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Microbial Manganese Oxidation

R. Schweisfurth

In Abhängigkeit von definierten pH- und Eh-Grenzen kann Mn(II) nur durch Mikroorganismen oxydiert werden. Diese Manganoxydation findet in Süß- und Meerwasser, in Thermal- und Mineralquellen, im Boden und in bestimmten Zonen von Manganlagerstätten statt. Zu den beteiligten Mikroorganismen gehören Bakterien (Arten von Chlamydobakterien, *Pseudomonas, Achromobacter, Arthrobacter, Pedomicrobium, Hyphomicrobium,* Streptomyceten und Nocardien sowie der Formenkreis um *Metallogenium)* und Pilze (mit Vertretern der Ascomyceten, Basidiomyceten, Fungi imperfecti und *Mycelia sterilia*). Von einigen Ausnahmen abgesehen sind Reinkulturen dieser Stämme erhältlich.

Die Grenzen der mikrobiellen Manganoxydation liegen derzeit nur für wenige Parameter bedingt fest: pH 5.5 - 8.0, Eh über +200 mV, O_2 über 3-5 mg/l, Temperaturbereich 10-44°C.

Mit Ausnahme von *Hyphomicrobium manganoxidans* besteht für die Mikroorganismen keine Abhängigkeit der Vermehrung vom Mn(II)-Gehalt, soweit dies aus Laboratoriumsuntersuchungen bekannt ist. Ein Energiegewinn aus der Manganoxydation ist bisher in keinem Falle eindeutig nachgewiesen worden.

Einzelheiten über Untersuchungen an *Pseudomonas manganoxidans, Hyphomicrobium manganoxidans* und *Metallogenium symbioticum* werden mitgeteilt.

Dependent on Eh and pH and some other factors, Mn(II) is oxidized by free or dissolved oxygen. Below pH 8 and above an Eh-value of +200 mV, manganese is only oxidized by microorganisms, under natural conditions. To what extent organic complexes with Mn(II) and Mn(III) shift the specified values is not known to me.

The number of strains at hand as pure cultures in laboratories has increased in the last years: while from the genera and species described up to 1968 (SCHWEISFURTH 1973a) all except *Sphaerotilus discophorus (Leptothrix discophora,* DONDERO 1975), *Pseudomonas manganoxidans* (SCHWEISFURTH 1973b) and manganese-oxidizing fungi (SCHWEISFURTH 1971) were no longer available or had ceased to oxidize manganese (a few genera as *Metallogenium, Kusnezovia, Caulococcus* or *Pedomicrobium* were at that time not obtainable). At present the following bacteria are available: *Arthrobacter* (EHRLICH 1963, 1968), *Arthrobacter* 216 (VEEN 1973), *Arthrobacter* (BROMFIELD 1974), *Arthrobacter siderocapsulatus* (DUBININA and ZHDANOV 1975), some cultures out of manganese nodules, by OTTOW and SCHÜTTE (pers. comm.), strains of *Pseudomonas putida* and *P. alcaligenes* with the capability of manganese oxidation (JUNG and SCHWEISFURTH 1976) and *Hyphomicrobium manganoxidans* (ELEFTHERIADIS 1976, ELEFTHERIADIS et al. 1976) as well as additional fungal species (TIMONIN et al. 1972). I hope that the list is complete but I beg for corrections.

The conditions for the occurrence of manganese-oxidizing microorganisms at the locations fresh water, salt water (KRUMBEIN 1971), thermal springs, soil and oxidized manganese deposits, have thus far been defined as: pH 5.5 to 8, Eh above +200 mV, 10 to 44°C, and a lower limit for O_2 (dissolved) of 3-5 mg/l. Measurements on the location of these Eh-values, for example, in the microzonal region, can only be accomplished when the Platinum electrodes are protected by a membran against the entry of manganese oxide; otherwise elevated Eh-values are recorded (SCHWEISFURTH 1976). Numbers for species and genera in the designated locations cannot yet be cited. In oxidized manganese deposits one can demonstrate through culture: *Metallogenium* (SOKOLOVA-DUBININA and DERJUGINA 1966) as well as *Hyphomicrobium species, Metallogenium personatum?, Pseudomonas, Achromobacter,* aerobic Actinomycetes and manganese-oxidizing fungi (SCHWEISFURTH and JUNG 1975).

It is conceivable that, by the microbial manganese oxidation, a specific enrichment of elements occurs, which differs quantitatively or typewise from those which appear in a chemical Mn(II) oxidation. During corresponding laboratory tests, in which one works with Mn(II) and bacteria on the one hand, and with identical O_2-containing solutions at a pH of 12 on the other, it was shown in provisional tests that, from the examined elements Co, Cu, Ni, Zn and Fe, Zn, Fe and Co as well as Cu were enriched in the microbially formed manganese oxide. Such processes are (possibly) suitable to clarify the enrichment of the above-mentioned elements in manganese nodules, especially since, e.g. in the "Valdivia"-cruises in January 1976 manganese-oxidizing bacteria were detected in manganese nodules in the Pacific (SCHÜTTE, pers. comm.).

The first culture of manganese-oxidizing microorganisms and their further cultivation as pure cultures under preservation of the manganese-oxidizing capacity requires moreover a great number of different media. Depending on genus or species different optima are shown for Mn(II)-concentration and for type and amount of organic carbon and energy sources. It is fundamental that the pH-value is not lowered too far as a result of acid production, e.g. from sugars. On the other hand pH may not rise above 8 (e.g. by too high concentrations of fatty acids) whereby either manganese oxidation ceases, or, e.g. *Cloaca cloacae* or *Pseudomonas fluorescens* with sodium citrate appear as manganese oxidizing bacteria (cf. BROMFIELD 1974, SCHWEISFURTH 1976). A buffering of the media could lead to difficulties: absence of manganese oxidation because of phosphate buffers, inhibition of bacteria propagation by the buffer, utilization of buffer substances or insufficient buffering capacity.

Experiments on pure cultures meet difficulties already during the recording of growth curves on the basis of protein and DNA determinations (HAJJ and MAKEMSON 1976) because Mn(II) or also Mn(IV) disturb the methods. Thus misunderstandings were caused by experiments on the biological effect of manganese oxidation. Indications of a positive effect of manganese oxidation on protein and DNA production were up to now only found by EHRLICH (1975). ELEFTHERIADIS (1976) found a multiplication of *Hyphomicrobium manganoxidans* during oxidation of Mn(II) only after addition of $NaHCO_3$ or organic C_1-compounds. With this organism, in contrast to *Pseudomonas manganoxidans* (ATCC 23483) or *Sphaerotilus discophorus* (HAJJ and MAKEMSON 1976), manganese oxidation starts immediately together with the exponential growth phase, not just at the end.

Additional results out of our research team can be summarized for some species as follows:

Pseudomonas manganoxidans (JUNG and SCHWEISFURTH 1976):
Mn(II) in concentrations of > 5-10 mg/l inhibits manganese oxidation. Ca^{++} promotes manganese oxidation, ammonium-ions inhibit it already below 5 mg/l. In flow cultures, although Mn(II) and its oxidation have no positive effect on the multiplication of bacteria, it comes to an enrichment of bacteria in manganese oxides which are produced. Cultures of *Pseudomonas manganoxidans* produce an intracellular, manganese oxidizing protein during the stationary phase of growth. The protein is heat labile, can be inactivated by protease and has a pH-optimum for manganese oxidation at pH 7. Mn(II) is oxidized only at concentrations below $3 \cdot 10^{-5}$ M = 0.5 mg/l. The occurrence of the protein is not dependent on the presence of Mn(II), but is clearly related to the ceasing of growth after the end of the exponential growth phase. Cell extracts contain several species of manganese oxidizing protein which show great variations in molecular weight. Oxygen, coenzymes and low molecular weight components of the cell extract are not involved in the reaction as electron acceptors. Continued manganese oxidation by manganese oxidizing protein results in a gradual decrease in activity which is corresponding to the amounts of formed Mn(IV). This behaviour suggests a direct, non-catalytic participation of the protein in the reaction.

Hyphomicrobium manganoxidans, new species (ELEFTHERIADIS 1976):
This involves a morphologically typical *Hyphomicrobium*, which multiplies at an optimal temperature of 37 to 44°C only in the presence of Mn(II) and its oxidation. The isolation succeeded from Mn(IV) - Fe(III)-deposits (Reissacherite) of the thermal springs in Badgastein. *H. manganoxidans* utilizes soil extract, methylamine, formiate, urea, methanol and sodium bicarbonate. Carbon-14 as bicarbonate is incorporated.

Metallogenium symbioticum (ZAVARZIN 1964, SCHWEISFURTH 1972, SCHWEISFURTH and HEHN 1972):
Thusfar we have succeeded neither to produce a fungus-free culture of *M. symbioticum* according to the instructions of DUBININA (1970) for culture on Mycoplasma media, nor could we obtain a host-free culture with Griseofulvin or Nystatin according to BOLOTINA et al. (1973). A manganese oxidation is occuring through fungus-free filtrate. It is dependent on the age of the culture, and begins already with the utilization of a 30 hours-old fungus mycel. The greatest number of manganese-oxidizing (fungus-free) units is reached after 48 hours. The manganese oxidation using filtrate is additionally dependent on the filtrate amount and the Mn(II)-concentration in the solution. The filtrate has protein character.

The positive effect of such jelling substances as gelatine, agar-agar or gummi arabicum on the manganese oxidation and thereby also the production of "arai" is based on a decrease of the Mn(II)-concentration in the medium by complexing.

In autoradiographical tests it was determined that tritiated Thymidin is not incorporated into the manganese oxide-containing filametous structures of fungi. Similar experiments with marked amino acids are not yet completed.

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Adresse

Prof. Dr. R. Schweisfurth
Fachrichtung Hygiene und Mikrobiologie
Medizinische Fakultät
Universität des Saarlandes

D-6650 Homburg/Saar

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