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## 主 論 文 の 要 旨

**論文題目**      **Chemical and Electrical Characteristics Supporting External Electron Mediating Function of Soil Humin**  
(土壤腐植ヒューミンの細胞外電子伝達機能を支える化学的および電気的特性)

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## 論 文 内 容 の 要 旨

Bio-electrochemistry is a future promising technology that may change the human's view on the environmental issue. Not only focus on pollution cleanup, it also can drive the waste-to-energy direction. Technically, for non-electroactive microorganisms, an electron mediator to help electron transfer among microorganisms or between microbe and electrode in reversible direction is required. Humic substances (HSs) are considered as natural electron mediating material in bio-electrochemical interactions. Over the past twenty years, the electron mediating function of HSs has been extensively studied. However, all studies mostly focused on soluble HSs, such as fulvic acids and humic acids, until the electron mediating function of solid phase humic substances was first announced in 2010. In the year of 2012, humin-dependent pentachlorophenol-dechlorinating culture was first announced, in which humin (HM, insoluble HS at any pH) functioned as an effective redox mediator. PCP is a broad-spectrum biocide and its use has been banned because of the toxicity, but it is still

persistent in the environment. Since 2012, in addition to PCP dechlorination, HM has been reported as an effective redox mediator for multiple microbial reactions, such as tetrabromobisphenol A debromination, reduction of ferric oxide, and nitrate reduction. Due to its insoluble nature, HM is able to meet the important requirement of mediator's selection in situ remediation: almost no losses. Due to its insolubility and heterogeneity, there is very less knowledge and less study about chemical structure, redox components and electrical properties of HM. Thus, the main objective of this study was to gain the knowledge about the chemical structure, electrical behavior, and redox-active components in HM. These characteristics would support the external electron mediating functionality of HM in anaerobic microbial reductive processes.

In Chapter 2, the electrical resistance and capacitance of soil HM was studied. HM extracted from five different soil sources were used. The results of electrochemical impedance spectroscopic (EIS) analysis using alternating current (AC) showed that HM resistance under saturated conditions ranged from 1100 to 2970 ( $\Omega$ ), and the capacitance from  $2.0 \times 10^3$  to  $1.1 \times 10^4$  ( $\mu\text{F/g-HM}$ ). Statistical analysis indicated the significant positive correlation between electrical capacitance and organic content in HM ( $P < 0.05$ ). Direct current (DC) measurement of conductivity and capacitance of HM at different moisture condition suggested that HM had water-mediated proton conductivity, which was similar to other humic substances, and the electrical capacitors were dispersed without conductive connections in HM.

In Chapter 3, a sample preparation method was developed to characterize carbon binding states in HM by synchrotron-radiation-based X-ray photoelectron spectroscopy (XPS). Because HM is a non-conductive and insoluble organo-mineral complex, the charge building up occurs on the sample, and it results in the shift in the XPS spectrum

which make the assignment of the carbon binding state confuse. Four sample preparation methods had been investigated in order to troubleshoot the charge buildup issue, which were: HM distributed randomly on (1) carbon tape, (2) indium sheet, (3) copper mesh (pore size of 100  $\mu\text{m}$ , diameter of 3 mm, thickness of 25  $\mu\text{m}$ , and (4) pellet of HM and copper powder (Cu, particle size of 75  $\mu\text{m}$ , purity of 99.9%) mixture. In method 4, three mixing ratios were investigated: HM:Cu = 1:1, 1:2, 1:6 v/v. The results showed that there was severe charge buildup in HM samples without Cu powder (three first methods). The binding energy of C1s XPS spectra shifted to higher than 296 eV made the deconvolution of the spectra impossible. Mixing HM with Cu powder increased the electric conductivity of the sample and reduced the charge buildup during the analysis. The C1s XPS spectrum of the pellet sample formed from the mixture of HM:Cu = 1:1 (v/v) had minimum shift, regardless to the detecting point. Thus, pellet sample of the mixture HM:Cu = 1:1 (v/v) was considered as the optimal sample preparation method. Various carbon binding state were detected in the chemical structure of HM, which were: C=C, C-C/C-H, C-O, C=O, O=C-O, and  $\text{CF}_x$ . Fluorinated carbon ( $\text{CF}_x$ ), this was the first discovery in the HM structure, not yet reported by any other studies. These advantageous information obtained from the pellet HM:Cu = 1:1 (v/v) help to gain more knowledge on the carbon binding states of the heterogeneous chemical structure of HM. C=C, C=O functional groups would involve in the external electron mediating (EEM) function of HM as parts of quinone structures. The results of this chapter also suggested that the extraction method of HM would influence the chemical structure of HM. This point was further examined in the Chapter 4.

In Chapter 4, a study was carried out on the chemical and electrochemical structures of associated with the external electron mediating function. The effect of

different extraction method on the EEM function of HM from the same soil source (Kamajima paddy soil) was carried out, by testing with HM-dependent pentachlorophenol (PCP) dechlorinating culture. The results showed that different PCP dechlorination rates were observed in the cultures used seven HMs extracted from the Kamajima paddy soil by different methods. Only HM extracted as soluble fraction by mixture of dimethyl sulfoxide and sulfuric acid did not support the culture activity, suggesting that its chemical structure did not contain redox active components (no EEM function). The contribution of quinone-type structure in to EEM function was minor. Predominantly, with the significant positive correlation, the carbohydrate carbons (functional groups of C=O, O=C-N, and O=C-O) contributed to EEM function of HM. Different HM extraction yields together with different chemical structures indicated that all the methods extracted only partial HM, and allowed to find the chemical structure associated with EEM function.

Chapter 5 provides a general discussion concerning on this study, which were (1) updating information of general characteristics and EEM functionality of HM, (2) advanced techniques to study HM, (3) electron transport through HM, (4) a messy literature about HM: definition and extraction, (5) time factor in humification process; and (6) future application prospects of HM.

Chapter 6 summarizes all the results obtained in this study. The future works are proposed, as follows: (1) characterization of redox-active structures in HM, (2) discovery of other materials which have similar function to HM, (3) development of a method to measure cyclic voltammetry of HM, (4) establishment a holistic approach to study the whole HM in soil, (5) particle size fractionation of HM associating with its EEM function, and (6) Widening the application of EEM function of HM.

In summary, two directions were conducted in this study, which were: (1) basic study on HM as a material and (2) applied study using HM as an external electron mediator. The obtained results indicated that HM has water-based conductivity, and the electrical capacitors were dispersed without conductive connections in HM. There is an association of peptidoglycan structure, together with O=C-O and O=C-N groups, with EEM function of HM. In contrast, quinone structure had minor contribution to this precious function. Overall, the redox-active components in HM had been narrowed down in this study.