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HUMIC ACIDS AND MICROORGANISMS STIMULATE THE PRECIPITATION OF IRON IN BOGS

HUMUSHAPOT EDISTÄVÄT MIKROBIAALISTA RAUTASAOSTUMIEN MUODOSTUMISTA SUOVESISSÄ

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Humic acids and certain thiobacilli and blue-green algae *(Beggiatoa* and *Oscillatoria* species) found in peat water stimulated the precipitation of oxidized iron products both in natural water and in laboratory cultures. The complex Fe (II)/Fe (III) humates had a considerably lower redox potential than Fe (II)/Fe (III) sulphate (488 vs. 782 mV at pH 2.0, 25°C). Oxygen consumption in excess of the endogenous rate occurring during the oxidation of Fe (II) to Fe (III) was significantly higher in the presence of humic acids and pyrophosphates than in that of sulphates and oxalates, for instance. The presence of microorganisms and high amounts of humic acids in some natural water samples may be the main reason for the iron precipitation, since no other significant differences could be observed between water containing high or low amounts of ferruginous iron oxidate deposits.

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INTRODUCTION

A number of bacteria and blue-green algae living in soil, springs and natural water are able to oxidize ferrous iron (Fe II) to ferric (Fe III) compouds, which are poorly soluble, especially at higher pH values (Ivarson et al. 1978, Tuovinen & Carlson 1979, Carlson et al. 1980, Lazaroff et al. 1982). These ferruginous deposits in peatlands and on the bottoms of lakes may be formed through pure chemical oxidation processes, but the role of microorganisms in their formation may also be considerable. The group of iron-oxidizing bacteria includes first of all *Thiobacillus ferrooxidans,* and probably also other thiobacilli, the closely related *Leptospirillum ferrooxidans* (Norris & Kelly 1978), and *Gallionella, Sphaerotilus* and *Metallogenium* species (Pringsheim 1949, Ivarson & Sojak 1978), while algae such as *Beggiatoa, Oscilla-* *toria, Thiotrix* and *Thioplaca* spp. (Pringsheim 1949) are probably important for the processes responsible for the precipitation of iron. The metabolism and bioenergetics of *Thiobacillus ferrooxidans* has recently been reviewed by Peck (1968), Kelly (1971), Lundgren & Tano (1978), Silver (1978) and Ingledew (1982).

The oxidation-reduction mid-point potential (E_m) of the Fe (II)/Fe (III) couple is approximately 780 mV, and that of the O_2H_2O couple about 850 mV at physiological (intracellular) pH. The difference in potential (ΔE_h) between these two oxidation-reduction systems is thus only about 70 mV, from which it appears that the oxidation of Fe (II) compounds is energetically unfavourable for these organisms. It has been calculated, however, that oxidation of two equivalents of Fe (II) produ-

ces one equivalent of ATP (Ingledew 1982), which is explicable through the creation and maintenance of a H^+ transmembrane electrochemical gradient. The acid substances which produce H^+ in order to lower the extracellular pH to about 2.0 differ in efficacy, however, the principal organism, *Thiobacillus ferrooxidans,* at least, requiring SO_4^{2-} ions for Fe (II) oxidation (Lazaroff 1977), while a number of organic and inorganic anions are inhibitory for this oxidation (Lazaroff 1963, Tuttle & Dugan 1976). This paper reports results concerning Fe (II) oxidation by a mixture of iron-oxidizing microorganisms isolated from natural water in the presence of certain organic and inorganic acids.

MATERIAL AND METHODS

Isolation, culture and identification of organisms. Samples collected from a spring (Haukipudas, Kalimeenkylä) where the groundwater comes into contact with the surface water, and where iron oxidate deposits are formed, were examined under a light microscope. They contained mixtures of bacteria and colourless blue-green algae, the principal organisms being *Thiobacillus ferrooxidans* and *T. acidophilus,* although some other unidentified thiobacilli were also present. The principal blue-green algae were the colony-forming *Beggiatoa* and *Oscillatoria* species. This mixture of organisms was grown in the laboratory in their natural water, to which yeast extract and $FeSO_4$ were added. The medium was acidified with H_2SO_4 (pH 2.0) where necessary. The cultures were gently aerated and the oxygen level monitored with an Orion 97—08 oxygen electrode. Equal amounts of microbial suspension were then transferred for later experiments at the exponential growth phase.

Preparation of half-oxidized Fe (II)/Fe (III) systems. Fe^{2+} and Fe^{3+} citrate, oxalate, acetate and pyrophosphate solutions, if not available commercially, were prepared from solutions of ferro- and ferrisulphates by adding an equivalent amount of the acid solution and precipitating the sulphates as $BaSO_4$. A humic acid fraction was prepared by lyophilizing peat water and dissolving the residue in a small amount of distilled water. The E_m potentials of the Fe (II)/Fe (III) couples thus formed were then measured in about 1 mM solutions at pH 2.0 containing equal concentrations of ferrous and ferric compounds.

Analytical procedures. Iron and the sulphates were determined gravimetrically, the for-

mer in the presence of ammonia $(Fe(OH)_3)$ or hydrogen sulphide and ammonia (FeS) (Erdey 1965), and the latter as $BaSO_4$. The redox potential was measured with a laboratory pH-E_h meter.

RESULTS

The peatland water contained large amounts of acidic humic substances, and its pH was somewhat lower than that of the pine forest water, but no other significant differences could be observed between these two samples (Table 1). The water channel of the pine bog contained prominent ferruginous deposits, while the water in the forest channel was clear and had no such deposits. The pH difference is apparently a consequence of the presence of humic or other organic acids, since the amount of sulphates present was equal in the two types of sample, and the amount of microorganisms present did not differ. The discharge of peatland water rich in humic substances and humic acids thus seemed to be the reason for the iron oxidate precipitation. Laboratory experiments with more "concentrated" microbial suspensions were therefore carried out in order to study the role of humic substances in the oxidation of ferrous iron compounds.

In the laboratory cultures the oxidation of Fe (II) compounds, measured as oxygen consumption, by a mixture of microorganisms isolated from the peatland water was most rapid in the presence of humic acids or pyrophosphates, and considerably slower in the acetate and citrate solutions and particularly so in the oxalate solution (Fig. 1).

Table 1. Chemical characteristics of water from a pine heath forest and a pine bog, being poor and rich in ferruginous deposits respectively.

Taulukko 1. Runsaasti rautasaostumia sisältävän suoveden kemiallisia ominaispiirteitä verrattuna mäntykankaalta tulevan veden vastaaviin ominaisuuksiin.

Constituent	Properties of	
	pine forest water	pine bog water
Fe	5.4 mg l^{-1}	5.8 mg l^{-1}
O_2	4.5 mg l^{-1}	4.3 mg l^{-1}
redox potential	380 mV	405 mV
pH	5.6	4.3
SO_4^{2-}	10.6 mg l^{-1}	11.2 mg l^{-1}
humic acids	sparsely	abundantly
	(A_{570}=0.15)	(A_{570}=0.45)
microorganisms	sparsely	sparsely

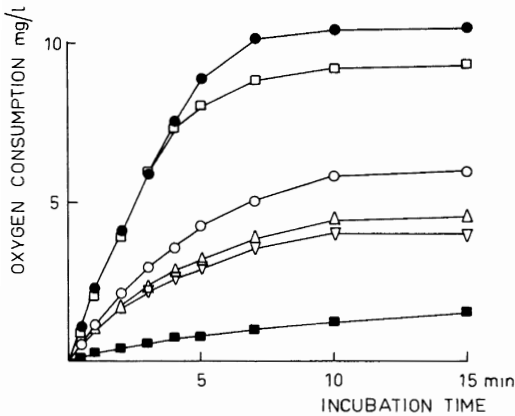Results are means from 4—8 experiments. S.D. varied from 3 to 6 %.

Fig. 1. Oxidation of Fe (II) compounds (oxygen consumption) by a mixture of microorganisms whose presence leads to the formation of iron deposits in natural waters and in the presence of various Fe^{2+} salts, ferrooxalate (■), ferrocitrate (▽), ferroacetate (△), ferrosulphate (o), ferropyrophosphate (□) or ferrohumates (●), in about 5 mM concentrations. The results, means from 5 experiments, are expressed in terms of oxygen consumption (mg x l^{-1} per equal amount of microbial suspension). The contribution of respiration was determined separately in each case without any substrate and subtracted from the actual readings.

Kuva 1. Muutamien ferroyhdisteiden hapettuminen (mitattuna hapenkulutuksena) mikrobisuspensiossa, joka aiheutti luonnossa voimakkaan rautasaostumien muodostumisen. Mittaukset suoritettiin noin 5 mM ferro-oksalaatti (■), ferrositraatti (▽), ferroasetaatti (△), ferrosulfaatti (o), ferropyrofosfaatti (□) ja ferrohumaatti (●) -liuoksissa. Hapenkulutus on ilmaistu my litrassa tiettyä, samankoista mikrobierää kohti. Hengityksen osuus eliminoitiin erikseen ilman substraattilisäystä, ilman mikrobisuspensiota hapenkulutusta ei havaittu.

These results show that the microorganisms present in the suspension favour humic substances and pyrophosphates in their iron oxidation reactions. The complexes of Fe^{2+}/Fe^{3+} ions and humic acids (iron

Table 2. Oxidation-reduction mid-point potentials of certain Fe^{2+}/Fe^{3+} couples in about 1 mM concentrations at 25°C and pH 2.0.

Taulukko 2. Eräiden Fe^{2+}/Fe^{3+}-yhdisteiden normaalipotentiaalit noin 1 mM liuoksissa 25°C:ssa, pH 2,0.

Compound	E_m, mV
Fe^{2+}/Fe^{3+}-sulphate	782 ± 41
Fe^{2+}/Fe^{3+}-acetate	650 ± 88
Fe^{2+}/Fe^{3+}-oxalate	622 ± 61
Fe^{2+}/Fe^{3+}-citrate	580 ± 38
Fe^{2+}/Fe^{3+}-pyrophosphate	540 ± 70
Fe^{2+}/Fe^{3+}-humic acids	488 ± 85

Results are means ($\pm$ S.D.) from 4—6 experiments.

humates) had the lowest oxidation-reduction mid-point potential among the iron compounds mentioned (Table 2). The potentials of iron acetate, oxalate and citrate were also considerably lower than that of iron sulphate, even though they were inhibitory for the oxidation of ferrous sulphate.

DISCUSSION

Precipitates of iron, in the form of highly insoluble complex iron (III) hydroxides, are common on the bottoms of lakes (Harriss & Troup 1969, Collins & Buol 1970), in peatlands (Harriss & Troup 1969, Carlson & Schwertman 1981), on the walls of springs (Carlson et al. 1977, 1980), in glaciofluvial material often exposed in the walls or on the floors of sand and gravel pits (Koljonen et al. 1976) or as concretions or surface coatings in stream sediments (Nowlan 1982). The oxidation of ferrous iron in natural waters may be a pure chemical reaction in certain cases, but the role of various microorganisms in speeding-up this oxidation process may also be considerable (Carlson et al. 1980, Ingledew 1982). *Thiobacillus ferrooxidans* requires an acidic environment (optimum pH 2.0) and sulphate anions, although several other organic acids, amino acids in particular, and inorganic anions also make good substrates (see Ingledew 1982). The anion requirements of the other organisms which also appear to oxidize ferrous iron are not known.

It is seldom possible to reach pH 2.0 in natural waters, as used in the present experiments. Those water samples studied here in which large quantities of iron hydroxides were precipitated gave pH values around 4 or 5. The peatland water was also found to have a slightly lower pH than the pine forest water, the difference perhaps partly originating from the presence of high amounts of humic acids, but had a slightly higher redox potential than the latter.

The addition of humic acids strongly stimulated the oxidation of ferrous iron, as was also the case with pyrophosphate. These two substances had relatively low redox potentials when compared with that of Fe^{2+}/Fe^{3+} sulphate. It is thus apparent that humic acids, and probably other organic acids present in peat water, are favourable for iron-oxidizing microorganisms. Iron-humic acid complexes may be energetically more efficient than ferrous sulphate, since they have lower redox

potential. The iron ions probably form complex chelates with the humic acids, and these chelates may then be bound to the bacterial membrane, allowing ferrous ions to pass to the oxidizing system inside the bacterial cells. Measurement of the oxidation-reduction potentials of the iron complexes confirmed the old hypothesis that iron-oxidizing organisms may first form complex compouds with iron and acid anions and thus lower the potential of the resulting ferrous-ferric couple and render the oxidation reaction energetically more efficient (Sokatch 1969, p. 195). The bioenergetic background of this hypothesis requires further study at the intracellular level, however.

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TIIVISTELMÄ:

HUMUSHAPOT EDISTÄVÄT MIKROBIAALISTA RAUTASAOSTUMIEN MUODOSTUMISTA SUOVESISSÄ

Eräät bakteerit ja sinilevät voivat käyttää rauta (II)-yhdisteiden hapettamista rauta (III)-yhdisteiksi energialähteenään. Rauta (III)-yhdisteet ovat yleensä hyvin vaikealiukoisia ja saostuvat tällöin vesistöjen pohjalle, suovesiin ja lähteiden reunoille usein hyvinkin monimutkaisina ruostesaostumina. Tunnetuin näistä organismeista on rikkibakteereihin kuuluva *Thiobacillus ferro-oxidans,* jonka bioenergetiikkaa on tutkittu paljon (Kelly 1971, Ingledew 1982). Vaikka Fe (II)/Fe (III) ja O_2/H_2O-systeemien normaalipotentiaalien ero fysiologisella pH-alueella on vain noin 70 mV, tuottaa yhden Fe (II)-moolin hapettuminen kuitenkin yhden moolin ATP:a, mikä on selitettävissä solukalvon eri puolilla ylläpidettävän H^+-ionien sähkökemiallisen gradientin avulla (Ingledew 1982). *Thiobacillus ferro-oxidans* vaatii rikkihappoa solun ulkopuolisen pH:n alentamiseen, mutta muille organismeille soveltuvat monet muutkin epäorgaaniset ja orgaaniset hapot tähän tarkoitukseen. Suoveden humushappojen osuuden rautasaostumien muodostumisen edistäjänä toi tämän tutkimuksen aiheeksi havainto, missä runsaasti humusaineita sisältävässä, suolta tulevassa purossa (Haukipudas, Kalimeenkylä) havaittiin huomattavasti runsaampia ruostesaostumia kuin viereisessä, mäntykankaalta tulevassa purossa, jonka vesi oli kirkasta.

Mikroskooppimäärityksissä suovedessä havaittiin monia rikkibakteerilajeja, muun muassa *Thiobacillus ferro-oxidans* ja *T. acidophilus.* Lisäksi joukossa oli kolonioissa kasvavia *Beggiatoa* ja *Oscillatoria* lajeja. Suoveden kemiallisessa koostumuksessa ei ollut kovin merkittäviä eroja mäntykankaalta tulevaan veteen verrattuna, ainoastaan pH oli edellisessä merkittävästi alhaisempi (4,3/5,5) kuin jälkimmäisessä (taulukko 1). Mikrobiseosta kasvatettiin laboratoriossa antamalla hiivauutetta ja $FeSO_4$ substraatiksi. Hapenkulutusta seurattiin Orionin happielektrodilla. Rauta ja sulfaatti määritettiin gravimetrisesti (Erdey 1965). Puoleksi hapettuneet ja pelkistyneet Fe (II)/Fe (III) systeemit valmistettiin kaupallisista kemikaaleista sekä lyofilisoimalla konsentroidusta suoveden humushappofraktiosta ferro- ja ferrisulfaatin kanssa. Hapetuspelkistyspotentiaalit mitattiin pH-E_h-mittarilla.

Humushappojen Fe^{2+}/Fe^{3+}-normaalipotentiaali havaittiin alhaisimmaksi ($E_m = 488$ mV, taulukko 1) niistä tutkituista orgaanisista ja epäorgaanisista $Fe^{2+}/Fe3^{3+}$-yhdisteistä, joita rautaa hapettavat mikrobit voivat käyttää substraatteina (Lazaroff 1977, Ingledew 1982). Kun ferroraudan hapettumista mikrobisuspensiossa seurattiin hapenkulutuksen avulla, havaittiin vilkkain hapenkulutus ferrohumaattien ja ferropyrofosfaatin läsnäollessa (kuva 1), yhdisteiden, joilla oli alhaisin hapetuspelkistyspotentiaali (taulukko 2). Ferrosulfaatin hyväksikäyttö sen sijaan oli nopeampaa kuin ferro-oksalaatin, ferrositraatin ja ferroasetaatin käyttö.

Näyttää siis siltä, että humushapoilla (kuten myös pyrofosfaatilla) on suotuisa vaikutus rautaa hapettaviin mikrobeihin. Kompleksiset humushappojen rautayhdisteet näyttäisivät olevan energeettisesti ferrosulfaattia edullisempia, koska näillä on alhaisempi hapetuspelkistyspotentiaali. Rauta muodostaa mahdollisesti humusaineiden kanssa kelaatteja, ja nämä rauta-kelaattiyhdisteet sitten vuorovaikutuksessa bakteerien seinämateriaalin kanssa antaisivat mahdollisuuden useampien ferroionien pääsyyn solun sisälle hapetussysteemien vaikutuspiiriin.