B−21 Biological-based Response Assessment of Membrane Reclamation System using Bioanalytical Tools

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1. INTRODUCTION

With many parts of the world suffering from water shortage, efforts have been made to address this increasing crisis through the exploration of wastewater reuse and reclamation process as a direct alternative for either raw water supply or potable water to meet water demand, continue water supply reliability and sustain existing resources [1,2]. Due to reclamation plant's limitless water supply and being near populated area, one biggest future application for reclaimed water is its potential as a raw supply for indirect and direct potable reuse. Such application is already operational in California Orange County, New Water Singapore, Nambia Africa and etc. [3].

An important consideration in potable reuse of reclaimed water is the presence of unregulated or emerging contaminants of complex micropollutants such as pharmaceuticals, personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) [4]. Though present in low concentration, many of these micropollutants are transformed in the environment and synergistic effects are highly potential to occur in different water matrixes.. In addition, chemical parameters and analysis (COD, BOD, GC-MS, etc.) are not enough to completely assess the performance of treatment processes due to full comprehensive chemical characterization being difficult because of the limitation of hazardous contaminants through targeted analysis; this causes for potentially toxic substances to be overlooked [5,6]. Biological-based testing and monitoring was therefore recognized as an essential additional practice for wastewater treatment and wastewater reuse in many countries [7].

The main objective of this study was to evaluate the toxicity of membrane based reclamation systems through the use of biological responses of water effluents from conventional bioassay and gene expression analysis.

2. MATERIALS AND METHODS

(1) Sampling

Table 1. Operational Condition of MBRs

Pilot-scale: Membrane Bioreactor (MBR)		
Properties	**AS-MBR I**	**S-MBR**
SRT (days)	40	50
HRT (days)	3.75	3.75
Flux (m/day)	2	0.4
Pore Size (um)	0.03	0.3
Material	PVPF tubular	PTFE hollow fiber

Fifteen (15) liters of water samples was taken from existing membrane bioreactor (MBR) pilot-plant at Sosei River Treatment plant with different operation condition (table 1): submerged MBR (S-MBR) and air-sparged side-stream MBR I (AS-MBR I). MQ was used as control while raw drinking water from Toyohira River (TR) was used as reference water sample and included at every sampling. S-MBR was sampled June 19, 2013 while AS-MBR I was taken last July 19, 2013.

(2) Laboratory Simultaed Tertiary Treatment

Tertiary treatment, nanofiltration (NF) and reverse osmosis (RO) were also explore in this study. Laboratory scale cross-flow membrane filtration was done to secondary effluents AS-MBR I and S-MBR using two system flow: MBR→NF→RO and MBR→RO.

Table 2. Chemical Analysis Result of Water Samples

Physical Data		DOC	EC	pH	UV_{254}	NH^{4+}(N ppm)	NO^{2-}(N ppm)	NO^{3-}(N ppm)	PO^{4-}(P ppm)
S-MBR	6月 19日	6.252	34.40	5.71	0.120	0.963	0.973	15.688	1.284
S-MBR→ NF	6月 19日	1.633	25.20	5.67	0.027	0.580	*ND	0.112	*ND
NF→RO	6月 19日	0.456	2.52	5.48	0.008	0.139	0.458	*ND	*ND
S-MBR→ RO	6月 19日	0.306	2.53	5.84	0.001	*ND	*ND	1.506	*ND
TR	6月 19日	0.745	7.30	6.15	0.069	0.404	*ND	1.088	*ND
AS-CMBR I	7月 19日	7.301	41.90	6.47	0.177	0.659	0.146	14.728	0.915
AS-MBR I → NF	7月 19日	1.479	33.00	6.49	0.018	0.502	0.269	13.547	0.102
NF→RO	7月 19日	0.643	1.19	7.16	0.003	*ND	*ND	0.575	*ND
AS-MBR I → RO	7月 19日	0.829	1.62	7.44	0.009	*ND	*ND	0.587	*ND
TR	7月 19日	1.272	16.28	7.11	0.036	*ND	*ND	0.411	*ND

**ND - Not detected

NF membrane used was polysulfone with a cut-off of 1000 Da. RO membrane used was polyamide with a salt rejection of more than 98%. RO effective surface area was 140cm^2 while kept in constant pressure of 2MPa. RO cross-flow speed and permeate flux were 0.1m/sec and 42L/m^2/h. Both membranes were commercially manufactured by GE. Effluents were kept in low temperature during filtration using a cooling bath at around 8°C.

(3) Pre-treatment of Water Samples

Collected water samples were concentrated following Macova's method of solid phase extraction (SPE) method. Organics were extracted through SPE using 200mg HLB cartridges and eluted using 1ml of methanol and 1ml hexane-acetone solution (1:1 ratio) evaporated under nitrogen gas until final volume of 250ul. Samples were x8000 concentrated.

(4) Bioanalytical Tools

Cells used in this study are HepG2, human hepatoma cell line, and MCF7, breast cancer cell line; both cells were obtained from RIKEN cell bank (Tsukuba, Japan). HepG2 cells have many similar features as normal liver cells which play an important factor in the human body while MCF7 cells has several characteristics able to process various hormone compounds.

Four conventional bioassays and gene expression analysis were conducted to all water samples. The conventional bioassays are as follows: cytotoxicity MTT and neutral-red uptake (NRU) assay; bacterial inhibition bioluminescence and estrogenicity E-SCREEN. Briefly, cytotoxicity assays are able to measure the viable cells after exposure of the water samples through viable cells absorbance of (NRU) or metabolization of (MTT) dye. Bioluminescence inhibition uses a marine bioluminescent bacterium *Vibrio fischeri* and measures the relative decrease of light output by the bacteria. Lastly, estrogenicity assyas measures the relative proliferation induced to the MCF7 cells after exposure to the water.

For gene expression analysis, qPCR and a kit developed in the laboratory called multiple endpoints gene alteration-based assay, or MEGA kit, was used. This kit includes 24 genes divided into 6 stress responses generally group into two major mode of action cytotoxicity (5 stress responses, 18 genes) and genotoxicity (1 stress response, 5 genes).

3. RESULTS AND DISCUSSION

(1) Chemical Analysis

Chemical analysis pH, EC, UV_{245}, TOC (measured as DOC), and IC (ion chromatograph) of several ions were done for each samples. Data gathered were only used as general data and are not a focus of this research. Results are shown in table 2. Parameters such as UV_{254} and ion content were shown to reduce during the serial treatment, some becoming negligible after RO treatment.

(2) Bioanalytical Tools

All water samples were concentrated 8000 times through SPE. However, only 1% of the extracted solution can be exposed to the cells to keep the effect of methanol negligible. All bioassays results are reported at the highest concentration 80, after normalizing with MQ. Results for S-MBR and AS-MBR I series treatment are shown in figure 1 and 2 respectively plotted against cell viability or relative proliferation for estrogenicity and DOC. Horizontal lines drawn across the figures represent the standard range set, ±20% change from 100%, taken from raw drinking water Toyohira river to be compared against the effluent samples.

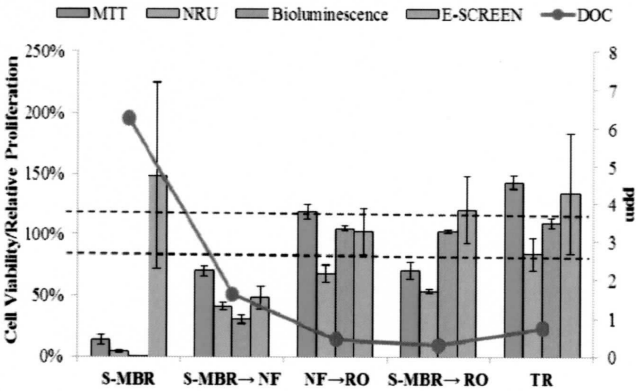

Figure 1. Bioassay Result for S-MBR Serial Treatment

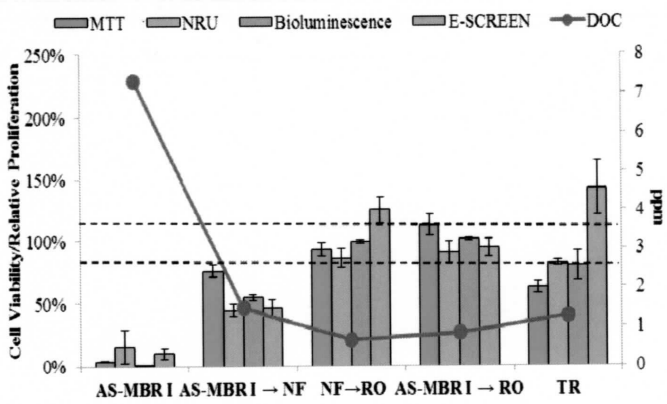

Figure 2. Bioassay Result for AS-MBR I Serial Treatment

Through pre-condition of water samples using HLB oasis, hydrophobic organics are extracted and dissolve in methanol. As shown in the figure 2, AS-MBR I showed very toxic to the cells, attaining almost zero cell viability. S-MBR, though showing similar toxicity response as AS-MBR I, showed high estrogenic acivity indicating presence of estrogenic compound present in the effluent after treatment.

Comparing both secondary treated effluent to the reference sample, it is clearly seen that these treatment is enough to be able to reuse as a direct source of raw drinking water and must be further treated. On the otherhand, NF and RO showed vast improvement of lessening the toxicity of the effluent. As seen in both figures, NF was able to lessen both effluents' toxicity by almost half while RO treatment showed responses were within the set standard range and is equal to or even better than the reference sample. In addition, there seems to be no significant difference between pre-treating effluent before RO or using RO directly.

In the case of gene expression analysis (figures not shown here), x40 concentration ratio was used, just before major cell death. Both secondary treated effluents showed many induced gene higher than 2 fold change. These indicate that even at x40 concentration ratio, eluted organics from the effluent is affecting the cell causing for genes to be induced higher than normal. These result reflect the same response as conventional bioassay.

Tertiary treatment, on the otherhand, showed different responses. NF showed as many induced genes as the MBRs, sometimes even higher. RO on the reducing the express genes to almost none. However, there is a difference response between pre-treating the effluent before RO and going straight to RO in terms of express genes. In S-MBR, pre-treating the effluent with NF before RO showed few induced genes while in the case of AS-MBR I, it is the process straight to RO that showed better response, registering few to almost no induced genes. This could indicate that different secondary treated effluent might need different tertiary treated flow. TR gene expression analysis on the otherhand showed no standard value and therefore a standard range for gene expression cannot yet be set.

3. CONCLUSION

Reclamation system was evaluated based on their biological response through the use of bioanalytical tools and compared to a raw drinking water source. Secondary treated effluent showed high toxicity as well as many genes expressed indicating that further treatment is required. NF and RO treatment showed a large improvement in lowering the toxicity with RO being the best end-treatment having equal or even better biological response than the raw drinking source. Lastly, though the MEGA kit showed more sensitively in detecting the difference between each system, the results however, are not conclusive and needs further experiments.

4. REFERENCE

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