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13. ABSTRACT *(Maximum 200 words)*

Humic substances are a class of yellow to black colored organic molecules that are recalcitrant and ubiquitous in the environment. Previous research has shown that humic substances can act as electron acceptors in the anoxic oxidation of acetate by Fe(III)-reducing microorganisms. The reduced humic substances were then able to transfer the excess electrons to Fe(III) abiotically. Our hypothesis was that quinone moieties within the humic substances are the primary reducible group. Using electron spin resonance, the organic radical concentration of the humic substances has been shown to increase up to six-fold after incubation with Geobacter Metallireducens. A direct positive correlation exists between the change in organic radicals and the molar concentration of Fe(III) reduced abiotically by the reduced humic substances from many environments. These findings support the hypothesis that quinone moieties are the electron accepting groups within the humic substances. These findings are very important in understanding the reduction of Fe(III) and other metals in anoxic environments, as well as the oxidation of organic contaminants in polluted environments such as marine sediments.

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FINAL REPORT

GRANT #: N00014-97-0235

PRINCIPAL INVESTIGATOR: Dr. Diane McKnight

INSTITUTION: Institute of Arctic and Alpine Research

GRANT TITLE: Humic Substances as Electron Acceptors and Electron Shuttlers in Anaerobic Marine Sediments

OBJECTIVE: To investigate the use of humic substances as a terminal electron acceptor by microorganisms in anaerobic marine sediments; to determine if the electron-accepting moieties within humic substances are quinone groups.

APPROACH: Humic substances were isolated and purified from marine and freshwater sediments. The semiquinone content of the two humic substances and several other humic substances from soils and water was measured using an electron spin resonance (ESR) technique. The electron-accepting capacity of the humic substances was measured by incubating the humics with a pure culture of *Geobacter metallireducens* and acetate, and then adding Fe(III) and measuring the resulting Fe(II) using the ferrozine assay. The semiquinone content was also measured at pH 7 before and after a 2-hour microbial incubation period to determine the increase in semiquinone content.

ACCOMPLISHMENTS: Our hypothesis was that quinone moieties within the humic substances are the primary reducible moiety within the humic substances. Using electron spin resonance, the organic radical concentration of the humic substances has been shown to increase up to six-fold after incubation with *Geobacter metallireducens*. A direct positive correlation exists between the change in concentration of organic radicals (#spins/g) and the molar concentration of Fe(III) reduced abiotically by the reduced humic substances from many different environments.

Our studies demonstrated that all of the humics evaluated had the ability to serve as electron acceptors in microbial respiration (Lovley et al.,1996a; Scott et al.,1998). This included microbially derived humics from marine sediments and other sources. Electron-accepting capacity increased with increasing molecular weight of the humics and was not directly related to aromaticity.

CONCLUSIONS: Direct evidence for quinones being the important electron-accepting moiety in humics was obtained with ESR studies (Scott, 1998). A direct correlation between the number of semiquinone radicals in a wide diversity of humics and their electron-accepting capability was observed. Microbial reduction of humics increased the number of semiquinone radicals detected with ESR, and the number of semiquinone radicals formed as the result of microbial reduction was also directly correlated with the electron-accepting capacity of the humics. These results clearly indicated that electron transfer to humics was associated with reduction of quinones.

Humic substances from a variety of environments, including sediments, can act as terminal electron acceptors in anaerobic microbial oxidation. The reduced humic substances can then reduce Fe(III) to Fe(II) abiotically.

SIGNIFICANCE: These findings support the hypothesis that quinone groups are the electron accepting moieties within the humic substances. These findings are very important in understanding the reduction of Fe(III) and other metals in anoxic environments, as well as the oxidation of organic contaminants in polluted environments such as marine sediments. Many different humic substances, including humic substances derived from algal and microbial material, can act as a terminal electron acceptor.

AWARD INFORMATION: Hired as a tenured associate professor at the University of Colorado in the department of Civil, Environmental and Architectural Engineering.

PUBLICATIONS AND ABSTRACTS:

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6. Cord-Ruwisch, R., D. Lovley and B. Schink. 1998. Growth of Geobacter sulfurreducens with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. Appl. Environ. Microbiol. 64: 2232-2236.

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8. Scott D.T., McKnight D.M., Blunt-Harris E., Lovley D.R. and Kolesar S.E., 1998. The ability of microorganisms to reduce quinone groups in humic substances. Presented at Humic Substances Seminar II, Northeastern University, March 1998.

9. Scott D.T., McKnight D.M., Blunt-Harris E., Lovley D.R. and Kolesar S.E., 1998. The reduction of quinone groups in humic substances by humics-reducing microorganisms. Presented at the Environmental, Population and Organismic Biology Symposium, April 1998.

10. Scott D.T., McKnight D.M., Blunt-Harris E., Lovley D.R. and Kolesar S.E., 1998. Quinone groups in humic substances as electron acceptors by humic-reducing microorganisms. Presented at the 216th National Meeting of the American Chemical Society, Environmental Chemistry Division, August 1998.

11. McKnight, D. M., University of Minnesota, Symposium honoring Prof. Eville Gorham, "Environmental Chemistry: New Tools for Limnology", November 1998.

12.McKnight, D. M., Limnological Laboratory, University of Uppsala, Sweden, March 1998, Oikos Workshop on Dissolved Organic Material, "Biogeochemistry of aquatic humic substances"

13.McKnight, D. M., Johns Hopkins University, Department of Geography and Environmental Engineering, Nov. 1997, invited seminar, "Biogeochemical processes involving humic substances and iron in natural waters".

14.McKnight, D. M., American Water Resources Association, Denver, CO, Nov. 1997, "Biogeochemistry of aquatic humic substances"

15.McKnight, D. M., American Society of Agronomy/International Humic Substances Society symposium, Anaheim, CA, Oct. 1997, "Biogeochemical processes involving humic substances and iron in natural waters".