WATER RESEARCH 52 (2014) 73–82

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Toxicity assessment of chlorinated wastewater effluents by using transcriptome-based bioassays and Fourier transform mass spectrometry (FT-MS) analysis

Toshikazu Fukushima [a], *Hiroe Hara-Yamamura* [a], *Makoto Urai* [b], *Ikuro Kasuga* [b], *Futoshi Kurisu* [b], *Taro Miyoshi* [c], *Katsuki Kimura* [a], *Yoshimasa Watanabe* [c], *Satoshi Okabe* [a,*]

[a] *Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, North-13, West-8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan*

[b] *Research Center for Water Environment Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

[c] *Center for Environmental Nano and Bio Engineering, Hokkaido University, Sapporo 060-8628, Japan*

ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form
24 December 2013
Accepted 1 January 2014
Available online 9 January 2014

Keywords:
Chlorination
Cytotoxicity
DBPs
DNA microarray
Fourier transform mass spectrometry
Wastewater effluents

ABSTRACT

Effects of chlorination on the toxicity of wastewater effluents treated by activated sludge (AS) and submerged membrane bioreactor (S-MBR_B) systems to HepG2 human hepatoblastoma cells were investigated. In addition to the cytotoxicity and genotoxicity assays, the DNA microarray-based transcriptome analysis was performed to evaluate the change in types of biological impacts on HepG2 cells of the effluents by chlorination. Effluent organic matter (EfOM) and disinfection by-products (DBPs) were also characterized by using Fourier transform mass spectrometry (FT-MS). Although no significant induction of genotoxicity was observed by chlorination for both effluents, the chlorination elevated the cytotoxicity of AS effluent but reduced that of S-MBR_B effluent. The FT-MS analyses revealed that more DBPs including nitrogenated DBPs (N-DBPs) were formed in the AS effluent than in the S-MBR_B effluent by chlorination, supporting the increased cytotoxicity of AS effluent. The lower O/C ratio of S-MBR_B EfOM suggests that a large number of organic molecules were detoxified by chlorination, which consequently decreased the cytotoxicity of S-MBR_B effluent. Integration of all the results highlights that both cytotoxicity and biological impacts of chlorinated wastewater effluents were clearly dependent on the EfOM characteristics such as DBPs and O/C ratio, namely, on types of treatment systems.

Abbreviations: AS, activated sludge; DAVID, Database for Annotation Visualized and Integrated Discovery; DBP, disinfection by-product; DCFH-DA, 2′7′-dichlorofluoroscein diacetate; DPD, *N N*-dimethyl-*p*-phenylenediamine; EfOM, effluent organic matter; FT-MS, Fourier transform mass spectrometry; NRU, neutral red uptake; MF, microfiltration; MOA, modes of toxic action; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PPCP, pharmaceuticals and personal-care product; ROS, reactive oxygen species; RT PCR, reverse transcription polymerase chain reaction; S-MBR_B, submerged membrane bioreactor; SMP, soluble microbial product; SPE, solid phase extraction; SRT, solid retention time; WWTP, wastewater treatment plant.

* *Corresponding author*. Tel.: +81 11 706 6266; fax: +81 11 707 6266.
E-mail address: sokabe@eng.hokudai.ac.jp (S. Okabe).

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http://dx.doi.org/10.1016/j.watres.2014.01.006

1. Introduction

Globally, water resources in various regions and countries are expected to face unprecedented pressures in the near future (Asano et al., 2007). Wastewater reclamation can be considered as an alternative urban water source. Disinfection is an essential component of many wastewater reuses. Although the most common method of disinfection is chlorination, chlorination of wastewater effluent has been known to result in the formation of toxic disinfection by-products (DBPs) (Buschini et al., 2004; Marabini et al., 2006; Wang et al., 2007; Bayo et al., 2009; Maffei et al., 2009). More than 600 DBPs had been identified, however, only a small number of DBPs has been assessed their toxicity and regulated (Richardson et al., 2007). Watson et al. (2012) demonstrated that toxicity of wastewater DBPs cannot be predicted by chemical monitoring of the regulated individual DBPs. It has been generally accepted that hydrophobic dissolved organic matter represents the major source of DBP precursor sites (Leenheer and Croué, 2003). Fourier transform mass spectrometry (FT-MS) has recently been applied to understand the reactivity of natural organic matter toward chlorine and to identify DBPs (Zhang et al., 2012a,b; Lavonen et al., 2013). The characteristics of effluent organic matter (EfOM) were changed by advanced biological treatments, such as membrane bioreactor (MBR) (Krasner et al., 2009; Mesfioui et al., 2012). Thus, it is assumed that treatment system influence on the DBPs production, which consequently determines the toxicity of wastewater effluent. The potential ecological impacts of DBPs in chlorinated wastewater effluents have been studied with various types of bioassays using ecotoxicology model organisms (Cao et al., 2009). The evaluation of potential human health risks becomes important when the reclaimed wastewater is used for any types of wastewater reuse practices with human body contacts. However, the potential human health risks of chlorinated wastewater effluents are not well understood.

The *in vitro* bioassays using human cell lines, such as genotoxicity and cytotoxicity assays, have been applied to assess the human health risks of DBPs in drinking water (Marabini et al., 2006; Maffei et al., 2009; Shi et al., 2009). However, since a variety of contaminants induce toxicity via different modes of toxic actions (MOAs), it is difficult to assess all MOAs by using those bioassays because each bioassay can assess only a single endpoint. On the other hand, the DNA microarray analysis enables us to examine global gene expressions in human, and has been used to evaluate biological impacts of hazardous chemicals (Kawata et al., 2007, 2009a,b) and wastewater effluents (Hara-Yamamura et al., 2013) on human cell lines.

The aim of this study was to investigate the effects of chlorination on the toxicity of wastewater effluent using HepG2 human hepatoblastoma cell lines. The wastewater effluents were collected from activated sludge (AS) and submerged MBR systems (S-MBR_B) treating the same domestic wastewater to compare the chlorine effect by different treatment systems. The cytotoxicity and genotoxicity assays and DNA microarray-based transcriptome analysis were performed to investigate changes in the cytotoxicity, genotoxicity and types of biological impacts on HepG2 cells by chlorination. Furthermore, the EfOM and DBPs were characterized by using FT-MS to explain the difference in toxicity developed by chlorination between two wastewater effluents.

2. Material and methods

2.1. Sample collection

Sampling was carried out from December 2011 to June 2012, at the full-scale municipal wastewater treatment plant (WWTP) in Sapporo, Japan. Wastewater effluent samples were collected from an activated sludge system (AS), which consists of aeration tank and secondary sedimentation basin, and a pilot-scale submerged membrane bioreactor (S-MBR_B) installed at the WWTP plant, which equipped with hollow-fiber polytetrafluoroethylene membranes with 0.3 μm nominal pore size. The solid retention times (SRTs) of AS and S-MBR_B are 7 and 50 days, respectively. Other detailed operational conditions were described previously (Hara-Yamamura et al., 2013). Grab samples were taken around 10:00 am, from the effluents of the grid chamber (considered as raw wastewater; RAW), the secondary sedimentation basin of the AS, and the S-MBR_B. The effluent samples were transferred into glass bottles and stored in cold boxes with refrigerants during transportation.

2.2. Chlorination

Sodium hypochlorite solution (30%) was added to effluent samples. Initial concentrations of chlorine were fixed at 0, 2 or 5 mg/L, respectively. Chlorination was carried out in the capped amber glass bottle at room temperature for 24 h with continuous mixing using a magnetic stirrer. The duration of chlorination simulates the disinfection process and following water storage and distribution processes. After 24 h, residual chlorine was not quenched because dechlorination agents, such as sodium thiosulfate, have been reported to reduce some chlorinated DBPs (Freuze et al., 2004). Residual chlorine concentration was measured by using *N*, *N*-dimethyl-*p*-phenylenediamine (DPD) method.

2.3. Solid phase extraction (SPE)

For RAW and other effluent samples, 0.1 and 1 L of samples were subjected to solid phase extraction (SPE), respectively. To prepare negative control samples for toxicity assays, DNA microarray analysis and FT-MS analysis, 1 L of pure water was also subjected to SPE. Samples were filtered with glass fiber membrane (GB-140, Advantec, Tokyo, Japan). After filtration, the SPE was conducted according to the protocol described previously (Macova et al., 2010, 2011). The detailed procedure of SPE is found in supplementary information.

2.4. Cytotoxicity assay

The culture condition of HepG2 is found in supplementary information. Cytotoxicity induced by each wastewater effluent sample was assessed by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and neutral red