Assessing technological feasibility for wastewater reclamation based on early life stage toxicity of Japanese medaka (*Oryzias latipes*)

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Abstract

To assess technological feasibility for reuse of secondary effluent from sewage treatment plant, the effects of raw sewage (RW) water sample and effluents samples from secondary treatment (SS), ultrafiltration treatment after SS (SS-UF), micro-filtration treatment after SS (SS-MF), activated carbon column adsorption after SS (SS-AC), as well as reverse osmosis treatment after SS-UF (SS-UF-RO) were assessed using embryo larval development of Japanese medaka (*Oryzias latipes*). Samples of RW and effluents from secondary treatment were taken from Beixiaohe sewage treatment plant (STP) in Beijing, China. Newly fertilized embryos (<5-h-old) were exposed to these water samples at different concentration gradients (including 100, 50, 25, 12.5, 6.25% effluent concentrations) in a 24-h static renewal system at 25 ± 1 °C for 15 day post-hatch. The assay considered both acute toxicity using endpoint of 96-h larval morality and chronic toxicity using endpoints including the time to hatch, hatching success, deformity, growth rate, swim-up failure, accumulative mortality and sex ratio. In the 96-h larval mortality test, 96h LC50 for RW, and effluents from SS, SS-UF and SS-MF on medaka larval were 33.7, 62.9, 60.0, 64.4% concentrations, respectively. However, no acute toxicity was observed for effluents from SS-AC and SS-UF-RO. No embryos exposed to 100% RW effluent hatched. Most of the endpoints were alternated when the fish was exposed to effluents from SS, RW, SS-UF, SS-MF that were 50–100% concentration, except those of growth and time to hatch ($P < 0.05$). The sex ratio of the larvae was alternated when exposed to 25% effluents from RW and 50% effluents from SS, SS-UF and SS-MF. Sex ratios in treatments with effluents from SS-AC and SS-UF-RO were not significantly different with the controls. However, embryos lesions and swim-up failure were still significantly different from the controls for 100% effluent from SS-AC ($P < 0.05$) and swim-up failure and larval growth could be observed for 100% effluent from SS-UF-RO ($P < 0.05$). We conclude that acute toxicity was present in the sewage water and secondary treatment could remove only part of the toxicants. As advanced treatment technologies, membrane technologies could not further remove trace toxicants and endocrine disruptors. Activated carbon adsorption and reverse osmosis were effective in removing most of the toxicants, but discharge without dilution could still manifest potential ecological risk on

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receiving aquatic biology. We conclude that activated carbon adsorption and reverse osmosis are relatively feasible technologies for reuse of secondary effluents from sewage treatment plant.

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1. Introduction

Most of the sewage treatment plants (STPs) in China receive complex mixtures of urban and industrialized discharges. Even after secondary treatment, they still contain a vast number of diverse chemical substances, many of which are unknown, to the receiving water (Trevizo and Nirmalak, 1999). When the effluents are recycled for certain purposes such as irrigation, advanced treatment is generally required. Advanced treatment technologies for reclamation of effluents from sewage treatment plants have increased rapidly in recent years in China. Currently the available technologies mainly include membrane filtration, activated carbon absorption, chemical oxidation and combinational biological treatment. Previous work has demonstrated that different technologies have different features regarding the target pollutants. For example, membrane filtration has high virus removal efficiency and was increasingly applied in disinfection processes for raw water and municipal effluent reuse (Madaeni et al., 1995; Glucina et al., 2000; Mousa et al., 2003). Activated carbon is effective in the adsorbent removal of volatile organic compounds in sewage treatment (Bansode et al., 2003). With the wrong selection, some treatment technologies may result in the discharges of toxic chemicals and pathogenic microorganisms (Chaler et al., 2001). Therefore, the effluent should be evaluated depending on the intended application.

Difference of the effluent from various stages of treatment has traditionally been regulated using methods with unspecified responses and by measuring global parameters, such as the dissolved organic content (DOC) or the chemical oxygen demand (COD). Moreover, the effects of effluents are largely unknown and the ecological hazards of effluent reuse are still very lacking. The characteristics of different secondary effluents vary with categories and the effluent guideline for different categories needs to be identified independently. For example, a previous

study has demonstrated that effluents from sewage treatment plants (STPs) had effects on fish at cellular, organ, organism and community levels (Clint et al., 2003). In addition, some secondary effluents have been found to be estrogenic to juvenile fish (McArdle et al., 2000; Julia et al., 2002), while no significant effects were observed in maturing rainbow trout exposed to effluent of a pulp and paper mill (Heuvel et al., 2002). In recent years, some studies have shown the removal of endocrine disruptors from treated effluent using yeast estrogen screen and the ethoxy resorufin-*O*-deethylase (EROD) induction assay (Anders et al., 2003; Chunxia et al., 2003). Currently there is little work being carried out to determine the differences of effluents from various treatment technologies, especially membrane treatment technologies.

In recent whole effluent toxicity (WET) tests for short-term chronic toxicity, adverse effects were assayed using embryo-larval survival and teratogenicity of fathead minnow (*Pimephales promelas*) test method (USEPA, 2002). However, this test method is based on the total frequency of mortalities and gross morphological deformities. Many field studies suggest effects on fish in polluted habitats, including reduced reproduction success (Petersen et al., 1997), reduced the levels of plasma estrogens in males (Johnson et al., 1988) and alternation in sex ratio (Lang et al., 1995). In addition, M74 syndrome and early mortality syndrome (EMS) were observed in fry and embryo. Some studies have reported that effluent from sewage treatment plants have both teratogenicity and estrogenic activity (Fuerchker et al., 2001; Montsrrat et al., 2001). Estrogenic activity was not been included in the recent WET.

Medaka (*Oryzias latipes*) serves as an excellent fish model to determine acute and chronic toxicities, including the endocrine disrupting activity of chemicals. Many reports have suggested that medaka is sensitive to toxicants and endocrine disrupting chemicals (Scholz and Gutzeit, 2000; Metcalfe et al., 2001; Islinger et al., 2002), especially in early

life stage. In the present study, to assess technological feasibility for reuse of secondary effluent from sewage treatment plant, toxicity of effluents from various processes (i.e. treatment technologies) on medaka (*O. latipes*) was assessed based on a variety of endpoints including lethal, sub-lethal, growth, deformities and sexual ratio. The effects of the effluents are discussed in relation to the hazardous risk that they put into the ecological system.

2. Materials and methods

2.1. Sampling and processing

All effluent samples were taken from Beixiaohe STP in Beijing. Effluents samples were taken from the in situ pilot advanced treatment setups, including those from secondary treatment (SS), ultrafiltration treatment after SS (SS-UF), micro-filtration treatment after SS (SS-MF), active carbon column adsorption after SS (SS-AC), as well as reverse osmosis treatment after SS-UF (SS-US-RO) expect raw sewage water (RW). All effluents samples were outflow from the various stages of treatment including listed above. Samples of technical details of the treatment technologies will be described elsewhere (Zha and Wang, in preparation).

The effluent samples were collected over a 24-h period according to composite sampling methods (USEPA, 2002) and delivered to the laboratory at 0–6 °C within a few hours. Water quality parameters including pH, dissolved oxygen (DO), total dissolved solids (TDS) and conductivity (CON) were determined using a portable multi-parameter meter (HACH, USA) immediately after the effluent samples were carried to the laboratory. The effluents were filtered through 60-μm plankton net to remove indigenous organisms and the frozen. Prior to use, the effluents were then thawed.

2.2. Test fish

Japanese medaka (d-rR) was kindly provided by the Laboratory of Freshwater Fish Stock in Bioscience Center, Nagoya University, Japan. The brood stock was kept in dechlorinated tap water (using active carbon column) at a constant temperature (25 ± 2 °C)

with a photoperiod of 16:8 h (light:dark). The brood stock was fed newly hatched brine shrimp (*Artemia nauplii*) in the morning and flake food (Trea, Germany) in the afternoon. Eggs were collected daily from the females at less than 2 h after fertilization. Filaments attaching adjacent eggs were removed by using a sucker to pull the individual eggs from the rest of the clusters. Eggs were checked using a dissecting microscope. Eggs with the migration of oil globules to the vegetal pole were deemed to be fertilized (Kirchen and West, 1976). Eggs were disinfected by placing them in a 0.9% solution of hydrogen peroxide for 10 min (Marking et al., 1994) and then placed in embryo rearing medium (ERM) (Kirchen and West, 1976) until initiation of exposure.

2.3. Experimental design

2.3.1. 96-h larval mortality experiment

Medaka larvae were exposed to 0 (blank), 20, 30, 40, 50 and 60% of RW and to 0 (blank), 60, 70, 80, 90 and 100% of effluents from SS, SS-UF, SS-UF-RO, SS-MF and SS-AC. In each vial, 10 newly hatched larvae and 100 ml test solution were added. Three replicates were conducted for each concentration gradient and control. All the vials were stored in an incubator at 25 °C. Larvae were examined daily for mortality and were fed with newly hatched brine shrimp only once. The experiment was carried out in a static renewal assay procedure (24 h renewal).

2.3.2. Short-time chronic exposure experiment

2.3.2.1. Embryological phase. Exposure was initiated at <4 h after fertilization. Medaka embryos were exposure to 6.25, 12.5, 25, 50 and 100% effluent concentrations. In each treatment, 100 embryos were randomly separated into five groups with 20 embryos in each test chamber (cylindrical glass cups with diameter of 9 cm and depth of 6 cm). Each chamber then received 100 ml test solution and was thereafter incubated at 25 °C under a 16:8 h light:dark photoperiod. Five replicates were performed for each gradient and control. The developing embryos were observed under a stereoscopic microscope until all the living embryos had hatched. The test solution was renewed every second day until all the able embryos hatched. Any dead embryos were discarded as the assay

progressed. Embryos cultured in embryos rare medium (ERM) were used as the control.

2.3.2.2. Larval phase. Newly hatched larvae and 500 ml test solution were placed into another cup (1000 ml) at 25 °C under a 16:8 h (light:dark) photoperiod. Each gradient and control had five replicates. All larvae were fed an adequate amount of newly hatched brine shrimp (*A. nauplii*) three times each day. Deformity, swim-up failure and mortality until 15 days post-hatch were observed and recorded. The test solution was renewed each day. Larvae cultured in ERM were used as the control.

2.4. Endpoints

The embryological and larval stages of medaka development have already been well documented (Iwamatsu, 1994). The morphology of the embryos was checked using a dissecting microscope and recorded with a digital camera. The numbers of live, lesion and dead embryos were recorded daily. After hatching, the number of live, deformed and dead larvae was monitored.

At 15-day post-hatching, all surviving larvae were sacrificed in 500 mg/l MS222 buffer (Sigma, USA) and then the shape of the dorsal as well as anal fins and the color of external secondary sex characteristics were observed under a stereoscopic microscope. The fish were then blotted on filter paper, and their body weight and total length were measured.

2.5. Data analysis

All statistical analyses were performed using SPSS Base 11.5 J software (SPSS, Japan). To determine differences among treatment and control and different treatment groups, we conducted the following statistical analyses. Except for the data on sex ratios all the other data were checked for homogeneity of variances across treatments by Levene's test. When the assumptions were met, the data were subjected to one-way analysis of variance followed using the Dunnett's multiple comparison tests. When no homogeneity was observed in the data, the nonparametric Kruskal–Wallis test was used followed by the Mann–Whitney *U*-test with Bonferroni's adjustment. The data on sex ratios were assessed using the chi-

squared analysis. Differences were considered to be significant at $P \leq 0.05$. LC50 values were calculated by probit transformation using the computer software SOFTTOXTM distributed by WINDOWCHEMTM Fairfield, CA, USA.

3. Results and discussions

3.1. Water quality parameters

The pH of the effluents from various stages of treatment ranged between 7 and 8 except for the effluent from SS-UF-RO which had pH 6.48 (Table 1). The lower pH in SS-UF-RO could be related to lower TDS whereas the higher pH in effluent of SS-AC could be due to removal of metals ions (Dinesh and Kunwar, 2002). In all treatments except that for RW, dissolved oxygen (DO) was increased to levels of 5.8–6.4 mg/l to satisfy the requirement for fish test (USEPA, 2002). There were no significant differences in water quality parameters among treatments.

3.2. Acute toxicity

For newly hatched medaka larvae exposed, 96-h LC50s for effluents from RW, SS, SS-UF and SS-MF were 33.7, 62.9, 60 and 64.4%, respectively (Table 2). On contact, the effluents from SS-UF-RO and SS-AC had not manifested acute toxicity on newly hatched medaka larvae at 96-h exposure. Based on the data of 48-h LC50 (Table 2), SS-UF and SS-MF even concentrated the toxicants in comparison with that for SS.

Table 1
pH, TDS, CON and DO of water samples from various stages of treatment and controls^a

Treatment	pH	TDS (mg/l)	CON (μS/l)	DO (mg/l)
RW	7.65	488	994	0.7
SS	7.87	427	873	5.8
SS-UF	7.48	408	838	6.4
SS-MF	7.47	410	834	6.1
SS-MF-RO	6.48	26	54.1	6.2
SS-AC	7.91	401	821	6.2
control	6.97	545	1107	5.8

^a DO, TDS and CON represent dissolve oxygen, total dissolve solid and conductivity, respectively. Data are the mean of five replicates.

Table 2
96-h LC50 of the effluents from RS and various treatment progresses on newly hatch medaka larvae

Source of effluent samples	LC50 (% effluents concentrations)		
	24 (h)	48 (h)	96 (h)
RW	55.4	39.4	33.7
SS	nd ^a	nd	62.9
SS-UF	nd	81.5	60
SS-MF	nd	83.8	64.4
SS-AC	nd	nd	nd
SS-UF-RO	nd	nd	nd

RW, SS, SS-UF, SS-MF, SS-AC, SS-UF-RO showed outflow of raw sewage water, secondary treatment, ultrafiltration treatment after SS, micro-filtration treatment after SS, active carbon column adsorption after SS, as well as reverse osmosis treatment after SS-UF (SS-UF-RO), separately.

^a No acute toxicity detected.

The results showed that secondary treatment could remove most of the toxicants in raw water and that SS-UF-RO and SS-AC perform better compare to all the other technologies tested. Based on chemical measurements, the AC process has been shown to more efficiently remove organic compounds and heavy metals from waters (Bansode et al., 2003; Dinesh and Kunwar, 2002). Similarly, recent research has shown that UF-RO also effectively removed trace toxicants (Brehant et al., 2002). These findings are better confirmed based on the early life stage toxicity test performed in the present study. Mavrov et al. (1998) reported that both UF and MF could remove microorganisms and turbidity from the water. However, these technologies should not be recommended for case where the secondary effluent is intended for reuse because they did not effectively removed acute toxicity and TDS when used in combination with secondary treatment (Table 1).

3.3. Embryo development phase effect

3.3.1. Embryo lesions

Abnormalities of embryos in all effluents and controls were observed after day 4 (Fig. 1). Most embryos were normal in the controls, the effluents from SS-UF-RO and in SS-AC effluents. In previous works, medaka embryos exposed to 2,3,7,8-terachlorodibenzo-*p*-dioxin (TCDD, Wisk and Cooper, 1990), bisphenol A (BPA, Stephanie et al., 2001) and a polychlorinated naphthalene (PCN) mixture (Halowax

1014, Villalobos et al., 2000a) exhibited lesions after day 4 or 5, or the time of formation of the liver (Villalobos et al., 2000b). The lesions would, to some extent, affect hatching success of embryos and the growth and survival of the larvae (Kjorsvik et al., 1990).

Almost all embryos in 100% RW effluent were dead at day 3 and 100% of the few embryos that still existed in this effluents had lesions by 7 days. The lesion rates of embryos exposed to RW were significantly different ($P < 0.05$) from that in the effluents of other types of treatment and from the controls (Fig. 2). The lesion rates of embryos exposed to high concentrations SS, SS-UF, SS-MF effluents were slightly different ($P < 0.05$) with controls (Fig. 2). However, the lesion rates of embryos in SS-UF-RO and SS-AC effluents were not significantly different from the controls. These results further showed that SS-UF-RO and SS-AC technologies had a good performance on removal of toxicants.

3.3.2. Time to hatch and hatching rates

Time for embryos to hatch after exposing them to effluents from all the various stage of treatment was not significantly different from the controls (data not shown). However, no embryos hatched in the 100% RW effluent. At 50% RW effluent, hatching of embryos was delayed, but the delay was not statistically significant. There were not significant differences in the hatching time between the controls and the low RW effluent concentrations (25, 12.5 and 6.75%) (data not shown).

Hatching rates are an index that directly determines the quality of the larvae and affect the survival of a species. Hatching rates were sensitive to changes of the environment (Dettlaff et al., 1993). Hatching success of embryos exposed to 50% RW effluent was significantly less than in the controls and the other treatment ($P < 0.05$). The hatching rate of embryos exposed to 25 and 12.5% RW effluent were also statistically less than in the controls ($P < 0.05$) (Fig. 3). In previous studies, a reduction of hatching success, induced by an exposure to single chemicals, has been reported in a number of types of fish, such as in medaka when exposed to 4-nolypheanol (NP, Hirofumi et al., 2001) and fathead minnow when exposed to ethinylestradiol (EE2, Reinhard et al., 2001). Reduction of hatching success was also

Fig. 1. Different characters of lesions embryos and newly hatching deformity larvae: (A) normal embryos; (B) lesion embryo, arrow shows pericardial edema; (C) lesion embryo, arrow shows hemorrhage; (D) normal larvae; (E) deformity larvae, arrow shows swollen abdomen; (F) deformity larvae, arrow shows distortion of eyes.

Fig. 2. Embryo lesions of medaka embryos exposed to different diluted effluents from raw sewage, the controls and various stages of treatment. Error bars are standard deviations. Columns with the same letter above them are not significantly different ($P < 0.05$).

observed in polluted environment, such as M74 pollution (environmentally related disease found in 1974) in Baltic salmon and the equivalent EMS syndrome in salmonids in North American Great lakes (AFS, 1996).

Hatching rates of embryos exposed to 100% SS effluents were very low and significantly less than in the controls ($P < 0.05$) (Fig. 3). Although hatching rates of embryos in 100% SS-MF and SS-UF effluents were different from those of the controls

($P < 0.05$) (Fig. 3), they were not different from those of embryos exposed to 100% SS effluents. Hatching success of embryos exposed to 100% SS-AC effluent concentrations were significantly less than in the controls ($P < 0.05$) (Fig. 3). However, hatching success of embryos in SS-UF-RO effluents did not significantly differ from the controls. The results showed that effluents from SS-UF-RO posed a less hazardous risk on receiving aquatic biology.

Fig. 3. Hatching success of medaka embryos exposed to different diluted effluents from raw sewage, the controls and various stages of treatment. Error bars are standard deviations. Columns with the same letter above them are not significantly different ($P < 0.05$).

3.4. Larval phase effect

3.4.1. Deformities of newly hatching larvae

Deformities of newly hatching larvae in water from various treatment technologies were observed (Fig. 1). The tail of some larvae curled and distorted whereas the abdomen swelled. In a previous study, deformities were observed in rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) when exposed to 7-isopropyl-1-methylphenanthrene at concentrations of 32–320 µg/L (Sonya et al., 1998). In our recent work, there was blue sac when medaka embryos were exposed to a banknote printing plant (BPP) effluent (data to be published).

Deformity rates of larvae at 50% RW effluents were significantly higher ($P < 0.05$) than in the controls and other treatments (Fig. 4). In 25% RW and 100, 50, 25% SS effluent, they were significantly different ($P < 0.05$) from the controls (Fig. 4). Though deformity rates of larvae in 100% SS-UF and SS-MF effluent were lower than that in 100% SS effluent, they were significantly higher ($P < 0.05$) than in the controls (Fig. 4). The other treatments had no significantly different deformity rates from the controls although deformity rates of larvae exposed to 100% SS-UF-RO effluent were even lower than those of the controls. Based on deformities rates, the SS-UF-RO and SS-AC technologies performed quite well in removing toxicants.

3.4.2. Swim-up failure

Swim-up failure is the consequence of chronic intoxication and an index that directly determined the quality of the species. No larvae at 100% SS, SS-UF, SS-MF and 50% RW effluents survived. They were significantly different with controls and other treatments ($P < 0.05$) (Fig. 5). With 50% SS, SS-UF, SS-MF effluents, a significant number ($P < 0.05$) of the larvae failed to swim up (Fig. 5). There were also significant differences in the number of the larvae that failed to swim-up in the 50% SS, SS-UF, SS-MF effluents. The swim-up failures of larvae in high concentration SS-UF-RO effluents were significantly higher ($P < 0.05$) than in the controls (Fig. 5) possibly due to the removal of large amounts of TDS. Similarly, Charlton and Bergot (1991) reported that absence of some necessary salts would cause swim-up failure of the newly hatching larvae. However, the larvae in SS-AC effluents had no significantly different swim-up failure with the controls. The results suggested that SS-AC effluents supplied a good environment on swim-up of the fish.

3.4.3. Mortality rates after swim-up

Mortality rates after swim-up in effluents from RW and various treatment techniques were not significantly different with the controls. All mortality rates were less than 10% (data not showed) indicating that mortality rate after swim-up was not a sensitive

Fig. 4. Deformities of medaka larvae exposed to different diluted effluents from raw sewage, the controls and various stages of treatment. Error bars are standard deviations. Columns with the same letter above them are not significantly different ($P < 0.05$).

Fig. 5. Swim-up failure of medaka larvae exposed to different diluted effluents from raw sewage, the controls and various stages of treatment. Error bars are standard deviations. Columns with the same letter above them are not significantly different ($P < 0.05$).

indicator for assessing early life stage exposure to assessment the effluent from various treatment technologies.

3.4.4. Larvae growth

Larvae growth was always an index that determined the quality of the water. In this experiment, we measured the total body length (TBL) and wet body weight (WBW) of the larvae at 15 days after hatching.

The results showed that TBL and WBW of medaka larvae grown in the effluents from SS, SS-UF, SS-MF, SS-AC had not significant difference from the controls. Similarly, TBL and WBW of the larvae that were exposed to 25% RW did not significantly differ with the controls (Table 3). TBL and WBW of larvae grown in 100, 50, 25% SS-UF-RO treatment technologies was significantly less than the controls and the other treatments except 25 RW ($P < 0.05$)

Table 3

Total body length (TBL), wet body weight (WBW) and sex ratio(♀:♂) of larvae at 15-day post-hatching in different diluted effluents from raw sewage, controls and various stages of treatment progresses

Treatment	Concentrations (%)	Total body length (mm)		Wet body weight (mg)		Sex ratio (♀:♂)
		Mean	S.E.	Mean	S.E.	
RW	25	15.9 a	2.4	38.2 a	15.6	20:10 ^a
SS	50	17.3 abc	1.6	58.4 e	19.5	18:9*
	25	17.3 abc	1.8	51.7 de	18.4	25:19
SS-UF	50	17.2 abc	1.7	49.1 bcd	20.5	19:10*
	25	17.6 bc	2.9	49.6 bcd	24.7	25:25
SS-UF-RO	100	15.3 a	1.5	39.3 a	11.8	20:20
	50	15.1 a	1.5	37.2 a	11.3	24:26
	25	15.4 ab	1.8	39.9 a	11.6	31:35
SS-MF	50	17.7 bc	1.1	49.8 bc	18.1	11:6*
	25	18.0 bc	0.9	50.6 cd	16.6	30:24
SS-AC	100	17.6 bc	1.1	49.9 bcd	8.3	30:27
	50	17.8 bc	1.9	50.5 bcd	12.4	28:26
	25	17.3 bc	1.4	52.5 cd	12.8	36:32
Controls	0	18.3 c	1.8	54.1 cde	14.2	40:39

Data expressed as mean $\pm$ S.E. of five replicates for each treatment. Within columns, treatment means followed by different letters are significantly different ($P \leq 0.05$).

^a Significantly different from the control: * $P < 0.05$; the statistic methods was chi-squared analysis.

(Table 3). Surprisingly, the 12.5% SS-UF-RO treatment also significantly reduced TBL and WBW in larvae compare to the controls ($P < 0.05$) possibly because this treatment technology removes many TDS. Removal of TDS may also get rid of some useful components (Table 1), causing a difficulty in assimilating nourishment, slowing down the growth of the fish. These results are in agreement with those of previous studies. For example, Nimrod and Benson (1998) reporting that exposed newly hatched medaka fry to 0.01–1.66 $\mu\text{g/L}$ of E2 for 28 days followed by a post-dosing recover period of 28 days reduced the total body length and weight. In other studies, the decreasing of total body length and weight of zebrafish were observed after exposure to EE2 (Van et al., 2003), cadmium (Bresch, 1982) and TCDD (Elonen et al., 1998).

3.5. Sex ratios

The sex ratio was a good indicator of endocrine disruption in the fish. For example, an increasing ratio of males to females was found in many types of fish including medaka after exposure to estrogen (E2) (Scholz and Gutzeit, 2000), EE2 (Metcalfe et al., 2001) and 4-NP (Hirofumi et al., 2001). In the present study, the sex ratio of the larvae was alternated when exposed to 25% effluents from RW and 50% effluents from SS, SS-UF and SS-MF (Table 3). Females were significantly ($P < 0.05$) more than males at higher concentrations of RW, SS, SS-UF and SS-MF displaying a feminization phenomenon. This was similar with the result of BPP effluents (Zha and Wang, 2005). However, the sex ratios in treatments with effluents from SS-AC and SS-UF-RO did not significantly differ from the controls. Both SS-AC and SS-UF-RO did not affect the sex ratio indicating that both techniques were quite well in removing of endocrine disruptors.

4. Conclusions

Present study suggested that both acute, chronic toxicities and endocrine disrupting could be observed for raw sewage and effluent from secondary treatment. Secondary treatment could remove part of toxicants from raw water. Among advanced treatment technol-

ogies, micro-filtration and ultrafiltration do not manifest efficiencies regarding to the removal of trace toxicants and endocrine disruptors. Active carbon adsorption and the combination of ultrafiltration and reverse osmosis are the most effective ones in removal of most toxicants. In addition, the proposed procedure based on the early life stage toxicity of Japanese medaka is valuable to screen acute and chronic toxicities and endocrine disrupting in effluents from different advanced treatment technologies for wastewater reclamation.

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