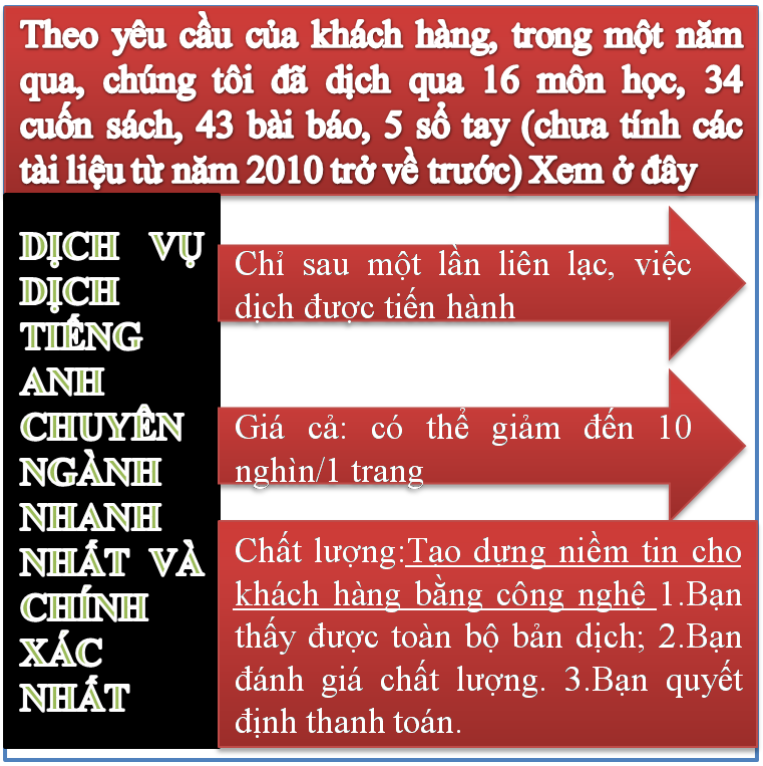

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Review Applications of pulsed EPR spectroscopy to structural studies of sulfite oxidizing enzymes ABSTRACT Sulfite oxidizing enzymes (SOEs), including sulfite oxidase (SO) and bacterial sulfite dehydrogenase (SDH), catalyze the oxidation of sulfite (SO_3^{2-}) to sulfate (SO_4^{2-}). The active sites of SO and SDH are nearly identical, each having a 5-coordinate, pseudo-square-pyramidal Mo with an axial oxo ligand and three equatorial sulfur donor atoms. One sulfur is from a conserved Cys residue and two are from a pyranopterindithiolene (molybdopterin, MPT) cofactor. The identity of the remaining equatorial ligand, which is solvent-exposed, varies during the catalytic cycle. Numerous in vitro studies, particularly those involving electron paramagnetic resonance (EPR) spectroscopy of the Mo(V) states of SOEs, have shown that the identity and orientation of this exchangeable equatorial ligand depends on the buffer pH, the presence and concentration of certain anions in the buffer, as well as specific point mutations in the protein. Until very recently, however, EPR has not been a practical technique for directly probing specific structures in which the solvent-exposed, exchangeable ligand is an O, OH^-, H_2O, SO_3^{2-}, or SO_4^{2-} group, because the primary O and S isotopes (^{16}O and ^{32}S) are magnetically silent ($I=0$). This review focuses on the recent advances in the use of isotopic labeling,	Tổng quan Các ứng dụng của quang phổ EPR xung để nghiên cứu cấu trúc của các enzyme oxy hóa sulfite Tóm tắt Các enzyme oxi hoá hợp chất sunfite (SOEs), bao gồm sulfite oxidase (SO) và vi khuẩn dehydrogenaza sunfite (SDH), xúc tác cho sự oxy hóa sulfite (SO_3^{2-}) thành sunfat (SO_4^{2-}). Các vị trí hoạt động của SO và SDH gần như đồng nhất, mỗi vị trí có 5 phối vị, hình tháp vuông giả Mo với một trục nối oxo và ba nguyên tử donor lưu huỳnh dạng vòng. Một nguyên tử lưu huỳnh từ một dư lượng dự trữ Cys và hai nguyên tử lưu huỳnh từ pyranopterindithiolene (molybdopterin, MPT) đồng yếu tố. Sự đồng nhất của các liên kết xích đạo còn lại, là dung môi tiếp xúc, thay đổi trong suốt chu kỳ xúc tác. Trong nhiều thử nghiệm khoa học, những thử nghiệm đó đặc biệt liên quan đến quang phổ cộng hưởng thuận từ điện tử (EPR) của các trạng thái Mo (V) của các SOE, đã chỉ ra rằng sự đồng nhất và sự định hướng của liên kết xích đạo có thể trao đổi được này phụ thuộc vào độ pH đệm, sự hiện diện và nồng độ của các anion bất định trong chất đệm, cũng như trong các đột biến điểm cụ thể trong protein. Tuy nhiên, cho đến gần đây, EPR không phải là một kỹ thuật thực tế để trực tiếp thăm dò các cấu trúc cụ thể, trong đó các dung môi tiếp xúc, liên kết trao đổi là nhóm O, OH^-, H_2O, SO_3^{2-}, hoặc SO_4^{2-}, bởi vì các đồng vị O và S ban đầu (^{16}O và ^{32}S) có tính chất từ tĩnh (I

variable-frequency high resolution pulsed EPR spectroscopy, synthetic model compounds, and DFT calculations to elucidate the roles of various anions, point mutations, and steric factors in the formation, stabilization, and transformation of SOE active site structures. …… ……	= 0). Tổng quan này tập trung vào các tiến bộ gần đây trong việc sử dụng đánh dấu đồng vị, quang phổ EPR xung biến đổi tần số với độ phân giải cao, sự tổng hợp các mô hình hợp chất, và các tính toán DFT để giải thích vai trò của các anion khác nhau, các đột biến điểm, và các yếu tố không gian trong quá trình hình thành, độ ổn định, và sự chuyển đổi các cấu trúc vị trí hoạt động SOE. ....
Asymmetric Dimeric Structure of Ferredoxin-NAD(P)+ Oxidoreductase from the Green Sulfur Bacterium Chlorobaculum tepidum: Implications for Binding Ferredoxin and NADP+ Ferredoxin-NAD(P)+ oxidoreductase (FNR) catalyzes the reduction of NAD(P)+ to NAD(P)H with the reduced ferredoxin (Fd) during the final step of the photosynthetic electron transport chain. FNR from the green sulfur bacterium Chlorobaculum tepidum is functionally analogous to plant- type FNR but shares a structural homology to NADPH-dependent thioredoxin reductase (TrxR). Here, we report the crystal structure of C. tepidum FNR to 2.4 A resolution, which reveals a unique structure- function relationship. C. tepidum FNR consists of two functional domains for binding FAD and NAD(P)H that form a homodimer in which the domains are arranged asymmetrically. One NAD(P)H domain is present as the open form, the other with the equivalent NAD(P)H domain as the relatively closed form. We used site-directed mutagenesis on the hinge region connecting the two domains in order to investigate the	[redacted]

importance of the flexible hinge. The asymmetry of the NAD(P)H domain and the comparison with TrxR suggested that the hinge motion might be involved in pyridine nucleotide binding and binding of Fd. Surprisingly, the crystal structure revealed an additional C-terminal sub-domain that tethers one protomer and interacts with the other protomer by n-n stacking of Phe337 and the isoalloxazine ring of FAD. The position of this stacking Phe337 is almost identical with both of the conserved C-terminal Tyr residues of plant-type FNR and the active site dithiol of TrxR, implying a unique structural basis for enzymatic reaction of C. tepidum FNR.
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Bacteria involved in sulfur amendment oxidation and acidification processes of alkaline ‘alperujo’ compost
Compost acidification efficiency Olive mill waste Horticultural use Paracoccus thiocyanatus Halothiobacillus neapolitanus
Eight strains of sulfur oxidizing bacteria were isolated from alkaline ‘alperujo’ compost, seven being identified as Paracoccus thiocyanatus and one as Halothiobacillus neapolitanus. This was the first time that P. thiocyanatus was isolated from mature compost. Acidification capability of isolated strains was compared with type strains H. neapolitanus CIP104769, Thiobacillus denitrificans CIP104767and Thiomonas intermedia CIP104401. Indigenous P. thiocyanatus strains were as much as or

more efficient for acidifying compost than type strains. Sulfur oxidizing population naturally occurring in compost showed maximum acidification efficiency and no extra effect was found with the help of type strains. pH reduction caused by S° was paralleled by a decrease in $CaCO_3$ and an increase in $CaSO_4$ and salinity levels. A remarkable increase in cultivable sulfur oxidizing bacteria population along with the acidification process was also recorded. Amended compost showed a range of chemical and biological characteristics suitable for use as container media constituent.

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Invited review

Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries

ARTICLE INFO ABSTRACT

Bacteria of the sulphur cycle, in particular sulphate reducing and sulphide oxidizing bacteria, are of immense importance from the industrial and environmental point of views. While biogenic production of H_2S by sulphate reducing bacteria creates severe processing and environmental problems for the petroleum industry and agriculture sector, when used in a properly designed and controlled bioreactor sulphate reducing bacteria could play an instrumental role in the treatment of acid mine drainage, a major environmental challenge faced by the mining industry. Biooxidation of sulphide and intermediary sulphur compounds carried out by sulphide oxidizing bacteria are crucial in

biotreatment of acid mine drainage and in the bioleaching of refractory minerals. Moreover, sulphide oxidizing bacteria are known as major players in the in situ removal of H2S from the onshore and offshore oil reservoirs and are used in the ex situ processes for the treatment of sour gas and sulphide laden waters. Owing to the numerous environmental and industrial applications, the bacteria of the sulphur cycle have been subject of numerous studies. The present article aims to provide an overview of the microbiology, biokinetics, current and potential applications of the bacteria of sulphur cycle and the reactions which are carried out by these versatile microorganisms. Special consideration is given to the role of these bacteria in the biotreatment of acid mine drainage, oil reservoir souring and the treatment of H2S-containing gaseous and liquid streams.

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Bioleaching mechanism of Co and Li from spent lithium-ion battery by the mixed culture of acidophilic sulfur-oxidizing and iron-oxidizing bacteria

ARTICLE INFO

The bioleaching mechanism of Co and Li from spent lithium-ion batteries by mixed culture of sulfur-oxidizing and iron-

oxidizing bacteria was investigated. It was found that the highest release of Li occurred at the lowest pH of 1.54 with elemental sulfur as an energy source, the lowest occurred at the highest pH of 0. 69 with FeS2. In contrast, the highest release of Co occurred at higher pH and varied ORP with S + FeS2, the lowest occurred at almost unchanged ORP with S. It is suggested that acid dissolution is the main mechanism for Li bioleaching independent of energy matters types, however, apart from acid dissolution, Fe2+ catalyzed reduction takes part in the bioleaching process as well. Co2+ was released by acid dissolution after insoluble Co3+ was reduced into soluble Co2+ by Fe2+ in both FeS2 and FeS2 + S systems. The proposed bioleaching mechanism mentioned above was confirmed by the further results obtained from the experiments of bioprocess-stimulated chemical leaching and from the changes in structure and component of bioleaching residues characterized by XPS, SEM and EDX.
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Bioleaching of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria: Effects of substrate concentration

Abstract

The aim of this study was to determine the effect of substrate concentration (elemental sulfur) on remobilization of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria. Also, the variation in the binding forms of heavy metals before and after bioleaching was explored. This work showed the laboratory results of bioleaching experiments on Pb-Zn-Cu mine tailings. The results showed that 97.54% Zn, 97.12% Cu, and 44.34% Pb could be removed from mine tailings by the bioleaching experiment after 13 days at 2% w/v substrate concentration. The results also indicated that substrate concentration 2% was found to be best to bacterial activity and metal solubilization of the five substrate concentration tested (0.5%, 1%, 2%, 3%, and 5%) under the chosen experimental conditions. The bioleaching had a significant impact on changes in partitioning of heavy metals.

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Comparison of reactive porous media for sulfur-oxidizing denitrification of high nitrate strength wastewater

Three packing materials for sulfur oxidizing denitrification packed bed systems seeded with acclimated anoxic sludge were evaluated. Two porous media were prepared via thermal fusion with sodium bicarbonate as porogen: sulfur

fused with powdered (1) calcium carbonate ($CaCO_3$)(SCa) and (2) oyster shell (SCr). Randomly packed sulfur and limestone granules (S + L) media were used as the control. Results revealed that SCr is the most suitable media as it exhibited the highest nitrate removals and lowest nitrite accumulation. It has macrovoidal pores which facilitated microbial attachment. Additionally, SCr had the highest $CaCO_3$ loading per unit volume and highest media dissolution rate which was favorable to avert pH decrease. But due to high denitrification activity, high sulfate levels in SCr may necessitate a post-treatment step prior to effluent discharge. Due to poor biomass attachment, S + L is most sensitive to change in fluid flow condition. As hydraulic retention time is decreased, S + L exhibited intensive and irreversible performance decline. Inferior denitrification performance of SCa was mainly due to low $CaCO_3$ loading per unit volume, low dissolution kinetics and low alkalinity consumption by denitrifiers. Using modified Stover-Kincannon kinetic model, overall performance and denitrification capacities can be arranged as SCr > S + L > SCa.

Composition and dynamics of sulfate-reducing bacteria during the waterflooding process in the oil field application

RESEARCH NOTES

Corrosion and Electrochemical Behavior of 316L Stainless Steel in Sulfate-reducing and Iron-oxidizing Bacteria Solutions*

Abstract Corrosion and electrochemical behavior of 316L stainless steel was investigated in the presence of aerobic iron-oxidizing bacteria (IOB) and anaerobic sulfate-reducing bacteria (SRB) isolated from cooling water systems in an oil refinery using electrochemical measurement, scanning electron microscopy (SEM) and energy dispersive atom X-ray analysis(EDAX). The results show the corrosion potential and pitting potential of 316L stainless steel decrease distinctly in the presence of bacteria, in comparison with those observed in sterile medium under the same exposure time. SEM morphologies have shown that 316L stainless steel reveals no signs of pitting attack in the sterile medium. However, micrometer-scale corrosion pits were observed on 316L stainless steel surface in the presence of bacteria. The presence of SRB leads to higher corrosion rates than IOB. The interactions between the stainless steel surface, abiotic corrosion products, and bacterial cells and their metabolic products increased the corrosion damage degree of the passive film and accelerated pitting propagation.

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Several of the thermophilic acidophilic sulfur-metabolizing archaebacteria lack rigid cell walls. Their irregular shapes are maintained by an interna! mechanism, presumably a cytoskeleton. Apparently this is an adaptation for respiration upon elemental sulfur, which requires cell contact since sulfur is insoluble in water. Also, we speculate that there could be additional functions of the cytoskeleton, such as prevention of osmotic cell lysis, thermal stabilization of enzymes, and improvements in metabolic efficiency through specific enzyme positioning. Such a well-developed cytoskeleton, evolving first in thermophilic archaebacteria, could have been a preadaptation for the evolution of eukaryotic cells.

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Desulfovibrio capillatus sp. nov., a novel sulfate-reducing bacterium isolated from an oil field separator located in the Gulf of Mexico

Abstract

A new spirilloid sulfate-reducing bacterium designated strain MET2t (T = type strain), was isolated from a Mexican oil field separator. Electron microscopy revealed a Gram-negative cell wall consisting of a 150 nm thick undulating outer membrane. Strain MET2t appeared singly or in long chains and was actively motile with a corkscrew-like motion. The isolate grew optimally at 40° C, pH 7.4 and 3% NaCl in a medium containing

lactate, thiosulfate and yeast extract. Sulfate, sulfite, thiosulfate, and elemental sulfur served as electron acceptors but not nitrate or fumarate. Lactate, pyruvate and H2 (with acetate as carbon source) were used as electron donors. Pyruvate was fermented. Desulfoviridin and cyt c were present. The G+ C content of the DNA was 58.7mol%. Phylogenetic analysis based on 16S rDNA sequencing showed that strain MET2t was a member of the genus Desulfovibrio with ‘‘D. gracilis” and D. longus being its closest relatives (similarities of 98.3% and 97.1%, respectively). However, DNA-DNA hybridization studies indicated poor homologies (values <70%) with both species. On the basis of genotypic, phenotypic, and phylogenetic characteristics, strain MET2t (= DSM14982t = CIP107483t) is proposed as the type strain of a new species, Desulfovibrio capillatus sp. nov. GenBank accession number for the 16S rDNA sequence for MET2t is AY176773.

Differential expression of genes encoding sulfur metabolism-related periplasmic proteins of Acidithiobacillus ferrooxidans ATCC 23270

Abstract: Reverse-transcription qualitative PCR (RT-qPCR) was used to analyze the changes in transcription levels of the sulfur metabolism-related periplasmic protein genes of Acidithiobacillus ferrooxidans ATCC 23270 grown on sulfur or ferrous. Seven periplasmic proteins with apparently higher abundance grown on

elemental sulfur than on ferrous sulfate were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Expression analysis of the corresponding genes by RT-qPCR shows that the constitutive expression of all those genes are more up-regulated grown on sulfur than those grown on ferrous (>10 folder). Study on the corresponding genes of the identified periplasmic proteins by RT-qPCR confirmed the results of two-dimensioned gel electrophoresis, indicating they may be related with sulfur metabolism in A. ferrooxidans.

[redacted]

Diversity and distribution of sulfate-reducing bacteria in four petroleum reservoirs detected by using 16S rRNA and dsrAB genes

ABSTRACT

Microbial sulfate reduction, an important metabolic process in petroleum reservoirs, is widely known as a major contributor to microbial influenced corrosion and deterioration of oil quality. To better control oil field corrosion and oil degradation caused by the sulfate-reducing bacteria (SRBs), the community structure and composition of SRBs in four oil reservoirs were investigated in this study by comparing clone libraries of 16S rRNA and dissimilatory sulfate reductase (dsrAB) genes. In addition, canonical correspondence analysis (CCA) was also employed to find relationship between biodata and physi- ochemical information. More information on SRB communities was obtained from nested-PCR-

[redacted]

phylogenetic analyses of 16S rRNA genes and PCR primer sets amplifying six groups of SRBs frequently detected in oilfields all over the world were used. Amplified sequences belonging to Desulfotomaculum and Desulfobacter were the most dominant in all four reservoirs. The diversity of SRB communities increased while the temperature of the four oil reservoirs decreased from 63 to 21. Correlations between environmental variables and species distribution indicated that Desulfotomaculum was correlated with temperature, depth, and the concentration of acetate, propionate and sulphate. Desulfomicrobium, Desulfobacter and Desulfobulbus showed positive correlation with sulphur and salinity. Desulfobacterium was influenced by both salinity and the concentration of acetate. The results of this study provided important information on the microbial ecology of sulfate-reducing bacteria in different petroleum reservoirs.

Effect of ore solid concentration on the bioleaching of phosphorus from high-phosphorus iron ores using indigenous sulfur-oxidizing bacteria from municipal wastewater

Dephosphorization of high-phosphorus iron ore is an unsolved problem worldwide so far. Biotechnology could be a cost-effective and environment-friendly way to solve this problem. A novel method for bioleaching of phosphorus from high-phosphorus iron ores using indigenous sulfur-oxidizing bacteria from municipal wastewaters was first reported in this

work. Before bioleaching, the contents of phosphorus and iron from the high-phosphorus iron ore used were 1.04 and 47.89% (w/w), respectively. The effects of ore solid concentration on the phosphorus bioleaching were investigated. It was found most of phosphorus existed in the form of apatite in the iron ore. After bioleaching for 41 days, the final ore slurry pHs at all solid concentrations 10-300 g/L were between 0.09 and 0.63. The average contents of phosphorus and iron in the bioleaching solid residues were 0.21 and 51.7% (w/w), respectively. The average removal percentage of phosphorus and percentage of iron lost were 82.3 and 1.7%, respectively. After bioleaching, the high-phosphorus iron ore was suitable to be used in the manufacture of iron and steel. The optimal ore solid concentration for bioleaching of phosphorus was 250g/L under the bioleaching conditions. Thus, this bioleaching process seems to be economic and effective.

Effects of lead upon the actions of sulfate-reducing bacteria in the rice rhizosphere Microbe—mineral interactions play an important role in affecting geochemical transformations of heavy metals in the soil environment. The formation of metal sunfua, which is mediated by sulfate-reducing bacteria (SRB) through contributing to sulfate reduction is an important pathway for heavy metal

stabilization in anoxic soil. In oxic rice rhizospheres, there are abundant sulfur oxidizing bacteria (SOB) which can enhance sulfur oxidation and hence the availability of heavy metals, resulting in the uptake of such metals by the plant and a potential risk to human health. In this study, the potential existence of SRB in oxic rice rhizospheres, their contribution to sulfate reduction, and potential to reduce the availability of heavy metal was investigated. PCR-DGGE fingerprinting and real-time PCR results showed increasing numbers of SRB with Pb addition, which corresponded with increases in soil pH and reduction in Eh, suggesting the enhancement of sulfur reduction and SRB activity. Sulfur K-edge XANES, which characterized sulfur speciation in situ, revealed reduced states of sulfur. The SRB mediated the sulfate reduction and contributed to the formation of reduced sulfur which interacted with Pb, leading to the formation of stable metal sunfua and reduction of Pb availability. In return, acclimated SRB populations developed in Pb-polluted conditions. Hence stabilization of reduced sulfur by Pb enhanced the activity of SRB and sulfate reduction in rice rhizosphere.	[redacted]
Enhanced elementary sulfur recovery in integrated sulfate-reducing, sulfur-producing rector under micro-aerobic condition Biological treatment of sulfate-laden wastewater consists of two separate	[redacted]

reactors to reduce sulfate to sunfua by sulfate-reducing bacteria (SRB) and to oxidize sunfua to sulfur (S0) by sunfua oxidation bacteria (SOB). To have SRB + SOB in a single reactor faced difficulty of low S0 conversion. This study for the first time revealed that dissolved oxygen (DO) level can be used to manipulate SRB + SOB reactions in a single reactor. This work demonstrated successful operation of an integrated SRB + SOB reactor under micro- aerobic condition. At DO = 0.10-0.12 mg l 1, since the activities of SOB were enhanced by limited oxygen, the removal efficiency for sulfate reached 81.5% and the recovery of S0 peaked at 71.8%, higher than those reported in literature. At increased DO, chemical oxidation of sunfua with molecular oxygen competed with SOB so conversion of S0 started to decline. At DO > 0.30 mg l 1 activities of SRB were inhibited, leading to failure of the SRB + SOB reactor.

FACTORS AFFECTING TOXIC METALS REMOVAL FROM DIGESTED SEWAGE SLUDGE BY ENRICHED SULPHUR-OXIDIZING MICROORGANISMS

Abstract

Enriched sulphur-oxidizing microorganisms were applied to remove metals from anaerobically digested sewage sludge in laboratory reactors. The pH drop and rise in oxidation-reduction potential (ORP) of the sludge by microorganisms were mainly responsible for the metal

solubilization. There was a significant effect of the sulphur amount per unit volume of sludge, the initial pH ofthe sludge, the inoculum size and the sludge solids concentration on the pH, the sulphate production rate, the ORP, the maximum metal solubilization rate, and the time required to achieve the acceptable level of Cu for sludge land application. The enriched microorganisms were able to grow and reduce the pH at a wide range of initial sludge pH values. Metals (Cu, Zn) were solubilized efficiently as a result of decreased pH.

Fractionation of multiple sulfur isotopes during phototrophic oxidation of sunfua and elemental sulfur by a green sulfur bacterium

Abstract

We present multiple sulfur isotope measurements of sulfur compounds associated with the oxidation of H2S and S0 by the anoxygenic phototrophic S-oxidizing bacterium Chlorobium tepidum. Discrimination between 34S and 32S was +1.8 ± 0.5& during the oxidation of H2S to S0, and —1.9 ± 0.8& during the oxidation of S0 to SO42—, consistent with previous studies. The accompanying A33S and A36S values of sunfua, elemental sulfur, and sulfate formed during these experiments were very small, less than 0.1& for A33S and 0.9& for A36S, supporting mass conservation principles. Examination of these isotope effects within a framework of the metabolic pathways for S oxidation suggests that the observed effects are due to the flow of sulfur through the metabolisms, rather than abiotic equilibrium isotope exchange

alone, as previously suggested. The metabolic network comparison also indicates that these metabolisms work to express some isotope effects (between sunfua, polysunfuas, and elemental sulfur in the periplasm) and suppress others (kinetic isotope effects related to pathways for oxidation of sunfua to sulfate via the same enzymes involved in sulfate reduction acting in reverse). Additionally, utilizing fractionation factors for phototrophic S oxidation calculated from our experiments and for other oxidation processes calculated from the literature (chemotrophic and inorganic S oxidation), we constructed a set of ecosystem-scale sulfur isotope box models to examine the isotopic consequences of including sunfua oxidation pathways in a model system. These models demonstrate how the small d34S effects associated with S oxidation combined with large d34S effects associated with sulfate reduction (by SRP) and sulfur disproportionation (by SDP) can produce large (and measurable) effects in the A33S of sulfur reservoirs. Specifically, redistribution of material along the pathways for sunfua oxidation diminishes the net isotope effect of SRP and SDP, and can mask the isotopic signal for sulfur disproportionation if significant recycling of S intermediates occurs. We show that the different sunfua oxidation processes produce different isotopic fields for identical proportions of oxidation, and discuss the ecological implications of these results to interpreting minor S isotope patterns in modern systems and in the geologic record.

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FUNGAL SULPHUR OXIDATION: EFFECT OF CARBON SOURCE AND GROWTH STIMULATION BY THIOSULPHATE

Elemental sulphur oxidation by Aspergillus niger and Trichoderma harzianum using acetate, sugars and amino acids as carbon sources was determined. Glucose and sucrose were the best carbon sources for sulphate production by A. niger while amino acids supported the production of larger amounts of sulphate by T. harzianum. Both fungi used acetate as sole carbon source to support the oxidation of elemental sulphur to thiosulphate and sulphate. Mycelial dry weight yield increased when fungi were grown on thiosulphate with low (o i % (w/v) C as sucrose), but not high (1-2% (w/v) C) carbon concentrations, suggesting that these fungi may grow chemolithoheterotrophically, using both thiosulphate and sucrose as energy sources.

Identification of two inactive forms of the central sulfur cycle protein SoxYZ of Paracoccus pantotrophus

Abstract The central protein of the sulfur-

oxidizing enzyme system of Paracoccus pantotrophus, SoxYZ, reacts with three different Sox proteins. Its active site Cys110Y is on the car- boxy-terminus of the SoxY subunit. SoxYZ ‘‘as isolated’’ consisted mainly of the catalytically inactive SoxY-Y(Z)2 heterotetramer linked by a Cys110Y-Cys110Y interprotein disunfua. Sunfua activated SoxYZ ‘‘as isolated’’ 456-fold, reduced the disunfua, and yielded an active SoxYZ heterodimer. The reductant tris(2-carboxyethyl)phosphine (TCEP) inactivated SoxYZ. This form was not re-activated by sunfua, which identified it as a different inactive form. In analytical gel filtration, the elution of ‘‘TCEP-treated’’ SoxYZ was retarded compared to active SoxYZ, indicating a conformational change. The possible enzymes involved in the re-activation of each inactive form of SoxYZ are discussed.

Isolation and characterization of acidophilic bacteria from Patagonia, Argentina

Abstract

Three acidophilic, chemolithotrophic and ferrous oxidizing bacteria were isolated from the Agrio River in the geothermal system of Copahue volcano in Neuquen (Patagonia, Argentina) using 9K enrichment medium and then purified on solid ferrous-agarose medium. Amplified ribosomal DNA restriction enzyme analysis (PCR/ARDRA) showed that these strains can be considered as Acidithiobacillus ferrooxidans. Ferrous

iron oxidation of these strains, including a collection strain was compared at different temperatures (20, 25, 30, 35 and 400C) and pH values (1.6, 1.8 and 2.3). Isolated bacteria proved to be less susceptible to low temperatures or high medium acidity. These strains showed adequate growth in iron-free 9K medium using sulphur as a sole energy source. Iron(II) oxidation inhibition in the presence of different heavy metals (Ag+, Cd2+, Cu2+, Zn2+) or different anions (NO3 -, Cl-) were also investigated.

Isolation and characterization of ferrous- and sulfur-oxidizing bacteria from Tengchong solfataric region, China

Abstract

Microbial oxidation and reduction of iron and sulfur are important parts of biogeochemical cycles in acidic environments such as geothermal solfataric regions. Species of Acidithiobacillus and Leptospirillum are the common ferrous-iron and sulfur oxidizers from such environments. This study focused on the Tengchong sofataric region, located in Yunnan Province, Southwest China. Based on cultivation, 9 strains that grow on ferrous-iron and sulfuric compounds were obtained. Analysis of 16S rRNA genes of the 9 strains indicated that they were affiliated to Acidithiobacillus, Alicyclobacillus, Sulfobacillus, Leptospirillum and Acidiphilium. Physiological and phylogenetic studies indicated that two strains (TC-34 and TC-71) might represent two novel members of Alicyclobacillus.

Strain TC-34 and TC-71 showed 94.8%-97.1% 16S rRNA gene identities to other species of Alicyclobacillus. Different from the previously described Alicyclobacillus species, strains TC-34 and TC-71 were mesophilic and their cellular fatty acids do not contain ^-cyclic fatty acids. Strain TC-71 was obligately dependent on ferrous-iron for growth. It was concluded that the ferrous-iron oxidizers were diversified and Alicyclobacillus species were proposed to take part in biochemical geocycling of iron in the Tengchong solfataric region.

Kinetic model for simultaneous leaching of zinc sunfua and manganese dioxide in the presence of iron-oxidizing bacteria

Abstract

The effect of iron-oxidizing bacteria on the simultaneous leaching of zinc sunfua and manganese dioxide was studied. Some researchers have reported the enhancement of the leaching rate during the simultaneous leaching of metal oxides and metal sunfuas. In the present study, we examined the effect of the presence of Thiobacillus ferrooxidans in the simultaneous leaching. We also examined the reaction rates during the simultaneous leaching in the presence of the bacteria in order to study the reaction kinetics. The rate equation was obtained for each reaction: the oxidation of zinc sunfua by ferric iron, the oxidation of zinc sunfua by ferric iron and by the bacteria, the reductive dissolution of manganese dioxide by ferrous iron, and the simultaneous dissolution of zinc sul"de

and manganese dioxide. A kinetic model based on these equations was proposed and the extent of leaching during the simultaneous dissolution with T. ferrooxidans was determined.

Leaching of petroleum refinery ash by acidophilic sulfur-oxidizing microbial cultures

ARTICLE INFO ABSTRACT

Sulfur-oxidising acidophilic bacteria were obtained from weathered sulfur piles produced by a petroleum refinery. When grown on commercial sulfur the yield was 1010 cell/g S. Sulfur conversion to sulfate was about 95% after 17 days. Cultures were also grown together with ash obtained from incinerated refinery sludge, which contained high amounts of iron. Cultures grown in ash plus sulfur gave somewhat higher values for the growth parameters (Y =1.6 x 1010 cell/g S). The sulfur conversion was about 70% (after 17 days) and more than 90% of the iron present in the ash was also leached. The sulfur-reduced compound thiosulfate, determined using ion pair HPLC, was found in the presence and absence of ash although the profile was different in each case. Sulfite was only found in non-ash containing cultures, whereas tetra- thionate was only formed in the presence of ash. These results are discussed with reference to the substrates used by the micro-organisms.

Microbial communities involved in electricity generation from sunfua oxidation in a microbial fuel cell

ARTICLE INFO

Simultaneous electricity generation and sunfua removal can be achieved in a microbial fuel cell (MFC). In electricity harvesting from sunfua oxidation in such an MFC, various microbial communities are involved. It is essential to elucidate the microbial communities and their roles in the sunfua conversion and electricity generation. In this work, an MFC was constructed to enrich a microbial consortium, which could harvest electricity from sunfua oxidation. Electrochemical analysis demonstrated that microbial catalysis was involved in electricity output in the sunfua-fed MFC. The anode-attached and planktonic communities could perform catalysis independently, and synergistic interactions occurred when the two communities worked together. A16S rRNA clone library analysis was employed to characterize the microbial communities in the MFC. The anode-attached and planktonic communities shared similar richness and diversity, while the LIBSHUFF analysis revealed that the two community structures were significantly different. The exoelectrogenic, sulfur-oxidizing and sulfate-reducing bacteria were found in the MFC anodic chamber. The discovery of these bacteria was consistent with the community characteristics for electricity generation from sunfua oxidation. The exoelectrogenic bacteria were found both

on the anode and in the solution. The sulfur-oxidizing bacteria were present in greater abundance on the anode than in the solution, while the sulfate-reducing bacteria preferably lived in the solution.

NITRIFICATION AND SULPHUR OXIDATION BY ASPERGILLUS FLAVUS GROWING IN MEDIUM CONTAINING REDUCED NITROGEN AND SULPHUR

Aspergillus flavus oxidized elemental sulphur in vitro to thiosulphate, tetrathionate and sulphate, and mineralized peptone oxidizing the liberated ammonium to nitrite and nitrate. When elemental sulphur was added to peptone-containing medium the fungus oxidized sulphur but its ability to nitrify was inhibited. Such inhibition could have resulted from the reduction in medium pH following S-oxidation. Nitrification was still inhibited however, when S-oxidation occurred in medium buffered to pH 7 0, which suggests that pH was not the only factor inhibiting nitrification. Thiosulphate inhibited nitrification by A. flavus at concentrations similar to that produced by the fungus when oxidizing sulphur. Higher rates of nitrification by A. flavus occurred in medium containing ammonium sulphate and acetate, and in this medium thiosulphate (100 fig ml-1) prevented nitrification by A. flavus for one week, and reduced the rate of nitrification after three weeks. A. flavus appears to be incapable of simultaneously nitrifying and oxidizing sulphur due to the inhibitory effect of thiosulphate.

Oxygen uncouples light absorption by the chlorosome antenna and photosynthetic

electron transfer in the green sulfur bacterium Chlorobium tepidum

Abstract

In photosynthetic green sulfur bacteria excitation energy is transferred from large bacteriochlorophyll (BChl) c chlorosome antennas via small BChl a antennas to the reaction centers which then transfer electrons from cytochrome c to low-potential iron-sulfur proteins. Under oxidizing conditions a reversible mechanism is activated in the chlorosomes which quenches excited BChl c. We used flash-induced cytochrome c oxidation to investigate the effect of this quenching on photosynthetic electron transfer in whole cells of Chlorobium tepidum. The extent of cytochrome c photooxidation under aerobic conditions decreased to approx. 3%o of that under anaerobic conditions when BChl c was excited under light-limiting conditions. Photooxidation obtained by excitation of BChl a was similar under aerobic and anaerobic conditions. We interpret this drastic decrease in energy transfer from BChl c to the reaction center as a consequence of the quenching mechanism which is activated by O2. This reversible uncoupling of the chlorosome antenna might prevent formation of toxic reactive oxygen species from photosynthetically produced reductants under aerobic conditions. The green filamentous bacterium Chloroflexus aurantiacus also contains chlorosomes but energy transfer from the BChl c and BChl a antennas to the reaction center in this species was not affected by O2.

Phosphogypsum biotransformation in cultures of sulphate reducing bacteria in whey

Assemblages of anaerobic sulphidogenic microorganisms were isolated from soil polluted by oil-derived products and grown using the microcosms method. The cultures were grown in minimal and Postgate media with phosphogypsum (PG) as the sole electron acceptor and with lactate, casein or lactose as the sole carbon source. The most effective was the assemblage in Postgate medium with lactose as the sole carbon source. A reduction of 980 mg COD T1 (reduction of about 40%) and 790 mg SO4~ l_1 (reduction of 53% of phosphogypsum introduced to the medium) was noted in the culture. The lowest activity was observed for minimal medium with lactose as sole carbon source (reduction of 4.4% COD and 40% PG). The selected assemblage became an inoculum for a culture in Postgate, minimal and/or distilled water medium with PG (6 g l-1) and cheese whey (2.5 and 4.5 g l-1).

A percentage reduction of COD and SO4~ of PG was observed in all cultures. After growth, the residues were weighed and in all cases a distinct mass reduction of PG was observed in comparison to the 6 g l_1 introduced to the medium. Diffractometric studies of the residues confirmed the presence of calcite and apatite. The presence of these mineral phases in the residues allows their application as agricultural fertilisers.

Pitting corrosion behavior of 316L

stainless steel in the media of sulphate-reducing and iron-oxidizing bacteria Pitting corrosion behavior of 316L SS was investigated in the presence of aerobic and anaerobic bacteria isolated from cooling water system in oil refinery using polarization measurement, electrochemical impedance spectroscopy, scanning electron microscopy examinations and energy dispersive spectrum analysis. The results show the corrosion potential (Ecorr), pitting potential (Epit) and polarization resistance (RP) of 316L SS had a distinct decrease in the presence of bacteria, in comparison with those observed in the sterile medium for the same exposure time interval. Micrometer-scale pitting was observed on the 316L SS surface in the presence of bacteria. The combination of SRB and IOB demonstrated higher corrosion rates than SRB or IOB alone. The synergy of 0.01 M NaCl + SRB + IOB yielded the highest corrosion rate. The synergies between the metal surface, abiotic corrosion products, chloride anion, and bacterial cells and their metabolic products increased the corrosion damage degree of the passive film and accelerated pitting propagation.	[redacted]
Polysunfua reduction by Clostridium relatives isolated from sulfate-reducing enrichment cultures Sulfur is almost insoluble in water at ambient temperatures, and therefore polysunfua (S?,-) has been considered as a	[redacted]

possible intermediate that is used directly by bacteria in sulfur respiration. Sulfur-reducing reductases have been purified and characterized from a few sulfur reducers. However, polysunfua reduction has only been confirmed in Wolinella succinogenes. In our previous study, the direct production of hydrogen sunfua from polysunfua was confirmed by an enrichment culture obtained from natural samples under sulfate-reducing conditions. The present study attempted to isolate and identify polysunfua-reducing bacteria from the enrichment cultures. Almost all the isolated strains were classified into the genus Clostridium, based on 16S rRNA gene sequence analysis. The isolates, and some closely related strains, were able to reduce polysunfua to hydrogen sunfua. During production of 1 mol of hydrogen sunfua, approximately 2 mol of lactate was converted to acetate. Thus, dissimilatory polysunfua reduction occurred using lactate as an electron donor. The ability to reduce elemental sulfur was also examined with the isolates and the related strains. Although elemental sulfur reducing strains can reduce polysunfuas, not all polysunfua-reducing strains can reduce elemental sulfur. These results demonstrate that the conversion of elemental sulfur to polysunfua seems to be important in the reduction process of sulfur.	[redacted]
Production of Hydrogen Sunfua from Tetrathionate by the Iron- Oxidizing Bacterium Thiobacillus ferrooxidans NASF-1	

When incubated under anaerobic conditions, five strains of Thiobacillus ferrooxidans tested produced hydrogen sunfua (H2S) from elemental sulfur at pH 1.5. However, among the strains, T. ferrooxidans NASF-1 and AP19-3 were able to use both elemental sulfur and tetrathionate as electron acceptors for H2S production at pH 1.5. The mechanism of H2S production from tetrathionate was studied with intact cells of strain NASF-1. Strain NASF-1 was unable to use dithionate, trithionate, or pentathionate as an electron acceptor. After 12 h of incubation under anaerobic conditions at 30°C, 1.3 fmo\ of tetrathionate in the reaction mixture was decomposed, and 0.78 //mol of H2S and 0.6 /anol of trithionate were produced. Thiosulfate and sulfite were not detected in the reaction mixture. From these results, we propose that H2S is produced at pH 1.5 from tetrathionate by T. ferrooxidans NASF-1, via the following two-step reaction, in which AH2 represents an unknown electron donor in NASF-1 cells. Namely, tetrathionate is decomposed by tetrathionate-decomposing enzyme to give trithionate and elemental sulfur (S4062~—►S3062- +S°, Eq. 1), and the elemental sulfur thus produced Is reduced by sulfur reductase using electrons from AH2 to give H2S (S°+AH2—>-H2S+A, Eq. 2). The optimum pH and temperature for H2S production from tetrathionate under argon gas were 1.5 and 30°C, respectively. Under argon gas, the H2S production from tetrathionate stopped after Id of incubation, producing a total of 2.5 /onol of H2S/5 mg protein. In contrast, under H2

conditions, H2S production continued for 6 d, producing a total of 10.0 jumol of H2S/5 mg protein. These results suggest that electrons from H2 were used to reduce elemental sulfur produced as an intermediate to give H2S. Potassium cyanide at 0.5 mM slightly inhibited H2S production from tetrathionate, but increased that from elemental sulfur 3-fold. 2,4- Dinitrophenol at 0.05 mM, carbonylcyanide-m-chlorophenyl-hydrazone at 0.01 mM, mercury chloride at 0. 05 mM, and sodium selenate at 1.0 mM almost completely inhibited H2S production from tetrathionate, but not from elemental sulfur.

Refinement of Low-Grade Clay by Microbial Removal of Sulfur and Iron Compounds Using Thiobacillus ferrooxidans

The refinement of low-grade clay, of which impurities are mainly sulfur and iron compounds, is required because of the recent shortage of high-grade clay for manufacturing of structural ceramics. The major impurity compound contained in the low-grade clay we treated was identified as pyrite by X-ray powder diffraction and inductively coupled plasma analyses. The well-formed crystals of pyrite had a framboidal form of 1 jum-20 fim diameter. The microbial removal of pyrite from the low-grade clay was investigated by using a sulfur and iron-oxidizing bacterium, Thiobacillus ferrooxidans. About 82-90% of the pyrite was removed in 5-12 d for pulp densities up to 70% (w/v). The removal rate of pyrite ranged from 270 to 914 mg-pyritic sulfur//-d depending upon

clay pulp density. The rate of pyrite removal (r) could be expressed as a function of pyritic sulfur concentration (S): r (mg-pyritic sulfur//*h) = 1.96 x 10~2 S (mg-pyritic sulfur//). The logarithm of the amount of oxidized pyrite per unit volume and the final pH in the reaction medium were found to have a linear relationship which could be expressed as pH = 2.43-0.55 log [FeS2 (mM)]. With the refined clay no red color due to the presence of pyrite was developed after firing, and its whiteness was similar to that of a high-grade clay.

Structural and Molecular Genetic Insight into a Widespread Sulfur Oxidation Pathway

Many environmentally important photo- and chemolithoautotrophic bacteria accumulate globules of polymeric, water-insoluble sulfur as a transient product during oxidation of reduced sulfur compounds. Oxidation of this sulfur requires the concerted action of Dsr proteins. However, individual functions and interplay of these proteins are largely unclear. We proved with a AdsrE mutant experiment that the cytoplasmic a2p>2Y2-structured protein DsrEFH is absolutely essential for the oxidation of sulfur stored in the intracellular sulfur globules of the purple sulfur bacterial model organism Allochromatium vinosum. The ability to degrade stored sulfur was fully regained upon complementation with dsrEFH in trans. The crystal structure of DsrEFH was determined at 2.5 A resolution to assist functional assignment in detail. In

conjunction with phylogenetic analyses, two different types of putative active sites were identified in DsrE and DsrH and shown to be characteristic for sulfur-oxidizing bacteria. Conserved Cys78 of A. vinosum DsrE corresponds to the active cysteines of Escherichia coli YchN and TusD. TusBCD and the protein TusE are parts of sulfur relay system involved in thiouridine biosynthesis. DsrEFH interacts with DsrC, a TusE homologue encoded in the same operon. The conserved penultimate cysteine residue in the carboxy-terminus of DsrC is essential for the inter-action. Here, we show that Cys78 of DsrE is strictly required for interaction with DsrC while Cys20 in the putative active site of DsrH is dispensable for that reaction. In summary, our findings point at the occurrence of sulfur transfer reactions during sulfur oxidation via the Dsr proteins.

KẾT THÚC PHẦN LOAN DỊCH LẦN I

BẮT ĐẦU PHẦN QUÍ DỊCH LẦN I

Structural Basis for the Thermostability of Sulfur Oxygenase Reductases

Abstract The thermostability of three sulfur oxygenase reductases (SORs) was investigated from thermoaci- dophilic achaea Acidianus tengchongensis (SORAT) and Sulfolobus tokodaii (SORst) as well as the moderately thermophilic bacterium Acidithiobacillus sp. SM-1 (SORsb). The optimal temperatures for catalyzing sulfur oxidation were 80 °C (SORat), 85 °C (SORst), and 70 °C (SORsB), respectively. The half-lives of the three SORs at their optimal catalytic conditions were 100 min (SORAT), 58 min (SORst), and 37 min (SORsb). In order to reveal the structural basis of the thermostability of these SORs, three-dimensional structural models of them were generated by homology modeling using the previously reported high-resolution X-ray structure of SORAA (from Acidianus ambivalens) as a template. The results suggest that thermostability was dependent on: (a) high number of the charged amino acid glutamic acid and the flexible amino acid proline, (b) low number of the thermolabile amino acid glutamine, (c) increased number of ion pairs, (d) decreased ratio of hydrophobic accessible solvent surface area (ASA) to charged ASA, and (e) increased volumes of the cavity. The number of cavities and the number of hydrogen bonds did not significantly affect the thermostability of SORs, whereas the cavity volumes increased as the thermal stability increased.

[redacted]

[redacted]

Structural insight into SoxC and SoxD interaction and their role in electron transport process in the novel global sulfur

[redacted]

cycle in Paracoccus pantotrophus Abstract Microbial oxidation of reduced inorganic sulfur compounds mainly sulfur anions in the environment is one of the major reactions of the global sulfur cycle mediated by phylogenetically diverse prokaryotes. The sulfur oxidizing gene cluster (sox) of a-Proteobacteria comprises of at least 16 genes, which form two transcriptional units, viz., soxSRT and soxVWXYZABCDEFGH. Sequence analysis reveals that soxD gene product (SoxD) belongs to the di-heme cytochrome c family of electron transport proteins whereas soxC gene product (SoxC) is a sulfur dehydrogenase. We employed homology modeling to construct the three-dimensional structures of the SoxC and SoxD from Paracoccus pantotrophus. SoxD protein is known to interact with SoxC. With the help of docking studies we have identified the residues involved in the interaction of SoxC and SoxD. The putative active site geometries of these two proteins as well as the structural basis of the involvements of these proteins in electron transport process during the oxidation of sulfur anions are also investigated.	[redacted]
Structural study of two proteins SigE and ORF1 to predict their roles in the biochemical oxidation of sulfur anions via the global sulfur oxidation operon (sox)	[redacted]

Abstract

Microbial redox reactions involving inorganic sulfur compounds in the environment are one of the major reactions of the global sulfur cycle. These reactions are mediated by phylogenetically diverse prokaryotes containing the sulfur oxidizing gene cluster (sox). The sox gene cluster of a-Proteobacteria comprises of at least 15 genes, which form two transcriptional units. Recently two new orfs, which code for proteins named, SigE and ORF1, were identified in Starkeya novella. Sequence analyses reveal that SigE protein has the signature sequence of ECF-type sigma factors and a helix-turn-helix (HTH) DNA binding motif whereas ORF1 is possibly an anti ECF-sigma factor, which also has the signature sequence of the dsr family of sulfate ion binding proteins. We employed homology modeling to construct the three-dimensional structures of these proteins. The model of SigE was docked on to its promoter DNA to investigate the favourable binding modes of the protein. Interactions of SigE with its anti-sigma factor ORF1 were also reported after docking these proteins. We also identified the putative sulfate ion binding residues of ORF1 by docking sulfate ion on to it. Our study provides a rational framework for understanding of the structural as well as the molecular basis of the mechanism of the regulation of sulfur oxidation reactions by SigE and ORF1 proteins via the sox operon.

Structure of the cytochrome complex SoxXA of Paracoccus pantotrophus, a

heme enzyme initiating chemotrophic sulfur oxidation

Abstract

The sulfur-oxidizing enzyme system (Sox) of the chemotroph Paracoccus pantotrophus is composed of several proteins, which together oxidize hydrogen sunfua, sulfur, thiosulfate or sulfite and transfers the gained electrons to the respiratory chain. The hetero-dimeric cytochrome c complex SoxXA functions as heme enzyme and links covalently the sulfur substrate to the thiol of the cysteine-138 residue of the SoxY protein of the SoxYZ complex. Here, we report the crystal structure of the c-type cytochrome complex SoxXA. The structure could be solved by molecular replacement and refined to a resolution of 1.9 A identifying the axial heme-iron coordination involving an unusual Cys-251 thiolate of heme2. Distance measurements between the three heme groups provide deeper insight into the electron transport inside SoxXA and merge in a better understanding of the initial step of the aerobic sulfur oxidation process in chemotrophic bacteria.

Sulfur activation-related extracellular proteins of Acidithiobacillus ferrooxidans

Abstract: The fractions of the extracellular proteins of Acidithiobacillus ferrooxidans grown on two different energy substrates, elemental sulfur and ferrous sulfate, were selectively prepared with hot water treatment and distinctly shown by two-dimensional gel electrophoresis. Some protein spots with apparently higher abundance in sulfur energy substrate than in ferrous sulfate energy substrate were

identified by using MALDI-TOF/TOF. Based on peptide mass fingerprints and bioinformatical analysis, the extracellular proteins were classified according to their functions as conjugal transfer protein, pilin, vacJ lipoprotein, polysaccharide deacetylase family protein, Ser/Thr protein phosphatase family protein and hypothetical proteins. Several extracellular proteins were found abundant in thiol groups and with CXXC functional motif, these proteins may be directly involved in the sulfur activation by use of their thiol group (Pr-SH) to bond the elemental sulfur.

Sulfur oxidation in Paracoccus pantotrophus: interaction of the sulfur-binding protein SoxYZ with the dimanganese SoxB protein

Abstract

The central protein of the sulfur-oxidizing enzyme system of Paracoccus pantotrophus, SoxYZ, formed complexes with subunits associated and covalently bound. In denaturing SDS-polyacrylamide gel electrophoresis (PAGE) SoxY migrated at 12 and SoxZ at 16 kDa. SDS-PAGE of homogeneous SoxYZ without reductant separated dimeric complexes of 25, 29, and 32 kDa identified by the N-terminal amino acid sequences as SoxY-Y, SoxY-Z, and SoxZ-Z, and subunit cleavage by reduction suggested their linkage via protein disunfua bonds. SoxYZ was reversibly redox active between -0.25 and 0.2 V, as monitored by a combined electrochemical and FTIR spectroscopic approach. The dimanganese SoxB protein

(58.611 Da) converted the covalently linked heterodimer SoxY-Z to SoxYZ with associated subunits which in turn aggregated to the heterotetramer Sox(YZ)2. This reaction depended on time and the SoxB concentration, and demonstrated the interaction of these two Sox proteins.

Surface characteristics and aggregation of microbiologically produced sulphur particles in relation to the process conditions

Abstract

The effect of surface properties and the effects of several process conditions, e.g. loading rate, ionic strength and the presence of polymers, on the degree of aggregation of sulphur particles were studied. Sulphur is formed under oxygen-limiting circumstances during the partial oxidation of sulphide by a mixed culture of thiobacillus-like bacteria. Since the freshly excreted particles are in a colloidal state, with a diameter of approximately 100 nm, their aggregation is a prerequisite in order to obtain a satisfactory sedimentation. Titration experiments revealed that the negative sulphur surface charge is determined by the presence of multiple functional groups. Attention was also paid to the effect of the chain length, hydrophilicity and charge of a number of dissolved polymers on the degree of sulphur aggregation. The degree of polymer adsorption on the sulphur surface mainly depends on the hydrophobicity and charge of the polymer. Since the charge of

biologically produced sulphur is negative at pH 8.0, a highly charged cationic polymer like Qn-HEC inhibits the sulphur aggregation. For Perfectamyl and carboxymethylcellulose no clear effect was measured. Particularly for long-chain polymers, a distinct negative effect on the aggregation was found. Steric hindrance, apparently, is an important factor in the aggregation process. Upon increasing the sulphide loading rate, larger sulphur aggregates were formed while the opposite trend was observed for increasing salt concentrations. In practice, therefore, a sulphide-oxidizing bioreactor should be operated at high loading rates to enhance the settleability of the sulphur sludge. The behavior of nitrifying sludge in presence of sulfur compounds using a floating biofilm reactor The tolerance, kinetic and oxidizing capability of a nitrifying sludge exposed to different initial concentrations of sunfua (1.7 to 18 mg/L) was evaluated in batch experiments. A nitrifying sludge fed with ammonium and thiosulfate and produced in steady state conditions was used as inoculum. Sunfua induced a significant effect either on ammonium consumption rates or nitrite accumulation. In spite of the nitrifying kinetic was affected, the ammonium consumption efficiencies were close to 100%, with nitrate production yields around 1.0. The IC50 value for ammonium oxidizing-process was 13 mg/L of sunfua. Sunfua was oxidized in two steps: first sunfua was oxidized to elemental sulfur and afterward into sulfate. FISH oligonucleotide probes for	[redacted]

Thiobacillus denitrificans, Nitrosomonas spp., and Nitrobacter spp. were used in order to know if these bacteria were part of the microbial ecology. The obtained results showed that under nitrifying conditions are possible to carry out simultaneously two biological processes, nitrification and sulfur oxidation.

[redacted]

The Crystal Structure of the [NiFe] Hydrogenase from the Photosynthetic Bacterium Allochromatium vinosum: Characterization of the Oxidized Enzyme (Ni-A State)

The crystal structure of the membrane-associated [NiFe] hydrogenase from Allochromatium vinosum has been determined to 2.1 A resolution. Electron paramagnetic resonance (EPR) and Fourier transform infrared spectroscopy on dissolved crystals showed that it is present in the Ni-A state (>90%). The structure of the A. vinosum [NiFe] hydrogenase shows significant similarities with [NiFe] hydrogenase structures derived from Desulfovibrio species. The amino acid sequence identity is ~ 50%. The bimetallic [NiFe] active site is located in the large subunit of the heterodimer and possesses three diatomic non-protein ligands coordinated to the Fe (two CN- , one CO). Ni is bound to the protein backbone via four cysteine thiolates; two of them also bridge the two metals. One of the bridging cysteines (Cys64) exhibits a modified thiolate in part of the sample. A mono-oxo

[redacted]

bridging ligand was assigned between the metal ions of the catalytic center. This is in contrast to a proposal for Desulfovibrio sp. hydrogenases that show a di-oxo species in this position for the Ni-A state. The additional metal site located in the large subunit appears to be a Mg2+ ion. Three iron-sulfur clusters were found in the small subunit that forms the electron transfer chain connecting the catalytic site with the molecular surface. The calculated anomalous Fourier map indicates a distorted proximal iron-sulfur cluster in part of the crystals. This altered proximal cluster is supposed to be paramagnetic and is exchange coupled to the Ni3+ ion and the medial [Fe3S4]+ cluster that are both EPR active (S = 1/2 species). This finding of a modified proximal cluster in the [NiFe] hydrogenase might explain the observation of split EPR signals that are occasionally detected in the oxidized state of membrane-bound [NiFe] hydrogenases as from A. vinosum.

The reaction center of green sulfur bacteria

The sulfur oxygenase reductase from Acidianus ambivalens is an icosatetramer as shown by crystallization and Patterson analysis

Abstract

The sulfur oxygenase reductase (SOR) is the initial enzyme in the aerobic sulfur metabolism of the thermoacidophilic and chemolithoautotrophic crenarchaeote Acidianus ambivalens. Single colorless

polyhedral crystals were obtained under two crystallization conditions from SOR preparations heterologously overproduced in Escherichia coli. They belonged to space-group 14 and diffraction data were collected up to 1.7 A resolution. Their Patterson symmetry shows additional 4-, 3- and 2-fold non-crystallographic symmetry rotation axes, characteristic of the point group 432. Taking into account the molecular mass of SOR, the crystal unit cell volume, the non- crystallographic symmetry operators and previous electron microscopy studies of the SOR, it was deduced that the quaternary structure of the functionally active enzyme is an icosatetramer with 871 kDa molecular mass.

ARTICLE INFO ABSTRACT

Behind the versatile nature of prokaryotic energy metabolism is a set of redox proteins having a highly mod- 23 ular character. It has become increasingly recognized that a limited number of redox modules or building 24 blocks appear grouped in different arrangements, giving rise to different proteins and functionalities. This 25 modularity most likely reveals a common and ancient origin for these redox modules, and is obviously 26 reflected in similar energy conservation mechanisms. The dissimilation of sulfur compounds was probably 27 one of the earliest biological strategies used by primitive organisms to obtain energy. Here, we review 28 some of the redox proteins involved in dissimilatory sulfur metabolism, focusing on sulfate reducing organ- 29 isms, and highlight links

between these proteins and others involved in different processes of anaerobic res- 30 piration. Noteworthy, are links to the complex iron-sulfur molybdoenzyme family, and heterodisunfua 31 reductases of methanogenic archaea. We discuss how chemiosmotic and electron bifurcation/confurcation 32 may be involved in energy conservation during sulfate reduction, and how introduction of an additional 33 module, multiheme cytochromes c, opens an alternative bioenergetic strategy that seems to increase meta- 34 bolic versatility. Finally, we highlight new families of heterodisunfua reductase-related proteins from 35 non-methanogenic organisms, which indicate a widespread distribution for these protein modules and 36 may indicate a more general involvement of thiol/disunfua conversions in energy metabolism. This article 37 is part of a Special Issue entitled: The evolutionary aspects of bioenergetics systems.

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KẾT THÚC PHẦN QUÍ DỊCH LẦN I

BẮT ĐẦU PHẦN LOAN DỊCH LẦN II
(21/5/2013)

Adenylylsulfate reductases from archaea and bacteria are 1:1 ap-heterodimeric iron-sulfur flavoenzymes - high similarity of molecular properties emphasizes their

central role in sulfur metabolism

Abstract Highly active adenylylsulfate (APS) reductase was isolated under N2 /H2 from sulfate-reducing and sunfua-oxidizing bacteria and archaea. It was a 1 :1 KKLL -heterodimer of molecular massW95 kDa, and two subunits (KW75, L W20 kDa). The specific activity was 11^14 Wmol (min mg)31; cofactor analysis revealed 0.96 þ 0.05 FAD, 7.5 þ 0.1 Fe and 7.9 þ 0.25 S23. The photochemically reduced enzyme had a multiline EPR spectrum resulting from two interacting [4Fe^4S] centers. The properties of the different APS reductases were remarkably similar, although the enzyme is involved in different metabolic pathways and was isolated from phylogenetically far separated organisms. A structural model is proposed, with FAD bound to the K-subunit, and two [4Fe^4S] centers located in close proximity on the L-subunit.

Analysis of iron- and sulfur-oxidizing bacteria in a treatment plant of acid rock drainage from a Japanese pyrite mine by use of ribulose-1, 5-bisphosphate carboxylase/oxygenase large-subunit gene

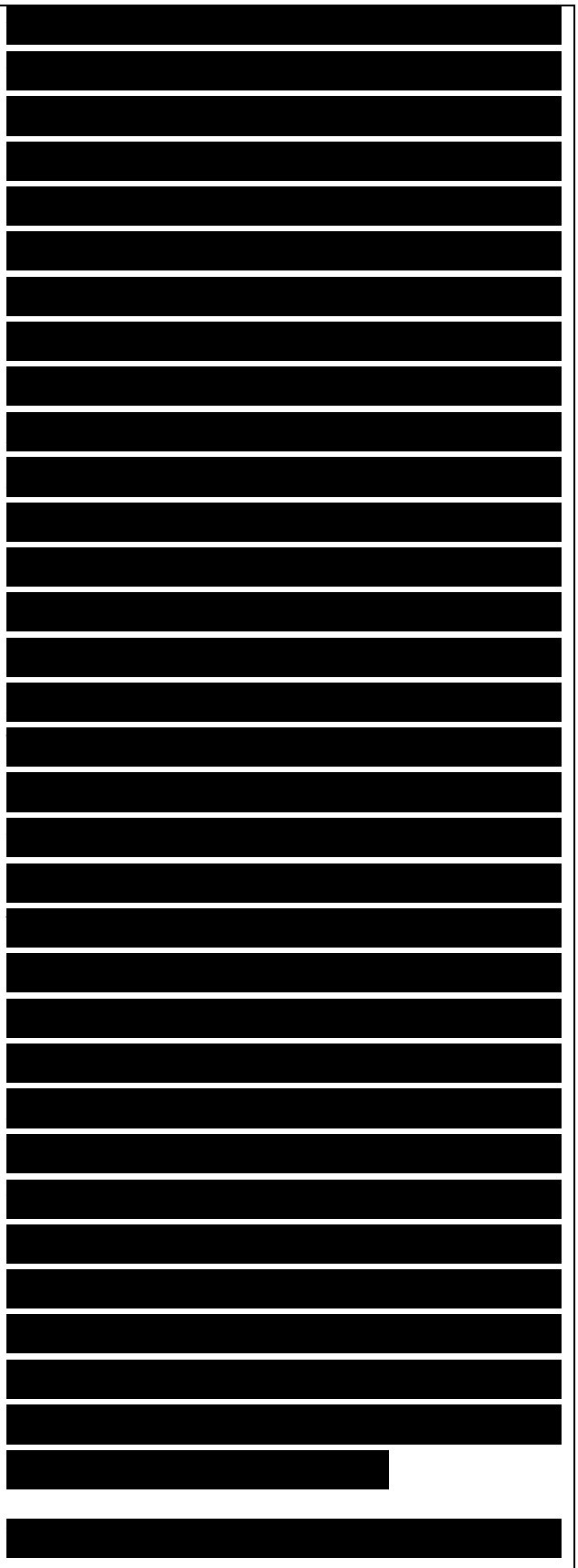
Iron- and sulfur-oxidizing bacteria in a treatment plant of acid rock drainage (ARD) from a pyrite mine in Yanahara, Okayama prefecture, Japan, were analyzed using the gene (cbbL) encoding the large subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO). Analyses of partial sequences of cbbL genes from Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Acidithiobacillus caldus strains revealed the diversity in their cbbL gene sequences.

In contrast to the presence of two copies of form I cbbL genes (cbbL1 and cbbL2) in A ferrooxidans genome, A thiooxidans and A. caldus had a single copy of form I cbbL gene in their genomes. A phylogenetic analysis based on deduced amino acid sequences from cbbL genes detected in the ARD treatment plant and their close relatives revealed that 89% of the total clones were affiliated with A ferrooxidans. Clones loosely affiliated with the cbbL from A. thiooxidans NB1-3 or Thiobacillus denitrificans was also detected in the treatment plant. cbbL gene sequences of iron- or sulfur-oxidizing bacteria isolated from the ARD and the ARD treatment plant were not detected in the cbbL libraries from the treatment plant, suggesting the low frequencies of isolates in the samples.

ATP requirements for growth and maintenance of iron-oxidizing bacteria

Abstract

A simple metabolic model of ferrous oxidizing bacteria based on biochemically structured balances of ATP and NAD(P)H is proposed in order to calculate maximum yield and maintenance on ATP. Similar values of growth yield and maintenance were obtained using data of ferrous iron and/or oxygen consumption in Acidithiobacillus ferrooxidans cultures on iron under different conditions. When pyrite was the sole energy source, growth yield was higher suggesting cells could obtain energy through the sulfur compounds oxidation. Values of growth yields for Leptospirillum ferrooxidans cultures on iron were a bit lower than those obtained for A. ferrooxidans



although within the experimental errors. The maintenance coefficients on ATP for both bacteria were similar and comparable to those observed in heterotrophic microorganisms. This fact is really surprising taking in account the high proton gradient that this kind of microorganisms should maintain.

Autotrophic denitrification and chemical phosphate removal of agro-industrial wastewater by filtration with granular medium

Abstract

A novel granular medium consisting (1.5-5 mm in diameter) of inert perlite particles as nuclei and an effective surface layer containing sulfur, CaCO3 and Mg(OH)2 was developed for advanced treatment of agro-industrial wastewater. The performance of the medium was examined with a laboratory-scale down-flow fixed-bed column reactor using piggery wastewater, which had been treated by an upflow anaerobic sludge blanket reactor and a trickling filter. The removal efficiency of NO“-N was more than 70% with a NO“-N loading rate of less than approximately 0.3 kg N m_3 d_1; the removal efficiency dropped due to the accumulation of nitrite when the loading rate exceeded that value. A significant drop of phosphate and Mg2+ concentrations occurred when the effluent pH exceeded 7.9. Ammonium was removed with an average removal efficiency of 12.4%. These results indicated that the crystalline reaction of PO4_, Mg2+ and NH^ (MAP reaction) under alkaline conditions contributed to the removal of phosphate. This medium

could be useful for the simultaneous reduction of nitrogenous and phosphorus compounds in biologically treated agro-industrial wastewater.

Bulk and surface characterization of crystalline and plastic sulphur oxidized by two Thiobacillus species

Abstract

This work studies the surface interaction between Thiobacillus ferrooxidans and Thiobacillus thiooxidans with crystalline and plastic elemental sulphur. The interaction mechanisms were analysed by fractal geometry which describes textural modifications of the substrate caused by bacterial action. The results demonstrated that the bacteria are able to produce two different effects depending on the substrates. Only surface smoothing (decrease on fractal dimension values) was detected on crystalline sulphur (this effect being stronger with T. ferrooxidans than with T. thiooxidans), but, perforation of the bulk was also observed in plastic sulphur

Comparative study on effects of Tween-80 and sodium isobutyl-xanthate on growth and sulfur-oxidizing activities of Acidithiobacillus albertensis BY-05

Abstract: Effects of two typical surfactants, Tween-80 and sodium isobutyl-xanthate (NaIBX), with different concentrations on the growth and sulfur-oxidizing activities of a new strain Acidithiobacillus albertensis BY-05, an acidophilic sulfur-oxidizing bacterium, were investigated. The results indicate that both surfactants can enhance the growth and sulfur-oxidizing activities of A.

albertensis BY-05 only at some special concentrations, e.g., 10~4-10~8 g/L for NaIBX and lower than 10~8 g/L for Tween-80, but were inhibited and even harmful at higher concentrations. Both surfactants can not be metabolized by A. albertensis BY-05. The contact between the bacteria and the sulfur particles may be dependent upon both the extracellular substance and the surfactants, both of which provide the amphiphilic environment improving the attachment for bacteria to the sulfur particles surface. These data could be significant for enlarging the applications of both A. albertensis BY-05 and some typical surfactants for industrial bioleaching of sunfuas minerals.

Comparison of reactive porous media for sulfur-oxidizing denitrification of high nitrate strength wastewater

Three packing materials for sulfur oxidizing denitrification packed bed systems seeded with acclimated anoxic sludge were evaluated. Two porous media were prepared via thermal fusion with sodium bicar¬bonate as porogen: sulfur fused with powdered (1) calcium carbonate (CaCO3)(SCa) and (2) oyster shell (SCr). Randomly packed sulfur and limestone granules (S + L) media were used as the control. Results revealed that SCr is the most suitable media as it exhibited the highest nitrate removals and lowest nitrite accumulation. It has macrovoidal pores which facilitated microbial attachment. Additionally, SCr had the highest CaCO3 loading per unit volume and highest media dissolution rate which was favorable to avert pH decrease.

But due to high denitrification activity, high sulfate levels in SCr may necessitate a post-treatment step prior to effluent discharge. Due to poor biomass attachment, S + L is most sensi¬tive to change in fluid flow condition. As hydraulic retention time is decreased, S + L exhibited intensive and irreversible performance decline. Inferior denitrification performance of SCa was mainly due to low CaCO3 loading per unit volume, low dissolution kinetics and low alkalinity consumption by denitrifiers. Using modified Stover-Kincannon kinetic model, overall performance and denitrification capacities can be arranged as SCr > S + L > SCa.

Determination of the intrinsic kinetic parameters of sunfua-oxidizing autotrophic denitrification in differential reactors containing immobilized biomass

Nitrogen removal coupled with sunfua oxidation has potential for the treatment of effluents from anaer¬obic reactors because they contain sunfua, which can be used as an endogenous electron donor for deni-trification. This work evaluated the intrinsic kinetics of sunfua-oxidizing autotrophic denitrification via nitrate and nitrite in systems containing attached cells. Differential reactors were fed with nitrified syn¬thetic domestic sewage and different sunfua concentrations. The intrinsic kinetic parameters of nitrogen removal were determined when the mass transfer resistance was negligible. This bioprocess could be described by a half-order kinetic model for biofilms. The half-order kinetic coefficients ranged from 0.425 to 0.658 mg N1/2 L 1/2 h 1 for

denitrification via nitrite and from 0.190 to 0.609 mg N1/2 L 1/2 h 1 for denitrification via nitrate. In this latter, the lower value was due to the use of electrons donated from intermediary sulfur compounds whose formation and subsequent consumption were detected.

Effect of pH on anoxic sunfua oxidizing reactor performance

Abstract

The effects of pH on the performance of anoxic sunfua oxidizing (ASO) reactor were evaluated. Performance was investigated under various operational conditions at influent pH range of 4-11. At the influent pH of 7-7.5 during loading tests and HRT tests, the sunfua oxidation was partial. In general, the amount of sulfate formed decreased with the increasing sunfua and nitrite loadings. The bacterial communities in ASO reactors were more sensitive to acidic pH compared with alkaline pH, as nitrite and sunfua removal rates dropped significantly when exposed to acidic pH 3. High dissolved bisunfua ions, nitrite and excess of sulfate (>300 mg/L) might have inhibited the sunfua oxidation under highly acidic and alkaline conditions in the ASO reactor. Based on sunfua and nitrite removal efficiencies, the ASO reactor can be operated in a wide range of pH, i.e. 5-11.

Effects of Cattle-Slurry Treatment on the Microorganisms of the Carbon- and Sulphur-Cycles in the Soil

ABSTRACT

The addition of cattle slurry to the soil brings about an increase in the number of microorganisms of the carbon and sulphur

cycles, though the levels attained do not exceed normal large population densities. The dynamics of the rise depend on the chemical and microbial composition of the slurry and the process by which it is incorporated into the soil. The aerobic cellulolytic and pectinolytic populations are favoured in the long term, whereas the numbers of anaerobic cellulolvtics and amylolytics increase rapidly on slurry treatment only to fall sharply shortly thereafter, and in the case of amylolytics these processes result in a net fall in population density. The rise in microbial populations after slurry treatment is due more to the added numbers of microorganisms present in the slurry itself than to the substrate stimulating a population growth, but whereas added elemental sulphur oxidizers survive, anaerobic microorganisms such as sulphate reducers and anaerobic organic sulphur mineralizers die off after a short time.

Iron-Oxidizing and Leaching Activities of Sulphur-Grown Thiobacillus ferrooxidans Cells on Other Substrates: Effect of Culture pH

The rate of iron (II) oxidation by sulphur-grown Thiobacillus ferrooxidans cells decreased when the pH of the original growth medium was lowered. This behaviour was observed even after shifting from the original growth pH to a higher pH. After being suspended in medium at a pH higher than the growth pH, sulphur-grown cells could leach covellite at a similar initial rate to iron-grown cells. Sulphur-grown cells exhibited a long lag phase when the original growth pH was

low. These results were correlated with the number of protons associated with the cell surface, rather than with cell hydrophobicity or cell capacity to attach to solid particles. Sulphur-grown cells grown in very acidic media (without pH control) were not able to oxidize iron (II) or leach covellite even after shifting to a high pH.

Isolation and Characterization of Acidophilic Heterotrophic Iron-Oxidizing Bacterium from Enrichment Culture Obtained from Acid Mine Drainage Treatment Plant

An acidophilic heterotrophic bacterium, designated as HIB4, having the ability to oxidize fer-rous ion was newly isolated from a sample of an enrichment culture for iron-oxidizing bacteria, using the modified washed agarose/yeast extract (WAYE) medium with ferrous sulphate. The iso-late HIB4 was an acidophilic, heterotrophic, mesophilic and gram-positive bacterium. Phyloge- netically, it was classified under the genus Alicyclobacillus and was the closest to Alicyclobacillus disulfidooxidans SD-11 with 99.7% 16S rDNA homology. It grew and oxidized ferrous ion in the medium containing 0.02% (w/v) yeast extract. Yeast extract was an essential substrate for this bacterium because it could not grow or oxidize ferrous ion without yeast extract. However, a higher concentration of yeast extract inhibited the growth of HIB4, so that the optimum concen-tration of yeast extract for this bacterium to grow was 0.02% (w/v) at 0.08 mol/l of ferrous ion. On the other hand, ferrous ion oxidation occurred almost at the end of the bacterium's logarithmic growth phase and

the isolate was able to grow without ferrous ion. These results denote that HIB4 did not obtain any energy from the ferrous ion oxidation and that HIB4 is an obligate hetero- trophic and aerobic bacterium even though it oxidized ferrous ion. Also, HIB4 could not utilize any organic compounds, among the several organic chemicals used in this study, as a carbon source except yeast extract. These characteristics were completely different from these of A. disul¬fidooxidans SD-11 so that HIB4 might be a different species.

Isolation of Iron-Oxidizing Bacteria from Corroded Concretes of Sewage Treatment Plants

Thirty-six strains of iron-oxidizing bacteria were isolated from corroded concrete samples obtained at eight sewage treatment plants in Japan. All of the strains isolated grew autotrophically in ferrous sulfate (3.0%), elemental sulfur (1.0%) and FeS (1.0%) media (pH 1.5). Washed intact cells of the 36 isolates had activities to oxidize both ferrous iron and elemental sulfur. Strain SNA-5, a representative of the isolated strains, was a gram-negative, rod-shaped bacterium (0.5-0.6 x 0.9-1.5 fan). The mean G + C content of its DNA was 55.9 mol%. The pH and temperature optima for growth were 1.5 and 30°C, and the bacterium had activity to assimilate 14C02 into the cells when ferrous iron or elemental sulfur was used as a sole source of energy. These results suggest that SNA-5 is Thiobacillus ferrooxidans strain. The pHs and numbers of iron-oxidizing bacteria in corroded concrete samples obtained by boring to depths of 0-1,1-3, and 3-5 cm below the concrete surface

were respectively 1.4,1.7, and 2.0, and 1.2 x 10®, 5 x 107, and 5 x 10® cells/g concrete. The degree of corrosion in the sample obtained nearest to the surface was more severe than in the deeper samples. The findings indicated that the levels of acidification and corrosion of the concrete structure corresponded with the number of iron-oxidizing bacteria in a concrete sample. Sulfuric acid produced by the chemolithoautotrophic sulfur- oxidizing bacterium Thiobacillus thiooxidansis known to induce concrete corrosion. Since not only T. thiooxidans but also T. ferrooxidans can oxidize reduced sulfur compounds and produce sulfuric acid, the results strongly suggest that T. ferrooxidans as well as T. thiooxidans is involved in concrete corrosion.

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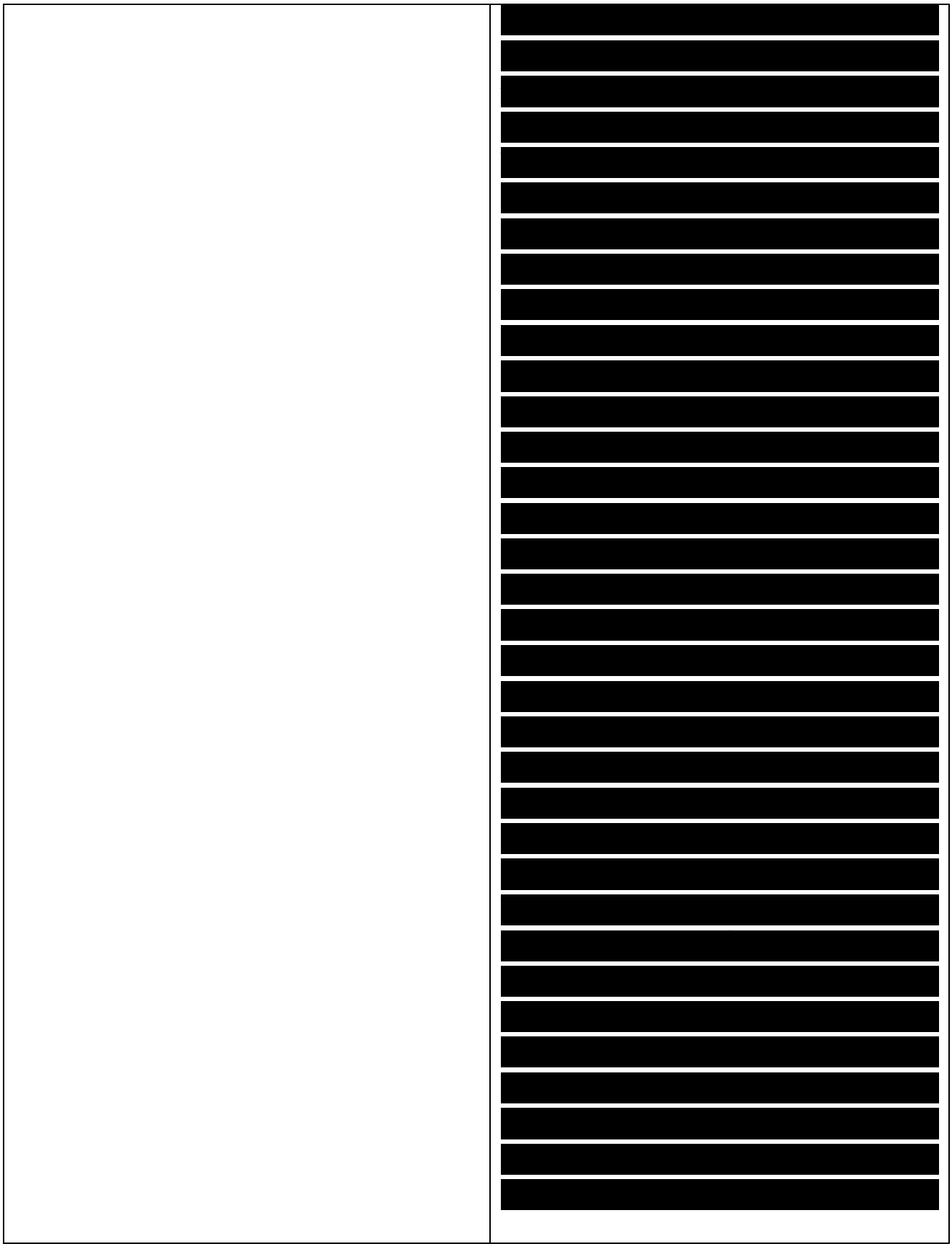

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Kinetics of the bio-oxidation of volatile reduced sulphur compounds in a biotrickling filter Mixtures of volatile reduced sulphur compounds (VRSCs) like hydrogen sulphide (H2S), methylmercaptan (MM), dimethyl sulphide (DMS) and dimethyl disulphide (DMDS) are found in gaseous emissions of several industrial activities creating nuisance in the surroundings. Hydrogen sulphide (H2S) decreases the	

removal efficiency of volatile reduced sulphur compounds (VRSCs) in biofilters but the kinetics of this effect is still unknown. Kinetic expressions that represent the rate of bio-oxidation of H2S, MM, DMS and DMDS are proposed. In order to observe and quantify this effect, equimolar mixtures of MM, DMS and DMDS were fed into a biotrickling filter inoculated with Thiobacillus thioparus at different H2S loads. Experimental results shown a good agreement with the simulations generated by the model considering the kinetic equations proposed. The estimated kinetic constants show that H2S and MM have a significant inhibitory effect on the bio-oxidation of DMS and DMDS, having the H2S the higher effect. Prokaryotic sulfur oxidation Recent biochemical and genomic data differentiate the sulfur oxidation pathway of Archaea from those of Bacteria. From these data it is evident that members of the Alphaproteobacteria harbor the complete sulfur-oxidizing Sox enzyme system, whereas members of the p and g subclass and the Chlorobiaceae contain sox gene clusters that lack the genes encoding sulfur dehydrogenase. This indicates a different pathway for oxidation of sulfur to sulfate. Acidophilic bacteria oxidize sulfur by a system different from the Sox enzyme	

system, as do chemotrophic endosymbiontic bacteria.	

Purification and characterization of sunfua dehydrogenase from alkaliphilic chemolithoautotrophic sulfur-oxidizing bacteria Abstract Extracts of the alkaliphilic sulfur-oxidizing autotroph strain AL3 contained	

sunfua:cytochrome c oxidoreductase. This was active above pH 8, and was associated with the cell membranes. Although up to 60% of the initial activity was lost during Triton X-100 extraction, further purification resulted in an enzyme that catalyzed sunfua oxidation with horse heart cytochrome c. This enzyme was a 41 kDa protein containing heme css4. The optimum pH of the membrane bound enzyme was 9.0, but after extraction this fell to 8.0. The enzyme catalyzed a single electron oxidation of HS—. Hydrosunfua radical is therefore the most probable product.	

Purification and Some Properties of Sulfur Reductase from the Iron- Oxidizing Bacterium Thiobacillus ferrooxidans NASF-1 Thiobacillus ferrooxidans strain NASF-1 grown aerobically in an Fe2+ (3%)-medium produces hydrogen sunfua (H2S) from elemental sulfur under anaerobic	

conditions with argon gas at pH 7.5. Sulfur reductase, which catalyzes the reduction of elemental sulfur (S°) with NAD(P)H as an electron donor to produce hydrogen sunfua (H2S) under anaerobic conditions, was purified 69-fold after 35-65% ammonium sulfate precipitation and Q-Sepharose FF, Phenyl-Toyopearl 650 ML, and Blue Sepharose FF column chromatography, with a specific activity of 57.6 U (mg protein)^1. The purified enzyme was quite labile under aerobic conditions, but comparatively stable in the presence of sodium hydrosulfite and under anaerobic conditions, especially under hydrogen gas conditions. The purified enzyme showed both sulfur reductase and hydrogenase activities. Both activities had an optimum pH of 9.0. Sulfur reductase has an apparent molecular weight of 120,000 Da, and is composed of three different subunits (Mr 54,000 Da (a), 36,000 Da (/S), and 35,000 Da (7-)), as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This is the first report on the purification of sulfur reductase from a mesophilic and obligate chemolithotrophic iron-oxidizing bacterium.	
Sewage sludge bioleaching by indigenous sulfur-oxidizing bacteria: Effects of ratio of substrate dosage to solid content	

The aim of this work was to study the effect of ratio of substrate dosage to solid content (Sd/SC) on sew¬age sludge bioleaching. The inocula - indigenous sulfur-oxidizing bacteria were enriched and cultured from the fresh activated sludge to a wastewater treatment plant. The results showed that Sd/SC signifi¬cantly influenced the sludge bioleaching process. With increase in Sd/SC the sludge bioleaching was enhanced, which was represented by the acceleration of sludge acidification, oxidizing environment for¬mation, and substrate (sulfur) utilization. Higher Sd/SC was more efficient to solubilize the heavy metals and total phosphorus (TP) than lower Sd/SC, while total nitrogen (TN) release was not influenced by Sd/ SC. Zinc and copper were efficiently bioleached because of sludge acidification and sludge oxidation, but lead was bioleached with a low efficiency because of the formation of low soluble PbSO4 precipitates. After bioleaching the biotoxicity of sewage sludge greatly reduced.	

Sulfur oxidation activities of pure and mixed thermophiles and sulfur speciation in bioleaching of chalcopyrite The sulfur oxidation activities of four pure thermophilic archaea Acidianus brierleyi (JCM 8954), Metal- losphaera sedula (YN 23), Acidianus manzaensis (YN 25) and Sulfolobus metallicus (YN 24) and their mixture in bioleaching chalcopyrite were compared. Meanwhile, the relevant surface sulfur speciation of chalco- pyrite leached with the mixed thermophilic archaea was investigated. The results showed that the	

mixed culture, with contributing significantly to the raising of leaching rate and accelerating the formation of leaching products, may have a higher sulfur oxidation activity than the pure cultures, and jarosite was the main passivation component hindering the dissolution of chalcopyrite, while elemental sulfur seemed to have no influence on the dissolution of chalcopyrite. In addition, the present results supported the former speculation, i.e., covellite might be converted from chalcocite during the leaching experi¬ments, and the elemental sulfur may partially be the derivation of covellite and chalcocite.	

SULPHUR OXIDATION BY FUNGI

Aspergillus niger oxidized elemental sulphur in vitro to form relatively large amounts of sulphate, initially producing thiosulphate, but not tetrathionate. Growth was inhibited by thiosulphate, but not by elemental sulphur, although both were oxidized to sulphate. Mucor flavus also oxidized elemental sulphur, but while it produced less sulphate than A. niger, it consistently formed both thiosulphate and tetrathionate. Substantial sulphur oxidation by Trichoderma harzianum occurred only when large amounts of sucrose were provided, but it was able to oxidize elemental sulphur in autoclaved soil and to use straw to support sulphur oxidation in

vitro. The relevance of these findings to the oxidation of sulphur in soils by fungi is discussed. Thiocyanate hydrolase, the primary enzyme initiating thiocyanate degradation in the novel obligately chemolithoautotrophic halophilic sulfur-oxidizing bacterium Thiohalophilus thiocyanoxidans Abstract Thiohalophilus thiocyanoxidans is a first halophilic sulfur-oxidizing chemolithoautotrophic bacterium capable of growth with thiocyanate as an electron donor at salinity up to 4 M NaCl. The cells, grown with thiocyanate, but not with thiosulfate, contained an enzyme complex hydrolyzing thiocyanate to sunfua and ammonia under anaerobic conditions with carbonyl sunfua as an intermediate. Despite the fact of utilization of the «COS pathway», high cyanase activity was also detected in thiocyanate-induced cells. Three-stage column chromotography resulted in a highly purified thiocyanate-hydrolyzing protein with an apparent molecular mass of 140 kDa that consists of three subunits with masses 17, 19 and 29 kDa. The enzyme is a Co,Fe-containing protein resembling on its function and subunit composition the enzyme thiocyanate hydrolase from the	

Betaproteobacterium Thiobacillus thioparus. Cyanase, copurified with thiocyanate hydrolase, is a bisubstrate multisubunit enzyme with an apparent subunit molecular mass of 14 kDa. A possible role of cyanase in thiocyanate degradation by T thiocyanoxidans is discussed.	