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DOCTORAL THESIS

TOXICOGENOMIC APPROACH TO IMPACT
ASSESSMENT OF WHOLE WASTEWATER EFFLUENTS
AND
DEVELOPMENT OF EFFLUENT-RESPONSIVE
BIOMARKER

HIROE HARA-YAMAMURA

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ABSTRACT

The reclaimed wastewater has been served as an alternative water source in some countries but uncertain impacts of wastewater residues including vast categories of micropollutants remains as a challenge to increase the public acceptance. Due to its comprehensive and rapid nature, transcriptome analysis such as DNA microarray and quantitative RT-PCR (qPCR) assay, was applied to human hepatocarcinoma cells (HepG2) exposed to the effluents from membrane bioreactors (MBRs), and the activated sludge process (AS), to better understand the whole effluent impacts on humans, and develop genetic markers for such impacts. The effective reclamation processes were further suggested for the selected wastewater effluents based on the toxicity reduction evaluation study using the genetic markers.

The DNA microarray was first applied to MBR and AS effluents without any enrichment processes. In parallel, the conventional bioassays (i.e., cytotoxicity tests and bioluminescence inhibition test), which were well-established for the evaluation of the overall effluent toxicity were also performed for the same samples. The transcriptome analysis identified 2 to 926 differentially expressed genes after exposure to the effluents and the raw wastewater, which were categorized to 0 to 225 biological processes. Among the tested effluents, the MBR operated at a relatively long solid retention time (i.e., 40 days) and small membrane pore size (i.e., 0.03 μm) was suggested to have the least impacts on the HepG2 even at the level comparable to tap water. The observed gene expression responses were in good agreement with the results of cytotoxicity tests, and provided additional molecular mechanistic information on adverse effects occurred in the sub-lethal region.

To select the effluent-responsive genetic markers, dose-response relationship between limited number of genes and effluent concentrations were studied by using MBR and AS effluents concentrated 10-15 times by reverse osmosis (RO). RO concentration successfully achieved sample enrichment with more than 80% recovery of organic content in the effluents. The qPCR assay demonstrated that four out of nine candidate marker genes, which were selected from the DNA microarray data obtained for the concentrated effluents (i.e., 30 mg/L), had clear concentration-dependency, and thereby suggested relevant as effluent marker genes.

Based on the reduction in gene expression levels of marker genes, effectivity of the selected physicochemical treatment processes were investigated for both simulation of actual reclamation system and identification of responsible fraction. The qPCR assay of four marker genes (i.e., AKR1B10, CYP1A1, GCLM, and GPX2) before and after four typical treatments (i.e., aeration, solid phase extraction with C18, chelating, and ion exchange) together with the detail chemical analysis suggested that hydrophobic organic content, which may be forming complex with metals were responsible for a part of gene expression response observed in HepG2 exposed to the effluents. In addition, it was indicated the possibility that the responsible fraction behave together with humic substances or their building blocks falling in a size range from 300 to thousands of Da. Thus, activated carbon adsorption or reverse osmosis were suggested as key processes in reclamation system of the selected MBR and AS effluents.

At the end, the arsenic-modulated gene expressions were investigated from inorganic arsenic exposure (5 nM to 40 μM as arsenic trioxide for 48 hours). The concentration dependent modulation of gene expression (induction of cell cycle genes, suppression of DNA repair genes, induction of cell cycle arrest genes and apoptotic genes) following exposure to arsenic was further supported by acceleration of cell proliferation, ROS generation, and cytotoxicity. These results indicated the potential pro-carcinogenic actions of inorganic arsenic occur in environmentally relevant exposures. Comparison with gene expression profiles between effluents and

model toxicants revealed the uniqueness of the gene expression profiles of the effluents were suggested.

In conclusion, the transcriptome analysis with human HepG2 cells is a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents. The effluent-responsive genetic markers were also proposed to quantitatively evaluate the treatability of the wastewater effluents. Based on the response of genetic markers, the activated carbon adsorption was suggested as an effective process to remove responsible fraction, and the importance of final polishing with RO was also supported. Since there is still a gap between the gene expression response and the manifestation of toxicities, the gene expression response should be considered as “potential” of toxicity and thereby, further accumulation of DNA microarray data with well-established toxicity data is indispensable for development of this technology. In addition, due to the change of raw wastewater quality, seasonal variations of effluent impacts are to be investigated based on the gene expression response.

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Supplemental Materials

Transcriptome-based Evaluation of Whole Wastewater Effluent and Development of Biomarkers for Wastewater Residues [SUMMARY]

Hiroe HARA-YAMAMURA

Introduction

Background

The reclaimed wastewater has been served as an alternative water source in some countries, for irrigation water, industrial water, recreational water, and even for indirect and direct potable water (1,2). 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 vast categories of micropollutants (e.g., pesticide, endocrine disrupters, pharmaceuticals) (3,4,5), poorly characterized or even unknown chemicals in wastewater effluents (e.g., degradation products) (6,7), remains as a challenge to increase the public acceptance. Due to such complex nature of wastewater effluents, it is not feasible that chemical-specific analysis covers all the constituents potentially appear in the effluent, and thereby, the importance of whole effluent evaluation has been recognized (8).

Literature Review

Various types of bioassays have been used to evaluate overall impacts of wastewater effluents. The test organisms used in the previous studies include Japanese medaka, *Daphnia magna*, algae, luminescent marine bacteria, yeast, and mammalian cells (9,10,11,12). However, most of the conventional bioassays targeted on the impacts on non-human organisms, and those on humans have not been fully developed for potable or any other type of reuse practice with a contact with human bodies. Furthermore, 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 (13,14). 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. Some researchers have applied this technology to characterize the toxicity of mixed chemicals or environmental samples such as polyfluorinated and perfluorinated compounds (15), diesel exhaust particles (16), oil contaminated waters (17,18), and wastewaters from factories (19,20). Since there is still a gap between gene expression responses and the manifestation of actual toxicity, accumulation of microarray data along with the well-established bioassay data is necessary.

From the environmental engineers' point of view, the characterization of physicochemical properties of responsible fractions is of larger importance than

identification of the specific chemicals since it is the information required in improvement of reclamation systems. So far, however, there has been no research reporting the relationship between certain gene expression responses and the physicochemical properties of the responsible fraction in the complex environmental samples except for Omura et al. (2009) (21), who revealed that n-hexane soluble fraction and n-hexane insoluble fractions of diesel exhaust particles regulated characteristic genes which respond to chemical properties of each fraction in rat alveolar epithelial cells. Thus, fractionation of complicated environmental samples was demonstrated effective to establish a relationship between gene expression responses and physico-chemical properties of responsible fraction.

Research Goals

The present doctoral research was carried out to provide a single rapid, sensitive and more comprehensive evaluation method of wastewater effluent impacts on humans, which increase reliability and efficiency of wastewater reclamation systems. To achieve this goal, the specific aims were set as:

1. To apply DNA microarray analysis to impact assessment of whole wastewater effluents
2. To select genetic markers for the impacts of wastewater effluents
3. To evaluate treatability of wastewater effluents using the selected genetic markers

Materials and Methods

Cell Culture

Human hepatoma HepG2 cells were provided by Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). HepG2 was selected because it has been frequently used in vitro model for human detoxification of chemicals, is easy to handle, and provides a reproducible human system. However, since it is known that expression of specific genes were extremely low in the cancer derived HepG2, the results could not be simply extrapolated into the normal cells and in vivo (22). Cells were grown in Eagle's minimal essential medium (MEM) (Nissui, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Cells were maintained at 37 °C in the 5% CO₂ humidified incubator.

Sampling site

Sampling was carried out at the pilot-scale MBR plant treating the real municipal wastewater collected via combined sewage system in Sapporo, Japan. Two pilot-scale MBRs were installed at the plant: the submerged MBR (S-MBR) equipped with hollow-fiber polytetrafluoroethylene (PTFE) membrane with 0.3 µm nominal pore size, and the air-sparged side-stream MBR (AS-MBR) equipped with

tubular polyvinylidene fluoride (PVDF) membrane with 0.03 μm nominal pore size (**Fig. 1**) (23). The operational conditions and general effluent quality of selected processes are found in elsewhere (13). Characteristics of the raw wastewater of this plant can be found elsewhere (24). Grab samples were taken from the two MBRs around 10:00 am.

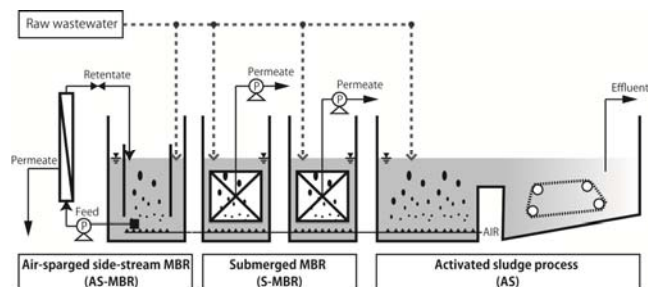


Fig. 1 Flow scheme of selected treatment processes. The raw wastewater and effluents were taken at a full-scale activated sludge process (AS) and three pilot-scale membrane bioreactors (MBRs) treating the same domestic wastewater

Treatment

The effluent samples were filtered for sterilization before exposure and organic content (dissolved organic carbon: DOC) was measured by the total organic carbon (TOC) analyzer (Shimadzu, Shiga, Japan). After adjustment of DOC to six levels from original DOC up to 50 mg/L using sterilized Milli-Q water, 46 mL of each dilution was combined with 4 mL of $\times 10$ concentrated MEM, and supplemented with 1% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Finally, pH was adjusted to 7.2-7.5 by adding 7.5% sodium hydrogen carbonate solution. Cells were seeded onto culture vessels in 105 cell/mL and incubated overnight to grow 40-50% confluent. The normal culture medium was replaced by the prepared samples and incubated for 48 h.

DNA microarray

Gene expression analysis was conducted only for the concentrated S-MBR effluent and for the selected model chemicals, gene expression profiles obtained in our previous studies were used (17,25). Cells exposed to the concentrated S-MBR effluent were lysed on the 60 mm culture dishes, and total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany). For duplicate, two dishes were prepared for each sample and individually treated in parallel. Total RNAs were quantified using the Nanoview (GE Healthcare, US). RNA with a A260/A280 ratio 1.8 or higher was used for DNA microarray analysis using Human Genome Focus Array (Affymetrix, Santa Clara, CA), which represents 8,795 verified human sequences from the NCBI RefSeq database. Target preparation and hybridization were carried out as described in the manufacturer's instruction. After hybridization, the array was washed, stained with streptavidin-phycoerythrin using the Affymetrix GeneChip Fluidics Station 450 (Affymetrix), and scanned on a GeneChip Scanner 3000 7G (Affymetrix). The raw data files [cell intensity (CEL) files] were created from scanned images by AGCC (GeneChip Command Console Software,

Affymetrix). The files are available on Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.gov/geo/>) of the National Center for Biotechnology Information (NCBI).

Cytotoxicity test

Viability of HepG2 cells after 48 hour was studied based on the mitochondrial dehydrogenase activity determined by MTT, which measured the amount of formazane resulted from reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT). MTT (Sigma-Aldrich, Japan) dissolved in PBS was added onto the treated cells seeded in 96-well plates and incubated for 4 hours. Supernatant was removed after centrifugation for 5 min at 3000 rpm, and the amount of formazane pigment in DMSO (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) elutes was measured at 540 nm by plate reader (multilabel counter ARVO MX, PerkinElmer, MA, US).

Quantitative Real-Time Reverse-Transcriptase PCR (qPCR)

Based on the microarray data, nine genes (i.e., CD3D, AKR1B10, GPR109B, GCLM, GPX2, AKR1C2, PLA2G1B, and PDZK1) were selected as candidate marker genes for effluent impacts. The expression levels were quantified by the quantitative real-time reverse-transcriptase polymerase chain reaction (qPCR). The primer sequences are shown in elsewhere. Data were analyzed by the relative quantification method using the Ct values normalized with the endogenous reference, GAPDH, which was measured in all runs. The fold induction/suppression of each target gene was calculated by dividing the normalized Ct value in a sample with that of the control. All assays were tested for presence of unspecific amplicons and primer dimmers by melting curve analysis.

Statistics

For microarray analysis, probe-level data in CEL files were normalized by MAS5 using AGCC and transferred to the Subio Platform ver. 1.15 (Subio, Inc., Tokyo, Japan). Reproducibility of the overall gene expressions of sample pairs were confirmed based on physical distance by principal component analysis (PCA). The features which were NOT flagged "Present" in any duplicates of the samples were rejected. We identified differentially expressed genes based on the two criteria: 1) a t-test p-value threshold of 0.05 and 2) a minimum fold change of 2 between the controls and exposed data sets. Gene ontology (GO) assignments and clustering into functional groups were performed using web-accessible programs provided by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>).

RO concentration for TIE study

Immediately after sampling, the MBR effluents were pre-filtered with 0.22 μm PTFE membrane (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) to remove suspended solids. Effluent concentration by reverse osmosis (RO) was conducted by the cross-flow filtration system for flat-sheet membranes (SEPA CF II Unit; SANKO Ltd., Yokohama, Japan), equipped with polyamide RO membrane (effective

surface area: 140 cm²; GE Water & Process Technologies, PA, US) under the constant pressure (2 MPa). The cross-flow speed and permeate flux were 0.1 m/sec and 42 L/m²/h, respectively. During the operation over two nights, the retentate was kept in cold water bath (8 °C), and the salt rejection rate more than 98% was monitored by the electronic conductivity meter.

Toxicity Identification Evaluation (TIE)

Toxicity Identification Evaluation (TIE) studies, whose guidelines are available from US-EPA (28,29), provides us with an approach to characterize the cause of observed effluent toxicity, where physical and chemical fractionation with biological toxicity testing allow the characterization and identification of key toxicants in various matrices, such as industrial wastewater, sediment, and leachate (30,31,32,33). Key toxicants identified can be reduced or eliminated by correcting treatment deficiencies or applying specific treatments. Immediately after sampling, the MBR effluents were pre-filtered with 0.45 µm glass fiber filter (GB-140, Advantec, Japan) membrane to remove suspended solids, and concentrated by reverse osmosis at the pressure-constant cross-flow mode (recovery of organic matters: >85%). All the concentrated effluents were adjusted to the same DOC level (i.e., 50 mg/L). Based on the TIE method (28), six different manipulations were applied to the concentrated effluents to examine the contribution of the target fraction removed by each treatment to the overall cytotoxicity: aeration with air (to remove the volatile, oxidizable, orcoagulable) and nitrogen gas (volatile, or coagulable),

solid phase extraction (SPE) with Sep-Pak C18 (hydrophobic) and ion exchange resin (cation/anion), and chelating with NOBIAS chelate-PA (metallic). In each manipulation, MQ water was treated in parallel and considered as a control.

Results and Discussions

Gene expression analysis using DNA microarray.

The DNA microarray analysis identified 2 to 926 genes which were differentially expressed after exposure to the effluents and raw wastewater. The numbers of the altered genes were far large in the raw wastewater (926 genes) compared to the effluents (<200 genes), indicating the reduction of cellular impacts after treatments. Among the effluents, however, the extent of alteration of the gene expression was relatively large for S-MBR_B (181 genes), moderate in S-MBR_A (39 genes) and AS (20 genes), and the lowest for AS-MBR (2 genes), which was even comparable to the tap water (TAP; 2 genes). For the bottled water (BOT), differentially expressed genes were not identified. More than half of the altered genes of S-MBR effluents were common with those of the raw wastewater.

Biological functional analysis was conducted by DAVID to connect altered genes and related biological processes, and identify the specific processes consisting of significant number of connected genes (**Table 1**). The smaller *p* value represents the higher significance of identification of the corresponding biological process. In AS-MBR effluent and the real drinking waters (i.e., TAP and BOT), any biological process was not identified at the statistical significance (*p*<0.05).

Table 1 Gene Ontology (GO) categories of differentially expressed genes. Biological functional analysis was conducted by the web-accessible program, DAVID (<http://david.abcc.ncifcrf.gov/>) to assign GO terms to the altered genes and cluster the related biological processes.

Related Process Groups	Biological Process	p value (log10)						
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW	TAP	BOT
Cell growth	regulation of cell growth				-2.37	-1.50		
	regulation of lipid metabolic process				-1.75	-1.16		
Lipid metabolism	lipid catabolic process		-1.88	-1.95				
	steroid metabolic process		-2.23	-3.02		-6.02		
	cholesterol metabolic process		-1.33	-1.05		-2.80		
	bile acid catabolic process			-2.87		-1.49		
	lipid transport			-1.73		-1.48		
Lipid transport	carboxylic acid transport		-1.95	-1.12				
	response to endogenous stimulus			-3.35	-1.39	-2.66		
Response to endogenous stimulus	response to hormone stimulus			-2.96		-2.77		
	response to corticosteroid stimulus			-3.56		-1.68		
	response to glucocorticoid stimulus			-3.07		-1.68		
	response to estradiol stimulus					-2.07		
	response to inorganic substance			-1.40				
Response to inorganic substance	leukocyte mediated immunity					-2.93		
Acute inflammatory response	cell death					-2.45		
Cell death	apoptosis					-2.49		
	response to nutrient					-2.45		
Response to nutrient	response to vitamin					-3.33		
	mitosis					-10.01		
Cell division	M phase					-9.37		
	cell cycle					-8.24		
	spindle organization					-3.47		
	microtubule-based process					-3.31		

Legend

	-2.0 < log[p] ≤ -1.0
	-3.0 < log[p] ≤ -2.0
	-4.0 < log[p] ≤ -3.0
	log[p] ≤ -4.0

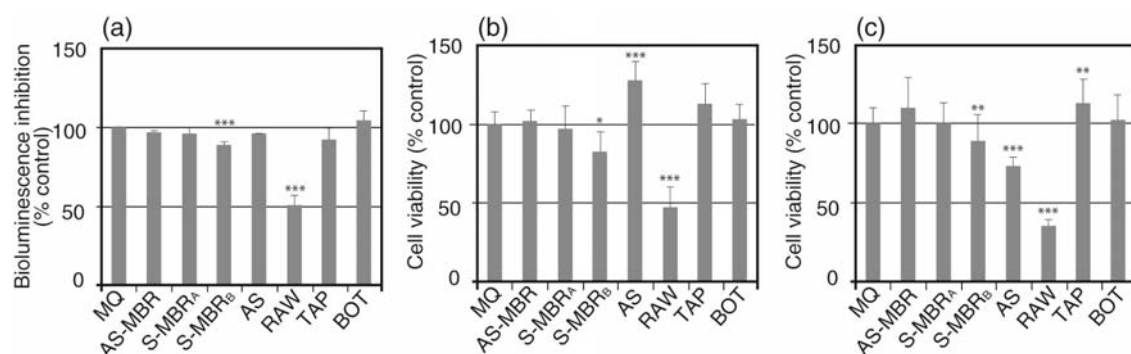


Fig. 2 Results of effluent toxicity evaluation by conventional bioassays: bioluminescence inhibition test (a) and the two cytotoxicity tests using direct cell counting (b) and MTT assay (c). Values were expressed as mean $\pm$ standard deviation of replicated experiments ($n > 3$). Statistical test was performed by using Tukey's multivariate analysis; *: <0.05 ; **: <0.01 ; ***: <0.001 .

For the rest of effluent samples and the raw wastewater, altered genes were categorized into 7 to 225 biological processes, which were further summarized into major nine groups of closely related processes: cell growth, cell division, lipid metabolism, lipid transport, response to endogenous stimulus, response to inorganic substance, acute inflammatory response, response to nutrients, and cell death. The biological processes identified for S-MBR_A exposure were common with those for S-MBR_B exposure, and the significance of alteration was mostly higher in the latter. The process groups such as “lipid metabolism” and “response to endogenous stimulus” were commonly identified in S-MBR effluents, AS effluent, and the raw wastewater exposures, while “response to inorganic substance” and “cell growth” were unique to S-MBR_B effluent and AS effluent, respectively. Although the significance of alteration of the gene expression was relatively high in “acute inflammatory response”, “cell death”, “response to nutrient” and “cell division”, these process groups were only identified in the raw wastewater.

The altered genes in the selected process groups and their fold change (FC) (relative expression ratios to the control) for the effluents and the raw wastewater are listed in Table 4. Most of the genes related to “lipid metabolism” were shared between S-MBR effluents and raw wastewater, with the increase of FCs in the latter sample. In AS effluent, however, the altered genes such as *AVPR1A*, *IGFBP7*, and *PPARA* were unique to its exposure. The genes categorized in “response to endogenous stimulus” exhibited a similar trend as “lipid metabolism”, reflecting some overlaps of the genes and probably close relationship of the two functions in the cellular response to the effluents. In S-MBR_B effluent, the genes categorized in “response to inorganic substance” (i.e., *DUSP1*, *FGB*, *SERPINE1*, *GCLC*, and *AQP3*) were largely altered by 4- to 13-fold, while in AS effluent, the genes categorized in “cell growth”, (i.e., *IGFBP7*, *CYR61*, *OSGIN1*, and *AVPR1A*) were moderately altered by 2- to 4-fold.

Although we currently cannot exclude the genetic response derived from the innocuous matters, if any, the changes of biological processes which have been suggested as the response to the toxic chemicals or environmental stresses were considered as a potential indication for the toxic effects. Among the identified biological processes,

“lipid metabolism” was the only process commonly affected by the exposure to the effluents and the raw wastewater. The alteration of the genes in this biological process was also reported when HepG2 cells were exposed to the organic toxicants such as aflatoxin, polycyclic aromatic hydrocarbons (PAHs), cationic amphiphilic drugs, and also the common carp exposed to the oil contaminated water. Based on these results, it was inferred that the alteration of “lipid metabolism” might not be a chemical-specific response but a general response to a wide range of chemicals in the tested cells. The drastic alteration of the lipid metabolism-related genes in the raw wastewater supported this hypothesis since the raw wastewater was likely to contain the largest variety of chemicals as indicated in its highest organic content (total organic carbon (TOC): 34 mg/L).

Conventional bioassays

Bioluminescence intensity of *V. fischeri* after exposure to effluent samples, the raw wastewater and drinking water samples is shown in Fig. 2a. Clear bioluminescent inhibition was observed for raw wastewater ($> 50\%$ of the control). However, in AS-MBR, S-MBR_A, and AS effluents, the bioluminescence intensity was unchanged as compared to that of the control. Only S-MBR_B effluent showed a slight reduction (i.e., 10%). Similarly, significant bioluminescence inhibition was not observed for the drinking water samples. Cell viability was evaluated by direct cell counting with tripan blue dye exclusion method and the MTT assay (Fig. 2b and 2c). The direct cell counting exhibited a significant reduction of cell viability in S-MBR_B and raw wastewater by 13% and 55%, respectively, while AS-MBR and S-MBR_A effluents did not affect the cell viability. Cell viability increased by 26% after exposure to the AS effluent. The results of MTT assay were consistent to those of the direct cell counting except for AS, which on the contrary marked 23% reduction in the cell viability.

Quantification of selected marker genes.

The detection frequency of the genes described above, and their accuracy in the DNA microarray analysis were taken into consideration, and nine genes were selected as candidate marker genes of wastewater impact. To examine their

capability as biomarker, the gene expression levels were quantified by qPCR over six concentrations from original DOC in the effluent up to 50 mg/L. FCs (relative expression ratios to the control) of candidate marker genes, CYP1A1, AKR1B10, GPX2, GCLM, and PDZK exhibited a clear concentration-dependency in their expression level changes, while it was only partly observed for AKR1C2 and GPR109B, and the correlation was poor for CD3D and PLA201B. Since the extent of the fluctuation was relatively small for PDZK, four genes (i.e., CYP1A1, AKR1B10, GPX2, and GCLM) were determined as marker genes. The reproducibility of their expression was also confirmed using the concentrated effluent samples taken on different day.

Gene expression levels before and after manipulation

The expression levels of the selected marker genes, which represented effluent impacts, were measured for before and after manipulations by qPCR assay (Fig. 3). Although there was no significant change in FCs of AKR1B10 and GCLM before and after manipulations, the relative expression level of CYP1A1 dropped after C18 and chelating, and slightly decreased after anion exchange. Similar trend was also observed for GPX2, particularly in the cells exposed to S-MBR effluent. These results suggested that the selected marker genes represent the effluent fractions with different chemical properties. CYP1A1 and GPX2 responded to the hydrophobic fraction and/or those with anionic feature since their expression was significantly reduced after SPE with C18 and chelating and anion exchange. Whereas, it was hypothesized that AKR1B10 and GCLM responded to the neutral and hydrophilic fraction which was not be able to remove by any of the selected manipulations, or they are non-specific markers. The qPCR data was in good agreement with the DOC removal calculated for C18, chelating resin and anion exchange resin (Table 5). Interestingly, chelating with lower DOC removal (ca. 20%) than C18 (>50%) were more effective to mitigate the induction of CYP1A1 and GPX2. Thus, it was more likely that responsible organic fraction formed a complex with metals.

Cytotoxicity before and after manipulations

The viability of HepG2 exposed to the concentrated effluents before and after six different manipulations (i.e., aeration with nitrogen, aeration with pure air, SPE with C18, chelating, cation exchange, anion exchange). The result clearly demonstrated that the effluent both before and after manipulations were not cytotoxic. Significant increase of the cell viability after three manipulations (i.e., aeration with pure air, C18, and chelating) in AS effluent might be due to the generation of more biologically active components by oxidation or removal of masking agents by C18 and chelating resin. At present, however, it is difficult to tell if such increase of cell viability in cytotoxicity test represents innocuous or hazardous impacts.

Conclusions

Due to its comprehensive and rapid nature for detecting early response of human cells, transcriptome analysis such as DNA microarray and quantitative RT-PCR assay, was

applied to evaluate the whole effluent impacts and establish marker genes for the wastewater impacts on human cells (i.e., HepG2). Major findings are listed below:

- 1) DNA microarray-based transcriptome analysis with human HepG2 cells is a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents.
- 2) The four marker genes for effluent impacts (i.e., CYP1A1, AKR1B10, GPX2, and GCLM) were proposed and demonstrated useful in investigation of treatability of effluents.
- 3) For reclamation of selected MBR and AS effluent, activated carbon adsorption and RO filtration are expected to be key treatments

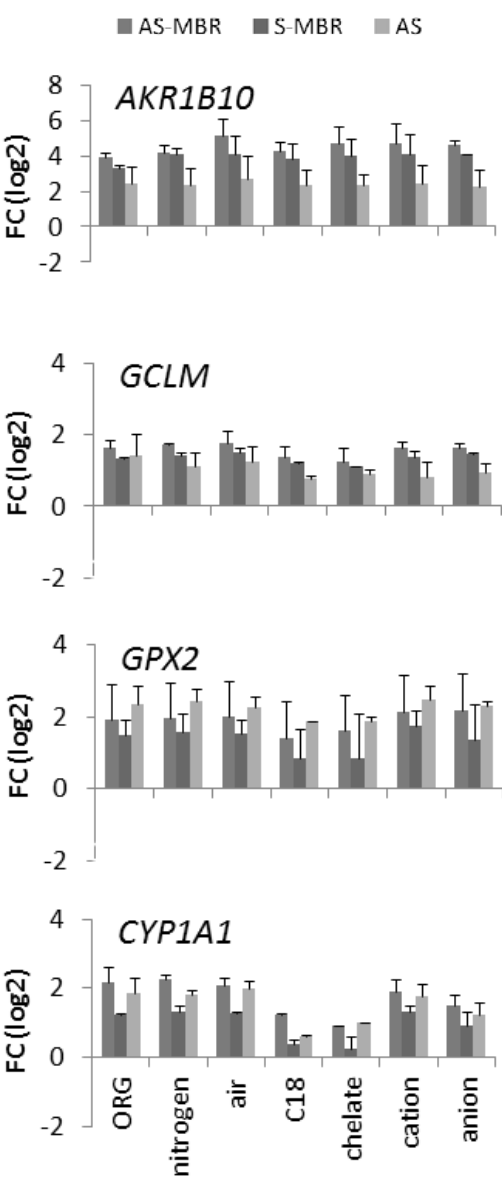


Fig. 3 Cytotoxicity before and after manipulations. (DOC before manipulation: 50 mg/L)

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