultúry, pravdepodobne v dôsledku interakcie hydrofóbnej síry a vzduchových bublín (flotačný efekt).

Počiatočná exponenciálna fáza (nelimitovaného) rastu prešla postupne do dlhotrvajúcej fázy s postupne klesajúcou špecifickou rastovou rýchlosťou. Lineárna fáza rastu zaznamenaná inými autormi [3, 6, 9] a ktorú by mohol zdanlivo indikovať priebeh nárastu koncentrácie síranov v tejto retardačnej fáze (Obr. 5) nebola pozorovaná. Bakteriálny rast a oxidácia síry sa zastavili pri poklese pH média na hodnotu 0,7. Na základe nameraných výsledkov možno H^+ protón označiť za dominantný inhibítor bakteriálnej rastovej a oxidačnej aktivity za daných kultivačných podmienok.

Abstract

Acidithiobacillus ferrooxidans oxidized elemental powdered sulfur to sulfuric acid in batch culture at aerobic conditions. The kinetics of bacterial growth and sulfur oxidation were evaluated from CO_2 and O_2 consumption rates, respectively. The culture achieved a maximum specific growth rate (μ) of 0.043 h^{-1} (doubling time 16 hours). The exponential growth phase was followed by the lasting phase with a gradual decrease of the specific growth rate. The bacterial growth and sulfur oxidation stopped at pH 0.7. The H^+ protons are considered as a main inhibitor of the bacterial growth and of the sulfur oxidizing activity.

Key words: Acidithiobacillus ferrooxidans, sulfur, oxidation, growth kinetics, oxygen, carbon dioxide, gas analysis, sulfate.

Introduction

Bacterial oxidation of reduced sulfur compounds plays an important role in many geochemical and industrial processes. Elemental sulfur formed during leaching of metal sulfides covers the particle surface and is often considered to be responsible for slowing down the rate of leaching [1]. Chemical oxidation of sulfur to sulfate is extremely slow. Elemental sulfur is a stable product in the oxidative dissolution mechanism [2]. *Acidithiobacillus ferrooxidans* oxidizes the elemental sulfur to sulfuric acid in the presence of oxygen according the following overall reaction:

$$2S + 3O_2 + 2H_2O \xrightarrow{A.\ ferrooxidans} 2H_2SO_4 \qquad (1)$$

Bacterial oxidation of sulfur and pyrite are dominant acidity producing reactions taking part in natural weathering of sulfide minerals and generation of acid mine drainage. A little work has been done on the growth kinetics of acidithiobacilli on elemental sulfur contrary to numerous papers dealing with ferrous iron or sulfide minerals oxidation kinetics. Most of the kinetic studies have suggested that the bacterial oxidation of poorly soluble solid sulfur particles implies the attachment of the bacteria to the sulfur surface [3, 4, 5]. Ceskova et al. [6] observed sulfur oxidation by both free and adsorbed bacteria considering that these bacteria probably utilize soluble sulfur compounds formed during the degradation of solid S_8.

This paper describes the kinetics of the autotrophic growth of *Acidithiobacillus*–like microorganisms in connection with sulfur oxidation in a batch reactor. The growth rate was determined from the CO_2 fixation rate using on-line off gas analysis. This experimental approach has successfully been applied in the study of the bio-oxidation kinetics of sulfide minerals [7]. The gas analysis enables the mass of total (free and adsorbed) bacteria in the slurry to be measured and the specific growth rate and substrate specific consumption rates to be calculated in the whole course of bacterial sulfur oxidation.

2. Materials and methods

2.1 Bacteria, culture medium and sulfur characterization

Iron and sulfur oxidizing strain of *Acidithiobacillus ferrooxidans* [CCM 3973] maintained on elemental sulfur was used in the study. The culture medium contained the following components (in grams per liter of water): $(NH_4)_2SO_4$, 0.2; KH_2PO_4, 3;0; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 6H_2O$, 0.25; (initial pH = 4). This basal medium was supplemented with 1.0 % w/v elemental sulfur. The average diameter of (poly) disperse sulfur particles was 30 μm and the specific surface area was 0.175 $m^2\ g^{-1}$.

2.2 Cultivation and analytical procedures

The experiment was carried out in a 1.5 L jacketed glass reactor tempered at 30 °C by the circulating water bath. Culture medium was stirred at 250 rpm and sparged continuously with air saturated with water vapor.

Bacterial growth and sulfur oxidation kinetics were determined from carbon dioxide and oxygen consumption rates using the on-line off-gas analysis [7]. The total concentration of bacterial cell was calculated from the sum of CO_2 fixed in the course of cultivation. To calculate the carbon and oxygen balance, the flow of air passed through reactor was controlled with a Cole & Parmer mass flow controller. The carbon dioxide in the off-gas and reference air was measured with a Guardian Plus infrared CO_2 monitor (Edinburg Sensors Ltd.). The concentration of oxygen was monitored using an electrochemical sensor. The concentration of soluble oxygen was measured with a Clark-type oxygen electrode. H^+ activity of the medium was measured using a glass pH electrode combined with the reference Ag/AgCl electrode. The suspension was periodically analyzed for free cells and SO_4^{2-}concentrations. The concentration of free bacteria in the liquid was determined turbidimetrically [8]. Sulfate concentration in solution was determined by the nephelometric method with $BaCl_2$.

3. Results

Most of the kinetic studies have centered on the adsorption of thiobacilli onto elemental sulfur. Bacterial adsorption to the sulfur particles can be recognized as a physical process in which the bacteria attached to the solid surface are in adsorption /desorption equilibrium with the free bacteria in the liquid phase [3, 8]. At a high concentration of free cells (X_L) in the solution, the concentration of adsorbed cells (X_A) approaches a limiting value. The Langmuir equilibrium adsorption isotherm has already been applied to describe the adsorption behavior of either non-growing cells [6, 8] or the growing cells onto sulfur over the course of cultivation [3, 8, 9].

The course of cultivation can be divided into four periods marked as A, B, C, and D (Fig. 1). The period A represents lag phase and exponential growth phase (unlimited growth). The rate of adsorption of the microorganisms onto sulfur is very rapid compared with the bacterial growth rate. Equilibrium is attained within a few minutes of exposure to sulfur particles. The duration of initial “lag phase” is therefore negligible in presented experiment lasting few weeks. The exponential growth of bacterial culture was followed by a longer phase (periods B and C) with a gradual decrease of the specific growth rate. The period D, when the increase of the specific growth rate can be again observed, will be discussed later.

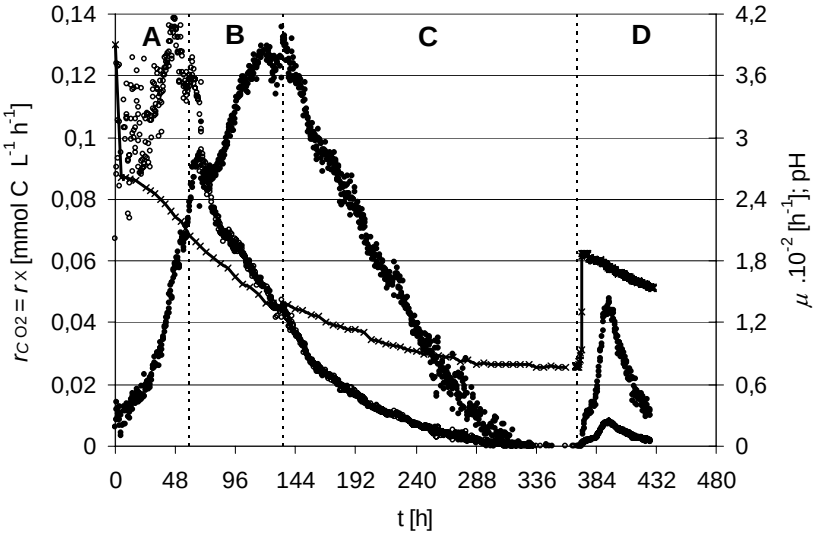

Fig.1 Bacterial growth rate r_X (●), specific growth rate μ (○) and pH of the medium (x) as a function of time in the course of cultivation of *Acidithiobacillus*–like microorganisms on elemental sulfur. Regions marked as A, B, C and D are discussed in the text.

Autotrophically growing bacterial culture used carbon dioxide as a sole carbon source. The production rate of bacteria (r_X) equals to the consumption rate of carbon dioxide (r_{CO2}). The total amount of biomass produced (X_T) can be derived from the total amount of CO_2 absorbed from air. The concentration of bacteria is expressed in moles of (organic) carbon per unit of volume and was calculated from the summation of carbon dioxide consumption rates in the course of cultivation (Fig. 2).

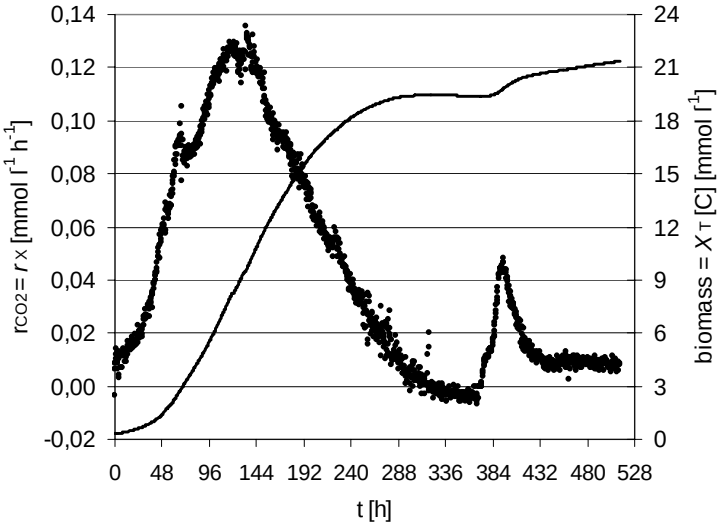

Fig.2 Bacterial growth rate r_X (●) equals to the carbon dioxide consumption rate $-r_{CO2}$ and biomass concentration X_T (curve) calculated from the summation of carbon dioxide consumption rates in the course of cultivation.

The gas analyses allowed us to derive the bacterial specific growth rate (related to unit of biomass) from the measured actual growth rate (r_X) and the actual biomass concentration (X_T) according to ($r_X = dX_T/dt = \mu X_T$). Similarly specific oxygen consumption rate (q_{O2}) and specific sulfur oxidation rate (q_S) related to unit of bacterial mass can be calculated.

Exponential behavior of the bacterial growth with an almost constant specific growth rate is apparent during the early stages (approx. 3 days) of the cultivation. A slight increase of the bacterial specific growth rate in this period (Fig. 3) can be a result of bacterial adaptation to the culture conditions and/or of the improved availability of utilizable sulfur due to the production of bacterial wetting agents. The maximum specific growth rate attains the value of 0.043 h^{-1} (1.0 day^{-1}).

The gas-liquid mass transfer of oxygen and carbon dioxide in the medium was considered to be sufficiently large for bacterial growth and sulfur oxidation. The increase of air-flow rate in this phase has surprisingly a negative effect on the bacterial specific growth rate (Fig. 1 period B). The retardation of specific growth rate in this period was probably caused by the depression of available sulfur surface due to the strong aeration and the hydrophobic nature of sulfur particles (flotation effect). On the other hand, turning off the reactor stirring, while air-flow rate still remained constant, caused immediate drop in bacterial growth and oxygen consumption rates. Next switching the stirrer on returned the culture back to the original state (Fig. 4).

The period B can be defined in general as growth phase with a continuing increase in the total bacterial growth rate and the sulfur oxidation rate but the specific rates (μ, q_{O2}, q_S), i.e. activities related to unit of bacterial mass decrease. The decrease indicates a limitation in the bacterial phase. The bacterial growth rate and sulfur oxidation rate gradually approached a limiting value, considering that maximum rates would be reached at a complete coverage of the sulfur particles` surface by the microorganisms. The culmination point, i.e. the maximum absorption capacity was attained at approx. 6-th day of the experiment (the end of the region B in Fig. 1). At this time the bacterial growth rate, and oxygen consumption rate were 0.13 mM C h^{-1} and 0.8 mM O_2 h^{-1} respectively.

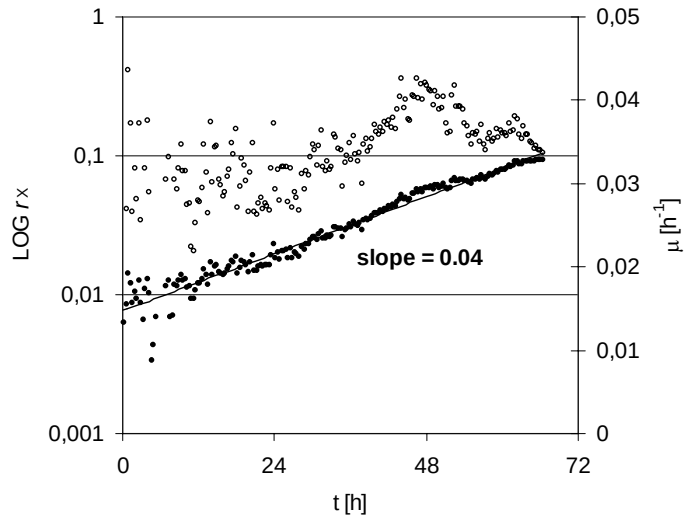

Fig.3 Logarithm of bacterial growth rate LOG r_X (●) and specific growth rate μ (○) as a function of time during the exponential growth phase of *Acidithiobacillus ferrooxidans* on elemental sulfur.

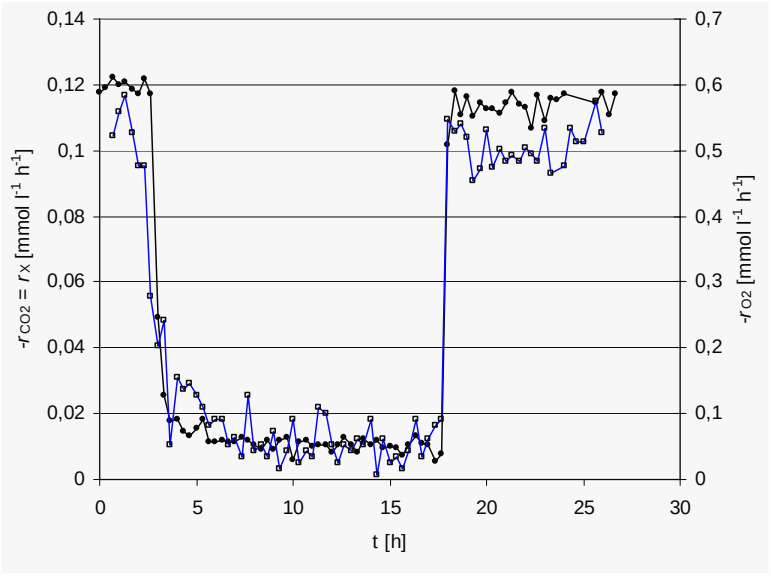

Fig.4 Sharp drop and recovery of the growth rate (●) and oxygen consumption rate (□) of the culture growing on elemental sulfur as a response to changes in culture conditions.

The period C stands for the cultivation phase with a descending growth rate. The decrease of growth rate can be explained by the fact that the bacterial surface oxidation process results in a reduced size of individual particles due to their progressive dissolution. A linear growth referred by other workers [3, 6, 9] was not observed here although the apparent linear behavior of sulfate concentration in the later phase (Fig. 5) might indicate it. Analysis of the on-line off-gas results shows that growth rate (Fig. 1), as well as the oxygen consumption rate (data not shown) gradually decreases. Fig. 5 shows concentration of sulfate produced and pH through the bacterial oxidation of elemental sulfur according to Eq. 1. The oxidation of S^0 to S^{6+} is considered to be complete without accumulating any intermediates in the medium. According to the results of other researchers [3, 10], partially oxidized products of elemental sulfur (thiosulfate, polythionates and sulfite) in similar systems were not detected. Due to bacterial sulfur oxidation, pH of the medium continuously decreased until the final value of 0.7, where the growth rate and the oxygen consumption rate reached zero. The adjustment of pH to 2, by the addition of 10 M KOH, caused an immediate recovery of cell growth and oxidation activity (Fig. 1, period D). In the next course of the cultivation, a similar gradual decrease of the bacterial growth rate due to H^+ accumulation in the culture medium is detected. Based on the above findings, we conclude that H^+ ions are the main inhibitory factor. Harahuk et al., [11] demonstrated that sulfate anions up to a concentration, of 0.2 M had very little effect on either the iron or sulfur oxidation activity in *T. ferrooxidans*. Beyond this point, iron oxidation was only marginally affected, while sulfur oxidation rate showed a dramatic drop. This apparent preferential inhibition of sulfur oxidation at high sulfate concentration was believed to be caused by changes in osmotic pressure, as it was observed in other anions, such as phosphate and chloride [11]. The concentration of sulfate anions did not exceed 0.2 M in the period recorded in Fig. 5.

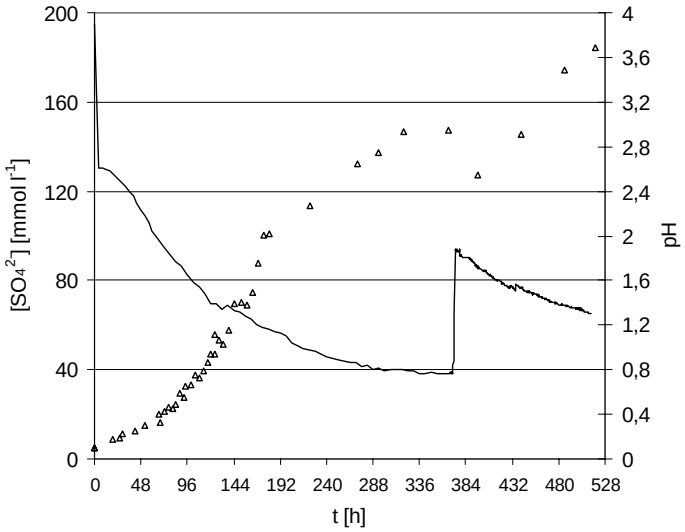

Fig.5 Sulfate concentration (Δ) and pH (curve) of the medium in the course of batch cultivation of *Acidithiobacillus ferrooxidans* on elemental sulfur

Conclusions

Bacterial oxidation of elemental sulfur represents a complex process, incorporating interactions of cells with the sulfur particle surface as well as interactions of cells with soluble products of the sulfur oxidation. Therefore, the specific growth rate is a function of many variables. Growth can be limited by the availability of sulfur as an energy substrate, oxygen as a terminal electron acceptor, carbon dioxide as a sole carbon source and the composition of mineral nutrients. The products of oxidation such as sulfate anions and hydrogen protons can inhibit growth. The methodology employed in this work is a useful tool for the immediate evaluating of the bacterial culture activity. In the next research, the above parameters will be kept constant in order to quantify their effects on the specific bacterial growth rate.

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