# Biomineralization of Leptothrix ochracea on the Electrode Surface



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# Biomineralization of *Leptothrix ochracea* on the Electrode Surface

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**Abstract - Iron-biomineralization of *Leptothrix ochracea* under an electric field were observed by electron microscopy. *L.ochracea* were inhabited both anode and cathode, and possessed different mineral sheath such a lepidocrocite on the anode and ferrihydrite on the cathode. *L.ochracea* is classified two-types by electric charge of their sheath surface: positive charged sheath and negative charged sheath. It is suggested that *L.ochracea* in different environment such an anode or cathode surface can form different iron mineral sheath with their metabolic activity.**

## I. Introduction

Bacterial cells, growing naturally in freshwater and marine environments or experimentally in culture, can precipitate a variety of authigenic iron minerals. With the vast majority of bacteria biomineralization is a two-step process: initially metals are electrostatically bound to the anionic surfaces of the cell wall and surrounding organic polymers, where they subsequently serve as nucleation sites for crystal growth [1]. Biologically induced mineralization is the dominant process among bacteria, and one particularly characteristic feature is that the type of mineral formed is a function of the environmental conditions in which the microorganism lives; conversely the same microorganism in different environments can form different minerals [2].

*Leptothrix ochracea*, one of the iron-oxidizing bacteria, deposit copious amounts of iron hydroxide as encrusted sheaths in the various environments where they grow [3]. The iron-oxidizing biomineralization are considered that the oxidation and hydrolysis of cell-bound ferrous iron, the binding of ferric species (e.g. $Fe(OH)^{2+}$, $Fe(OH)_2^{+}$) or cationic colloidal species (e.g. $Fe(H_2O)_5(OH)_5(OH)^{2+}$) to negatively charged polymers, and the alteration of local pH and redox conditions around the cell due to their metabolic activity can all induce the transformation to insoluble hydroxide forms [4][5]. Certainly, the biomeneralization of iron onto bacterial surface in the environment is well documented, yet the interaction between cell potential and biomeneralization are questions still open to debate.

In this study, iron-oxidizing bacteria such as *L.ochracea* was given an electric field. The results provide different biomineralization of *L.ochracea* under an electric field has been revealed by electron microscopy.

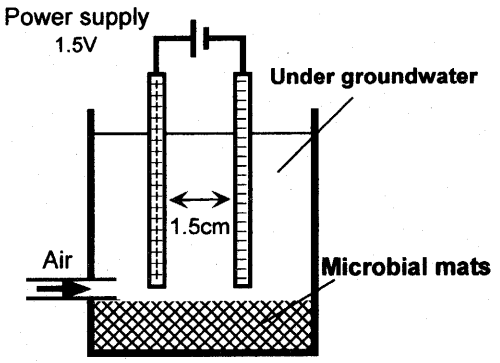


Fig. 1. Schematic representation of the experimental system in which gave an electric field to iron-oxidizing bacteria.

## II. Materials and Methods

The experimental system in which gave electric field to *L.ochracea* is described in Fig. 1. An electric field was applied using two iron plates connected to a power supply (HITACHI alkaline 1.5V). The distance between the electrodes is 1.5 cm. To keeping dissolved oxygen, the air was sending throughout experiment. Samples of *L.ochracea* contained in microbial mats and underground water were collected from controlling pond at Kanazawa University [6]. In comparison, microbial mats were sterilized by an autoclave at 121 °C temperature and high pressure during 20 min. Experiments were run in microbial mats containing and in sterilized microbial mats containing.

With the course of experimental aging time, brown incrustation was formed on the anode and the cathode surface. After 9 days experiments, these incrustation was observed by SEM (scanning electron microscope, SEM; JEOL JSM-5200LV) equipped with EDX (energy dispersive X-ray spectrometer, EDX; Philips-EDAX PV 9800 STD) and TEM (transmission electron microscopy, TEM; JEOL JEM-2000 EX) equipped with selected-area electron diffraction (SAED) analysis. Natural microbial mats were also observed. For SEM observation, incrustation on the each electrode surface were hand-picked onto brazen SEM stubs. After drying in air, the surface was coated with carbon and examined at an accelerating voltage of 15 kV. For TEM observation, the sample was suspended in distilled water. The suspension was treated with ultrasonic waves for one minute to detach bacteria from incrustation before these

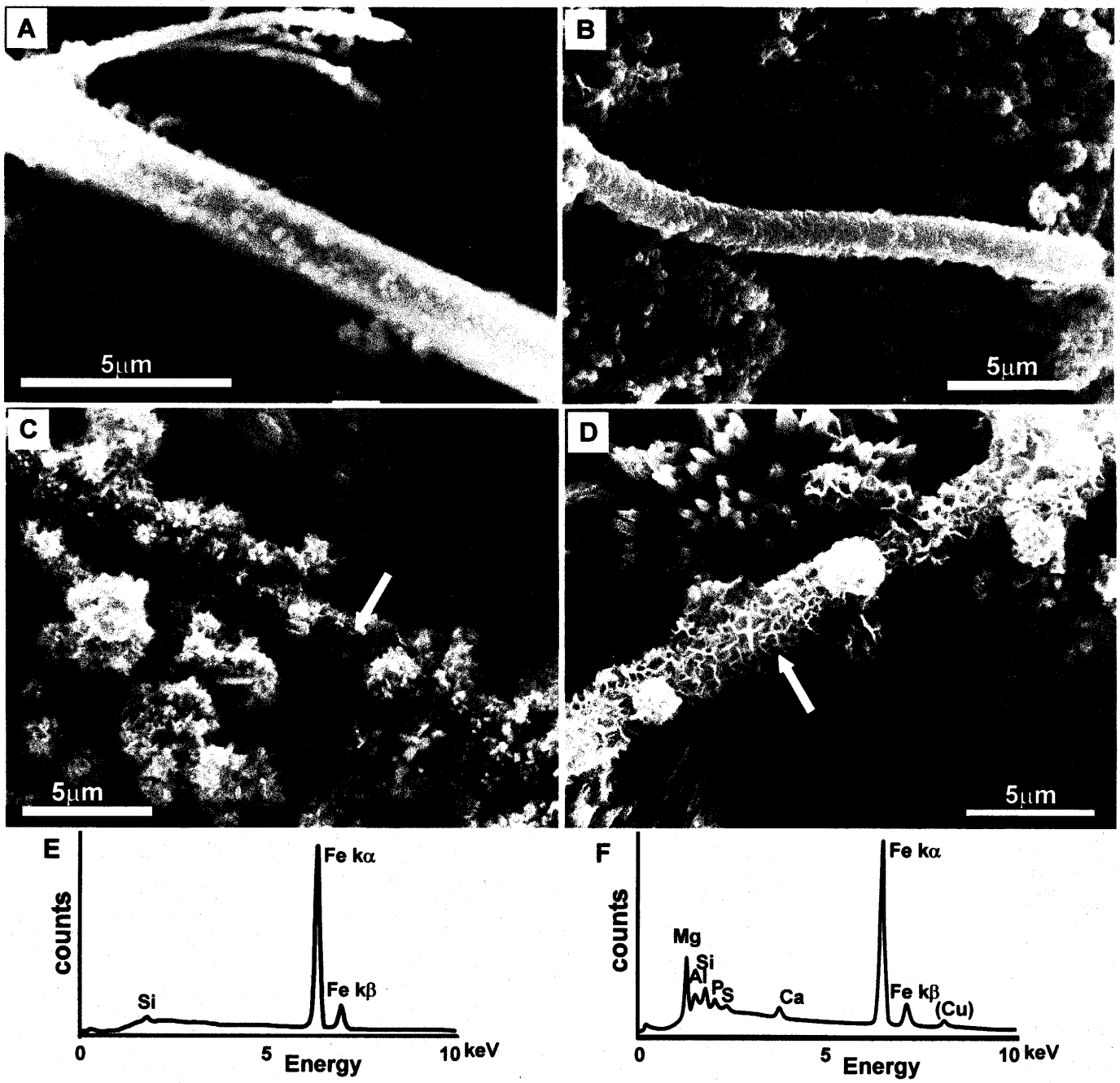


Fig. 2. Scanning electron micrographs of natural microbial mats and incrustation on each electrode showing several mineral particles attached on the sheath surface. A; the sheath of *L.ochracea* at natural environment, B; the sheath of *L.ochracea* on the cathode surface after sterilization, C; the sheath of *L.ochracea* on the anode surface, D; the sheath of *L.ochracea* on the cathode surface, E; EDX spectrum of the sheath of *L.ochracea* on the anode surface (C, arrow) shows a strong peak of Fe with a weak peak of Si, F; EDX spectrum of the sheath of *L.ochracea* on the cathode surface (D, arrow) shows strong peaks of Mg and Fe associated with weak peaks of Al, Si, P, S and Ca. Cu is due to brazen SEM stub.

were mounted on a micro grid. The accelerating voltage was 160 kV with different magnifications.

Carbon and nitrogen contents of incrustation on each electrode were analyzed by an automatic gas chromatographic elemental analyzer (CE Instruments NA 2500 NCS) at 1000 °C with 20 ml oxygen. All powder samples were analyzed after treatment with HCl.

## III. Results and Discussion

### *A. Observation of natural microbial mats and incrustation on each electrode by electron microscopy*

SEM observation of natural microbial mats and incrustation on each electrode revealed that the sheath of *L.ochracea* was composed of several mineral particles (Fig. 2). The sheath of *L.ochracea* at natural environment and

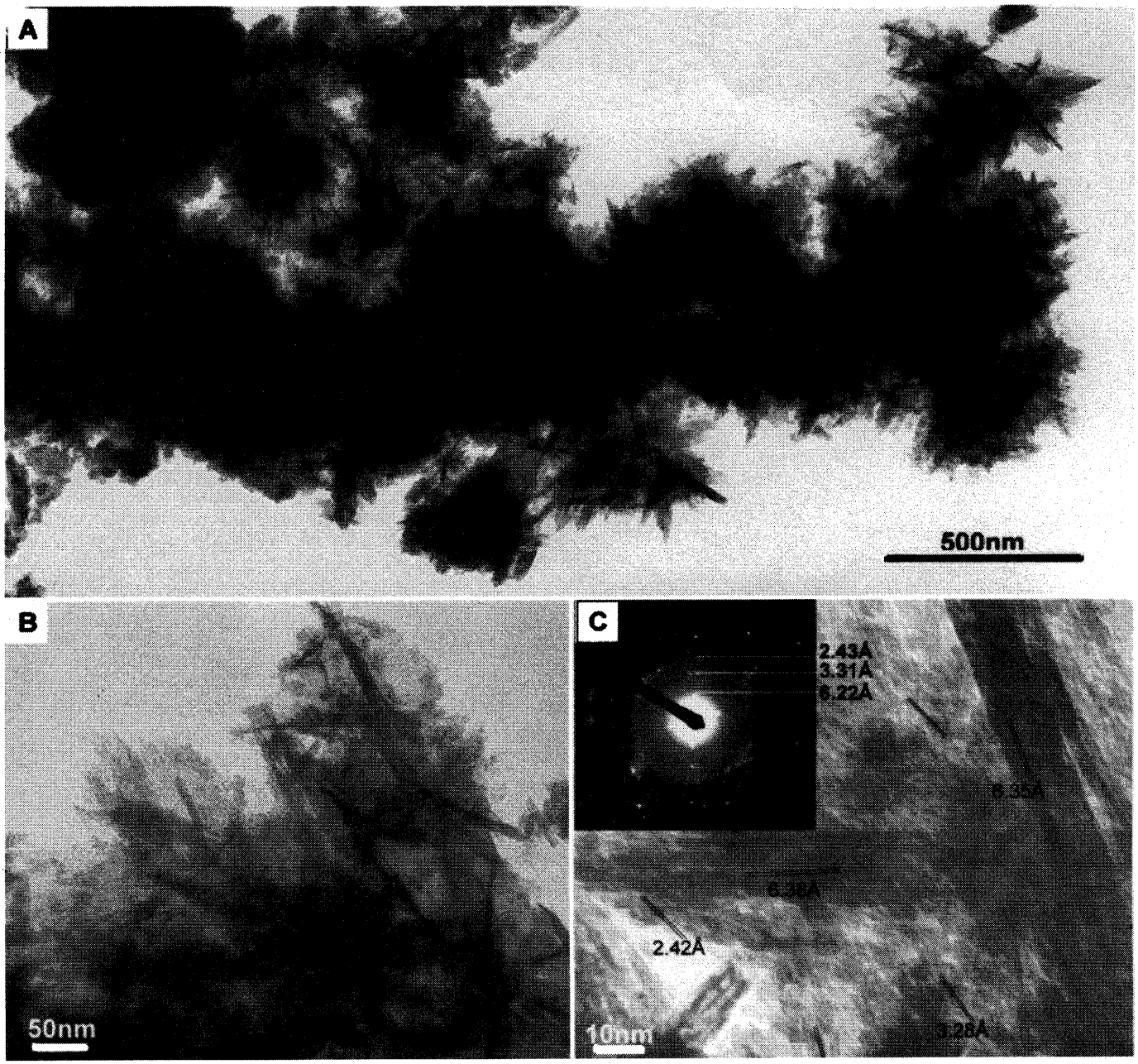


Fig. 3. Transmission electron micrographs of the sheath of *L.ochracea* on the anode surface. A; *L.ochracea* are covered with needle-like minerals. B; the needle-like minerals under high magnification. C; high-resolution transmission electron micrograph of needle-like minerals show variation in lattice images. The *d*-spacings represent of areas with lattice images. The SAED pattern of needle-like minerals show *d*-spacing of rings, which have 6.22 Å, 3.31 Å and 2.43 Å indicating lepidocrocite (inset).

sterilized sheath were composed of same mineral shape that is spherical mineral particles of less than 1μm in size (Fig. 2A and 2B). In contrast, the sheath of *L.ochracea* on anode and cathode were consisted of different mineral shape, respectively (Fig. 2C and 2D). Needle-like minerals composed the sheath of *L.ochracea* on the anode surface (Fig. 2C), and flakey minerals composed the sheath of *L.ochracea* on the cathode (Fig. 2D). EDX spectra of needle-like minerals and flakey minerals showed that both of minerals are mainly composed of Fe with trace of Si. Moreover, flakey minerals contain Mg, Al, P, S and Ca (Fig. 2E and 2F).

TEM micrographs of the sheath of *L.ochracea* and needle-like minerals on the anode surface are shown in Fig.3. This sheath and needle-like minerals showed a high density-contrast, suggesting that the sheath is thickly encrusted by needle-like minerals (Fig. 3A). High-resolution TEM micrographs give a more precise shape of needle-like minerals (Fig. 3B). The SAED pattern and HRTEM micrograph shows that needle-like minerals are identified as lepidocrocite with 6.22 Å, 3.31 Å and 2.43 Å (Fig. 3C). Whereas, TEM micrographs of the sheath of *L.ochracea* and flakey mineral on the cathode surface are shown in Fig. 4. Flakey minerals formed spherical particles of 500nm in

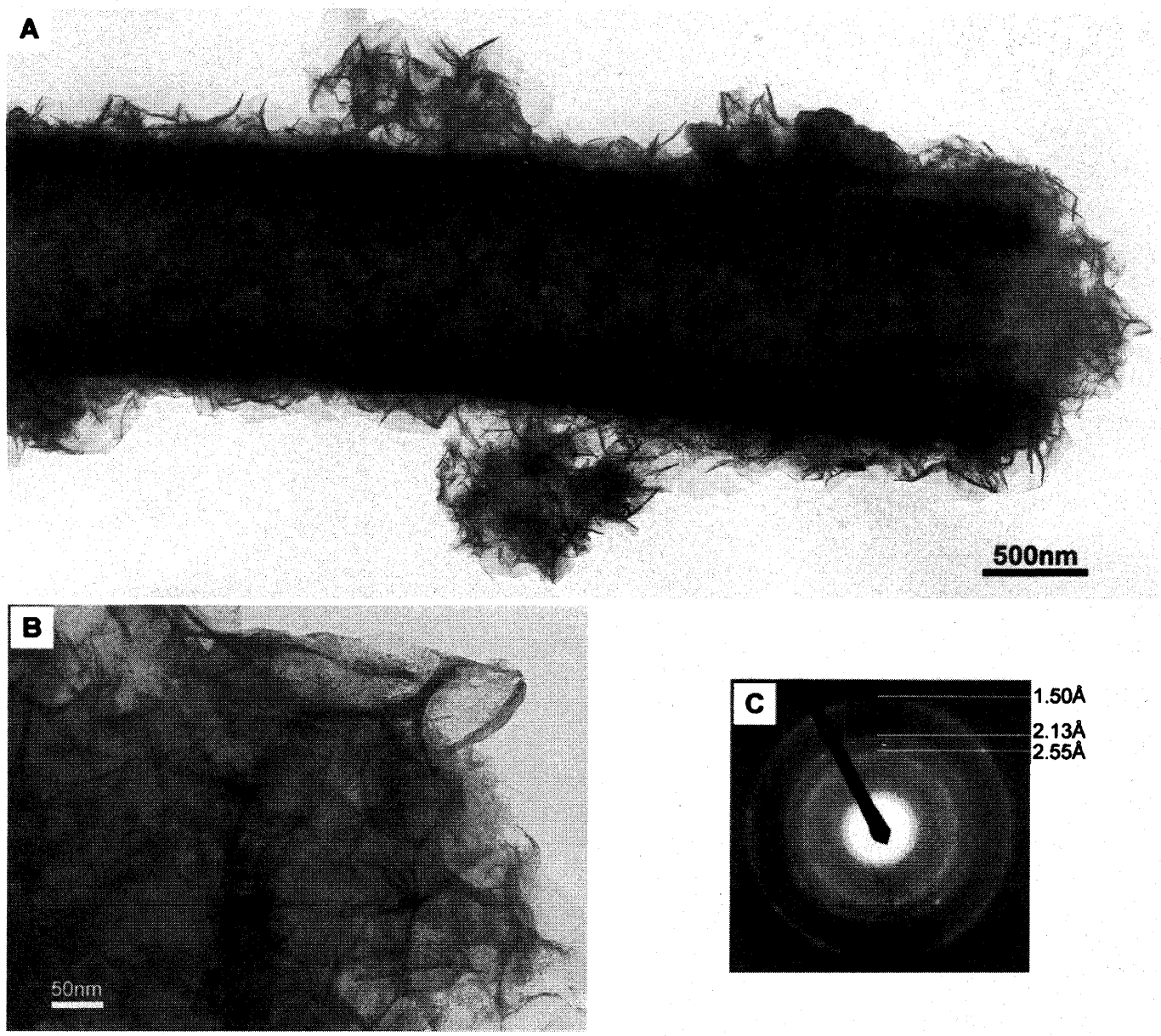


Fig. 4. Transmission electron micrographs of the sheath of *L.ochracea* on the cathode surface. A; *L.ochracea* are covered with flakey minerals. B; flakey minerals under high magnification. C; The SAED pattern of flakey minerals show *d*-spacing of rings, which have 2.55 Å, 2.13 Å and 1.50 Å indicating 2-line ferrihydrite.

size on their sheath surface (Fig. 4A). HRTEM micrograph gives a more precise shape of flakey minerals (Fig. 4B). The SAED pattern shows diffraction rings of 2.55 Å, 2.13 Å and 1.50 Å (Fig. 4C). According to Dawn et al. (2000), this diffraction rings indicate 2-line ferrihydrite [7].

The difference of the sheaths between in natural microbial mats and on the each electrodes is considered that the sheaths of *L.ochracea* on electrode such a lepidocrocite and ferrihydrite are formed by the affection of their metabolic activity. Usually, bacteria suspended in the water possessed negative charged surface, and migrate toward anode [8]. The surface charge of the sheath is attributed to the balance between negative charged cell surface and the positive charged sheath they formed. The inhabitation of *L.ochracea* on both anode and cathode surface indicate that the sheath of *L.ochracea* is classified two-types by electric charge surface: positive charged sheath and negative charged sheath. It is suggested that the sheaths of *L.ochracea* on the cathode surface possess a positive charged surface, and migrate toward cathode.

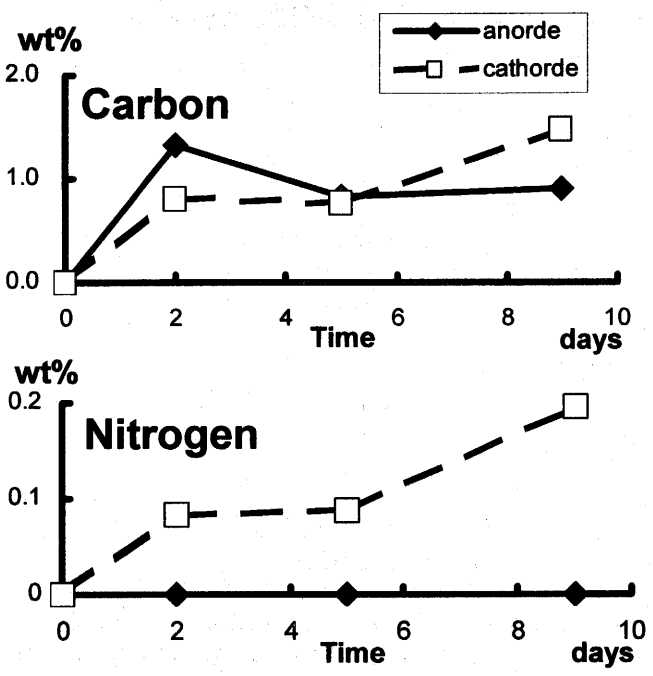


Fig. 5. Carbon and nitrogen analytical data of incrustation on each electrodes surface, showed that carbon content of incrustation on the anode are approximately steady after 2 day whereas nitrogen also steady throughout experiment. In contrast, carbon and nitrogen contents of incrustation on the cathode increase with aging time.

*B. Carbon and nitrogen analysis of the incrustation on each electrode surface*

Carbon and nitrogen analysis of incrustation on each electrode surface shows different tendency between anode and cathode. Carbon contents of incrustation on the anode are approximately steady after 2 day aging, and nitrogen also steady throughout experiments. For comparison, carbon and nitrogen contents of incrustation on the cathode increase associate with aging time. Carbon and nitrogen indicate organic material such a microorganism. The results indicate that bacterial contents of incrustation on the cathode were increased, and on the anode were steady. Microorganisms are highly amenable to the influence of electric charge, due to their small size and the large surface area. *L.ochracea* are also considered that their movement was controlled by electric field. Consequently, increasing of bacterial content in cathodic incrustation is suggested that *L.ochracea* possessed positive charged sheath were contained in microbial mats more than negative charged sheath, and formed flakey ferrihydrite with their metabolic activity.

## V. Conclusions

*L.ochracea* inhabited on the both anode and cathode, and possessed different mineral sheath such a lepidocrocite on the anode and ferrihydrite on the cathode, was revealed by electron microscopy. Bacterial contents on the cathode were increased associated with experimental aging time. *L.ochracea* is classified two-types by electric charge of their sheath surface: positive charged sheath and negative charged sheath. As results of these, *L.ochracea* in different environment such an anode or cathode surface can form different iron mineral sheath with their metabolic activity. Finally, characteristic of electrical charge of *L.ochracea* is suggested that *L.ochracea* possessed positive charged sheath were contained in microbial mats more than negative charged sheath.

## Acknowledgments

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