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Abstract

TITLE:	Development and Application of Supercritical Fluid Technology for the Comprehensive Analysis of Microbial Lipid Biomarkers in Environmental Samples

Knowledge of microbial community structure plays a significant role in environmental assessment. The development and application of a more powerful and cost-effective method will provide new opportunities to gain a comprehensive understanding of the complex microbial community structure in the environmental samples.

One of the most commonly used analytical methods in microbial community structure is the microbial lipid biomarker profiles. In this thesis, four lipid biomarkers, namely respiratory quinones (RQ), phospholipid fatty acids (PLFA), phospholipid ether lipids (PLEL), and polyhydroxyalkanoates (PHA) were selected as target compounds. These lipid biomarker profiles have been well recognized as a quantitative and sensitive method for determining the microbial community structures in environmental samples without the need for the laboratory isolation and cultivation. A combination of the profiles of different lipid biomarker profiles could provide a comprehensive understanding of microbial interactions in complex communities.

Conventionally, analysis of lipid biomarkers is performed using organic solvent extraction. However, increased awareness of the environmental, health and safety issues in the use of organic solvents has triggered to study supercritical fluid extraction (SFE). SFE using carbon dioxide ($scCO_2$) as a solvent is a green technology and offers numerous advantages for analytical purpose, mainly selectivity, rapidity and low solvent volume usage. In addition, current knowledge of the microbial lipid biomarkers analysis using SFE is mainly derived from the study of pure cultures.

Therefore, the main objective of this thesis was to develop and apply the SFE methods for the comprehensive analysis of microbial lipid biomarkers in real environmental samples. New strategies for SFE method were investigated. Evaluation of the reliability of the SFE method was performed by comparing to the conventional organic solvent extraction method. A statistical experimental design was also applied for the optimization of the SFE parameters.

First, SFE methods for single lipid biomarker were investigated. The use of $scCO_2$ extraction with a solid-phase trapping cartridge followed by ultra performance liquid chromatography (UPLC) proved to be a more effective and rapid for extracting microbial RQ biomarker. Simultaneous $scCO_2$ extraction and chemical derivatization with in-line a solid-phase trapping for the microbial PLFA biomarker analysis resulted in a 90% reduction in solvent usage and more than a ten-fold reduction in sample preparation time. Solid-phase trapping eliminated the need for purification and obtained reproducible results in the final gas chromatographic (GC) analysis. These methodologies led to a successful analytical procedure that involved a significant reduction in the complexity and sample preparation time. Second, the suitability of SFE method to simultaneously extract microbial RQ, PLFA, PLEL and PHA in environmental samples was investigated. The effects of several SFE parameters on the total amounts of the extracted lipid biomarkers were investigated using a statistical experimental design. This study showed the potential application of SFE as a routine method for the comprehensive analysis of microbial community structures in environmental assessment using the lipid biomarker profiles. Finally, application of the developed method was demonstrated to various environmental samples obtained from anaerobic digestion, wastewater treatment, and composting process.

The methodologies that developed in this thesis open the road to a superior method for analysis of microbial lipid biomarkers comprehensively for environmental assessment and monitoring, with the possibility of extended application and automation in the future. The findings of this thesis will also trigger and motivate scientists to work efficiently in this field, accelerating the data and information on microbial community structure in environmental samples to provide useful references for further development of ecologically relevant process.