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Abstract

論文内容の要旨 (博士)

Title of Thesis 博士学位論文名	Analysis of Lipoquinones by On-line SFE – HPLC for Monitoring Environmental Biological Processes (環境生物学的プロセスのモニタリングに向けたオンライン SFE-HPLC を用いたリポキノンの分析)
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(Approx. 800 words)

(要旨 1,200 字程度)

Microbial community has an important role in the biological processes. Monitoring the microbial community in biological processes is necessary to ensure the process is working properly, and to improve its performance. Therefore, the necessity of a simple, rapid and reliable method with low running cost and technical skills is a basic requirement in the analysis. One of the most simple, quantitative and high reproducible method to determine the microbial community structure is lipolipoquinone profile method. Lipolipoquinone is constituent of the bacterial plasma membrane that is essential for electron transport. Lipoquinone could be used as biomarker for microbial community because lipoquinone exist in almost all bacteria, and generally, a species or genus in a microbial community produces one dominant type of lipoquinone thus any change in the lipoquinone profile reflects a change in the microbial community. Lipoquinone profile provides not only information about composition of the microbial community, but also their biomass concentration.

The conventional method for lipoquinone determination is direct extraction from the matrix using organic solvent, which is usually a mixture of chloroform and methanol. Since the method requires long extraction time and large volume of organic solvent that against the principle of green chemistry, the supercritical fluid extraction (SFE) method is then introduced. Carbon dioxide which is used as the extraction solvent in SFE is environmentally friendly with high diffusivity, selectivity, and many other advantages.

The on-line SFE-HPLC could have several potential advantages for the qualitative and quantitative determination of lipoquinone. On-line system can improve the performance and cost-effectiveness of analysis. The main objective of this project is to develop on-line Supercritical Fluid Extraction-High Performance Liquid Chromatography (on-line SFE-HPLC) for microbial community analysis method based on lipolipoquinone profile. The SFE was connected to the solid phase trapping column (Zorbax SB-C18) which is used to collect the extracted lipoquinone. This trapping column is the interface between the SFE and

HPLC systems and associated to six-port valve in HPLC system. Under on-line SFE-HPLC, all the extracted lipoquinone could be directly transferred to the chromatography system without tedious sample pretreatment. Therefore, the on-line SFE-HPLC is not only reduce the analysis time but also the sample requirement. In addition, since lipoquinone and their derivate are photosensitive and susceptible to oxygen, the direct transferring of extracted lipoquinone from extraction step to chromatography system can minimize the lipoquinone loss through degradation. Consequently, those can improve the reproducibility of analysis.

Optimization of the extraction and trapping column conditions were investigated using activated sludge. The reliability of the method was evaluated by comparing to the organic solvent extraction method. The optimum conditions obtained on activated sludge were as follows: 45°C; 25 MPa; 15 min; 10% methanol with flow rate of 1 mL min⁻¹ and water flow of 0.04 mL min⁻¹ in the trapping column. The on-line SFE-HPLC has been proved to be a more effective and rapid method for lipolipoquinone determination in various activated sludges

The static extraction then was proposed to combine with the dynamic extraction and methanol spiked directly into the sample to simplified the system. The effect of static extraction on extraction efficiencies of the lipoquinone was then investigated in order to eliminate the water pump and methanol pump in the previous system. The best results in terms of extraction yield were obtained at 25 MPa, 45°C, 10 min static extraction with 500 µL methanol spiked, and 30 min dynamic extraction with 0.9 mL min⁻¹ CO₂ flow rate. The development of on-line SFE-HPLC method offers simplification for a rapid and routine analysis of lipoquinone to monitor the performance of environmental biological processes based on lipoquinone profile.