



# HOKKAIDO UNIVERSITY

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## 学 位 論 文 内 容 の 要 旨

### DISSERTATION ABSTRACT

博士の専攻分野の名称    博士（工学）    氏名   山村   宏江

## 学 位 論 文 題 名

Title of dissertation submitted for the degree

Toxicogenomic Approach to Impact Assessment of Whole Wastewater Effluents and

Development of Effluent-Responsive Biomarker

(トキシコゲノミクス的アプローチを用いた下水処理水の包括的影響評価及び下水応答性  
バイオマーカーの開発)

The reclaimed wastewater has been served as an alternative water source in some countries. Although growing water stress, cost and energy constraints have prompted a call for more widespread utilization of wastewater reclamation and reuse practices, uncertain impacts of wastewater residues including vast categories of micropollutants (e.g., pesticide, endocrine disrupters, pharmaceuticals) and poorly characterized chemicals (e.g., degradation products) remains as a challenge to increase the public acceptance. To evaluate overall impacts of wastewater effluents, various types of bioassays have been used. The test organisms used in the previous studies include Japanese medaka, *Daphnia magna*, algae, luminescent marine bacteria, yeast, and mammalian cells. However, since a set of bioassay methods targeting on different endpoints are usually adopted to evaluate the overall effluent toxicities, the test procedure tends to be laborious and time-consuming. Thus, a single more rapid, comprehensive, and sensitive approach needs to be developed. The recent advances in genomics have provided clues to understand the responses of biota to the environmental pollutants and its associated mechanisms of actions. The DNA microarray can detect the genome-wide gene expression. The patterns of such gene expression responses represent the primary interactions between the environmental contaminants and biota. Thus, it has already been widely applied in the field of medicine and pharmacology, as a rapid and comprehensive method for detection of early cellular response as gene expressions.

The present study was carried out to reveal the impacts of whole wastewater effluents on cultured human cells based on the gene expression response, and to develop effluent-responsive biomarkers. More specifically, gene expression patterns were analyzed and compared between treated and untreated cells by comprehensive gene expression analysis using the DNA microarray, and impacts of effluent impacts on the cellular function so of cultured human cells were identified. Furthermore, the biomarkers, the genes whose expression was

altered corresponding to the effluent exposure, were determined based on the dose-response relationship, and their expression were compared before and after physico-chemical manipulations.

This thesis is composed of 7 different chapters. Chapter 1 provides a general background of water reclamation practices and bioanalytical tools to assess the overall toxicity of environmental samples including toxicogenomic approaches. Chapter 2 provides a literature review on the past findings of the overall toxicity of aqueous samples, focusing on the used methods and the effectiveness of the water treatment technologies for the toxicity removal.

In Chapter 3, DNA microarray-based transcriptome analysis was applied to impact assessment of whole wastewater effluents from Membrane Bioreactors (MBRs) and Activated Sludge process (AS), on the biological processes of human hepatoma HepG2 cells. The conventional bioassays (i.e., cytotoxicity tests and bioluminescence inhibition test) were conducted in parallel since they were well-established evaluation methods for the overall effluent toxicity. The DNA microarray-based transcriptome analysis with human HepG2 cells was suggested as a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents.

In Chapter 4, based on their dose-response relationship in exposure of concentrated MBR and AS effluents from 10 to 50 mg-DOC/L, AKR1B10, CYP1A1, GPX2 and GCLM were selected as effluent-responsive marker genes among the commonly altered genes identified in the DNA microarray analysis of two or more effluents. The results altogether suggested RO concentration as an effective pretreatment step for DNA microarray of relatively "clean" effluents.

In Chapter 5, quantification of four selected marker genes (i.e., AKR1B10, CYP1A1, GPX2 and GCLM) before and after various manipulations suggested that hydrophobic organic content, which may be forming complex with metals, were responsible fraction for a part of gene expression response observed in HepG2 exposed to MBR and AS effluents (particularly CYP1A1-inducible fraction). The chemical analysis indicated that the responsible fraction would behave together with humic substances or their building blocks with fell in a size range from 300 to thousands of Da.

In Chapter 6, the change in the modes of toxic actions of inorganic arsenic at environmentally relevant concentrations to apparently toxic concentration was investigated by DNA microarray-based transcriptome analysis. Comparison of gene expression profiles between effluents and model toxicants revealed the uniqueness of the gene expression profiles.

Finally, Chapter 7 presents the main conclusions extracted from the work presented in this dissertation and a few recommendations for future work.