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(28) FATE OF 17ß-ESTRADIOL DURING BIOLOGICAL SAND FILTRATION: EFFECT OF FILTRATION RATE AND TEMPERATURES

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Fate of 17β-estradiol (E2) during biological sand filtration was investigated by a series of continuous flow experiments for treating surface river water spiked with E2. The likely impacts of filtration rates and temperatures were examined for combinations of these two variables at the filtration rates of 2.4, 4.8, 12 and 24 m/d and the temperatures of 5, 16 and 20 °C. Under all experiment conditions, estrone (E1) was detected as a biotransformation product of E2, indicating that there were bacteria species in the biofilms of the sand filters that can degrade E2. The half-life of E2 ($t_{1/2}$), which was estimated from the parameters of the first-order reaction equation combined into a complete mixing model, showed that the disappearance of E2 took place faster when the filtration rate was smaller and the temperature was higher. Compared to the biofilms attached onto the sand media in the top layers, the biofilms in the lower layers demonstrated slow degradation rate for E2, with the degradation rate revealing a profile in similarity with the density profile of either heterotrophic bacteria or general bacteria.

Key Words: *natural estrogens, 17β-estradiol, estrone, slow sand filtration, biofilms*

1. INTRODUCTION

Natural estrogens are a great concern and their occurrence and behavior in natural water resources has been widely investigated[1-6]. Tabata *et al.*[2] conducted an extensive survey of natural estrogens in 109 Japanese rivers and found 17β-estradiol (E2) in 222 samples out of the total 256 samples with a mean concentration of 1.8 ng/L. Koplin *et al.*[3] has completed an extensive reconnaissance of surface water across 30 states in the United States. Reproductive hormones were found in approximately 40% of the 139 streams studied. Recently, Kumar *et al.*[6] studied the free and conjugated estrogen loads discharged by eight major sewage treatment plants into the Yodo River basin, Japan in the winter and autumn seasons from 2005 to 2008 and estrone (E1) was detected in almost all samples discharged from seven STPs in the concentrations ranging from <0.3 to 180 ng/L and followed by E2 (means of 0.5-3 ng/L).

Natural estrogens are particularly concern by water quality regulators and drinking water production units due to their strong estrogenic impacts on humans and wild lives[7]. They are mainly consisted of E1, E2 and estriol (E3) excreted by humans and animals in conjugated and isolated forms[8]. Since the estrogenic activity of E2 is much stronger than E1 and E3 as revealed by the results of yeast assay[9] and feminization of male fishes[10] more researchers have focus their studies on this compound.

Slow sand (or biological) filtration is a process used to treat water containing low content of turbidity and organic matter by passing raw water through a bed of sand. During its passage the particulate impurities are brought into contact with the surface of sand grains and held in position there. On the surface of the sand, there is a thin slimy matting of material, largely organic in origin, known as the *schmutzdecke,* biofilms, through which water must pass before reaching the filter medium itself. The thickness and activity of the biofilms determine the overall performance of slow

sand filters. The biofilms that consist of numerous forms of organisms including algae, plankton, diatoms, protozoa, rotifers and bacteria, is intensely active; the various microorganisms entrapping, digesting and breaking down organic matter contained in the water passing through. For smaller impurities (like E2), the diffusion (Brownian movement) is most obvious process by which they are brought into contact with sand grains[11].

The slow sand filter is operated in wastewater and drinking water treatment for removal of carbon (C), pathogenic bacteria[12], protozoan parasite and suspended solids[13]. The high efficiency of water treatment achieved by slow sand filters is partly explained by the slow filtration rate (0.1–0.3 m/h) and fine effective size of the sand (0.1–0.3 mm), but is also attributed to biological processes in the layer of the slime material that accumulates above the sand surface (*schmutzdecke*) and within the upper layers of the sand bed[13].

So far, lots of research has been conducted on the removal of turbidity and biodegradable organic matter contained in less polluted surface water by slow sand filters; however, little is known about the fate of E2 in slow sand filters.

The first objective of this study was to investigate if the biofilms in the practical slow sand filters treating surface river water can degrade E2. Then the effects of filtration rates and temperatures were studied. For these objectives, continuous flow sand filtration experiments were conducted using columns packed with sand media collected from a practical slow sand filter used for treating river water with lower turbidity and lower content of natural organic matter.

2. MATERIALS AND METHODS

(1) Sand media and overlying water

Core sand samples and overlying water were collected from a practical slow sand filter basin, operated for treating river water with a turbidity of about 2 NTU and dissolved organic carbon (DOC) of 1.3 mg/L as shown in **Table 1**. Sand samples were collected using a core sampler that allows collecting sand layers for a vertical depth up to 20 cm from the bed surface of the slow sand filter without disturbing the stratification structure of the filter bed.

During the sand sampling, the river water entered the treatment plant was also sampled. The quality of the water is described in **Table 1**. The river water was filtered through 0.45 μm membrane filters (Toyo Roshi Kaisha Ltd., Japan) to remove suspended solid and microorganisms content[14]. The obtained filtrate was then stored at 5 °C before use.

Table 1. Characteristics of river water.

Parameter	Unit	Value
pH		6.94
DOC	mg/L	1.32
DO	mg/L	3.78
UV_{260}	m^{-1}	2.78
EC	mS/m	5.64
ORP	mV	257

DOC: dissolved organic carbon, DO: dissolved oxygen, UV_{260}: UV abs. at 260μm, EC: electrical conductivity, ORP: oxidation reduction potential.

(2) Stock solution of 17β-estradiol (E2)

A stock solution of E2 (about 1000 μg/L) was prepared by dissolving a weighted amount of E2 (Wako Pure Chemical Co., Osaka, Japan) in Milli-Q water. Organic solvent was not used in order to eliminate its effect on degradation of E2 as mentioned in previous studies[14,15]. After stirring for 24 hours after addition of the weighted E2 into the Milli-Q, the solution was filtered using a pre-washed 0.2 μm PTFE membrane filter to separate the fraction of E2 not yet dissolved, and the filtrate obtained was then used as the stock solution and refrigerated stored in the dark at 5 °C prior to use.

(3) Filtration experiments

The collected sand core was divided into ten layers, each with a thickness of 2 cm. For each divided sand layer, the biofilms attached on the sand media was subjected to measurements for SS, VSS, general bacteria and heterotrophic bacteria.

The experiment set-up for the continuous flow sand filtration experiments is displayed in **Fig.1**. Four columns each with the inner diameter of 2.5 cm and the length of 150 cm were used. Of the four columns, three columns were packed to a bed depth of 6 cm with three divided sand layers corresponding to the sand depths of 0-6, 6-12 and 12-18 cm from the bed surface of the practical slow sand filter, respectively. For the remaining one column, it was packed to a bed depth of 20 cm, corresponding to the sand depth of 0-20 cm of the practical slow sand filter. To all columns, water was introduced to levels that ensured the water depth above the sand surface was 70 cm. This water depth was maintained throughout all experimental runs by an overflow opening designed on each column.

Three series of continuous flow experimental runs were performed by varying the filtration rate at 2.4, 4.8, 12 and 24 m/d under three different water temperatures of 20, 16 and 5 °C, as displayed in **Table 2**. For each run, water containing 30 μg/L

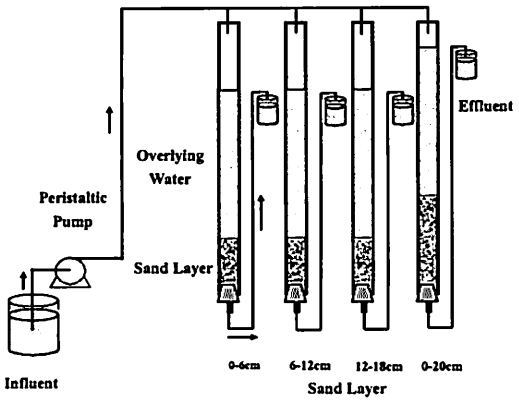

Fig.1 A schematic diagram of continuous flow sand filtration experiment.

of E2 was flowed through the column in a down flow mode and the effluent was collected from the column outlet for quality analysis.

Samples were taken 8, 16 and 24 hours after filtration started for each experiment run. Based on the filtration rate and depth of sand packed in each column, the time needed to completely replace water in the column with the influent water spiked with E2 differed. However, the time length of 8 hours for the first sampling was long enough to ensure that all data obtained were corresponded to the influent water spiked with E2.

(4) Analysis

In this study, E2 and E1 (by-product of E2) were analyzed using the Agilent 1100 series Liquid Chromatography/Mass Spectrometry (LC/MS) system (HP1100MSD; California, USA), the setting conditions of which are shown in **Table 3**. For every commencing of the analysis, calibration was performed using 10 and 50 μg/L of both E2 and E1 standard solutions with methanol content of 20% in v/v. To minimize measurement errors, two internal standards, namely 17β-estradiol-C4 and estrone-C4 (Hayashi Pure Chemical Ind., Co., Ltd) were added to all samples, and the identification and quantification were made in negative SIM mode by following the well-used internal standard methodology[14-17]. The target ions for E2 and E1 were 271 and 269, respectively. By adopting a large injection volume (i.e., 25 μL), the detection limits reached a level about 0.01 μg/L for both targeted species.

In addition to E2 and E1, DOC, UV_{260}, ORP, EC and pH were also analyzed using TOC analyzer (TOC-VWs, Shimadzu Co, Japan), spectrophotometer (UV 1600 series, Shimadzu Co., Japan), ORP meter (RM-20P DKK-TOA, Japan), EC meter (CM-20P, DKK-TOA, Japan) and pH meter (HM-20P, DKK-TOA, Japan), respectively. The density of general bacteria and heterotrophic bacteria on sand media of each divided sand layer was determined by quantifying the bacteria number concentration in the detached suspension of the biofilms of the corresponding sand layer following the conventional plate culture method[18-20]. For the culture method, 1 ml of each suspension, after numerous dilutions, was placed to a sterile plate and then pour with 10 ml of melted standard culture agar (a weighted amount of peptone, yeast extract, glucose, agar powder and Milli-Q water). The agar plates were then placed in an incubator at 36 °C for 24 hours (for general bacteria) and 20 °C for 7 days (for heterotrophic bacteria) before counting the colonies produced.

Table 2 Continuous flow sand filtration experiment conditions.

Series no.	Run no.	Temperature (°C)	Filtration rate (m/d)
1	1	20	2.4
	2		4.8
	3		12
	4		24
2	5	16	2.4
	6		4.8
	7		12
	8		24
3	9	5	2.4
	10		4.8
	11		12
	12		24

Table 3 Major LC/MS parameters used for detecting E2 and E1.

Instrument		HP1100MSD
LC	- Column	Zorbax Eclipse XBD-C8 (4.6Φ x 150 mm)
	-Effluent	Acetronitrile/ Methanol = 60:40
	- Flow-rate	0.4 mL/min
	- Column temperature	40 °C
	- Injection volume	25 μL
MS	- Ion source	ESI
	- Mode	Negative (SIM)
	- Capillary voltage	3000 V
	- Fragmentor	70 V
	- Nebulizer pressure	35 psi
	- Drying gas flow (N_2)	12 L/min

3. RESULTS AND DISCUSSION

(1) Behavior of E2 under different filtration rates

The concentration profiles of E2 in the continuous flow experiments, for series 1 (Run 1-4) are shown in **Fig.2.** The effluent E2 increased with time over the initial running period, with the period length being found to be equivalent to the designated filtration rate. After the initial period, apparent changes in the effluent concentration of E2 were not appeared, indicating that the biological reaction had reached the steady state. For the column with the sand layer of 0-6 cm (**Fig.2a**), the average concentrations of E2 in effluent after reaching the steady state were 3.88, 8.66, 19.92 and 25.80 μg/L for the filtration rate of 2.4, 4.8, 12 and 24 m/d, which represented residual E2 by about 12.96, 28.89, 66.45 and 86.09%, respectively. Similar results were also obtained for sand layers of 6-12, 12-18 and 0-20 cm, as shown in **Fig.2 (b, c and d)**, and for running series 2 (run 5-8) and 3 (run 9-12) (data not shown). The results indicated that as filtration rate increased, the residual E2 concentration increased as well, thus indicating a significant impact of filtration rates on degradation of E2. The decreasing profiles of the spiked E2 corresponded to those obtained in previous studies using sediment from a freshwater reservoir by batch experiments[14,21] and those using activated sludge from STPs[16,18].

For all runs, formation of E1, a well-known biotransformation product of E2, was also confirmed (as shown in **Fig.3** for an example), which indicated that there were microbial species in the sand media that could degrade E2. Similar results were also obtained for all sand layers (data not shown). Decreasing the concentration of E2 for each layer associated with the microbial density along vertical direction of sand layer as shown in **Fig.4**. Similar patterns in biomass distribution with depth in slow sand filters have also been reported by Campos[12], Duncan[20] and Yordanov *et al*[22]. Li *et al.*[23] examined the effect of bacterial population density on E2's degradation behavior using undiluted and diluted sludge liquors with different concentration (as MLVSS) under different temperatures. Their results indicated that the removal efficiency of E2 depended markedly upon the levels of MLVSS that directly reflected the role of bacterial populations.

Since E1 was probably the most important natural endocrine disrupting compound presents in most natural environment[18] because of: (1) the quantities of E1 discharged from STPs into receiving water bodies are 10 times larger than E2, (2) E1 possesses

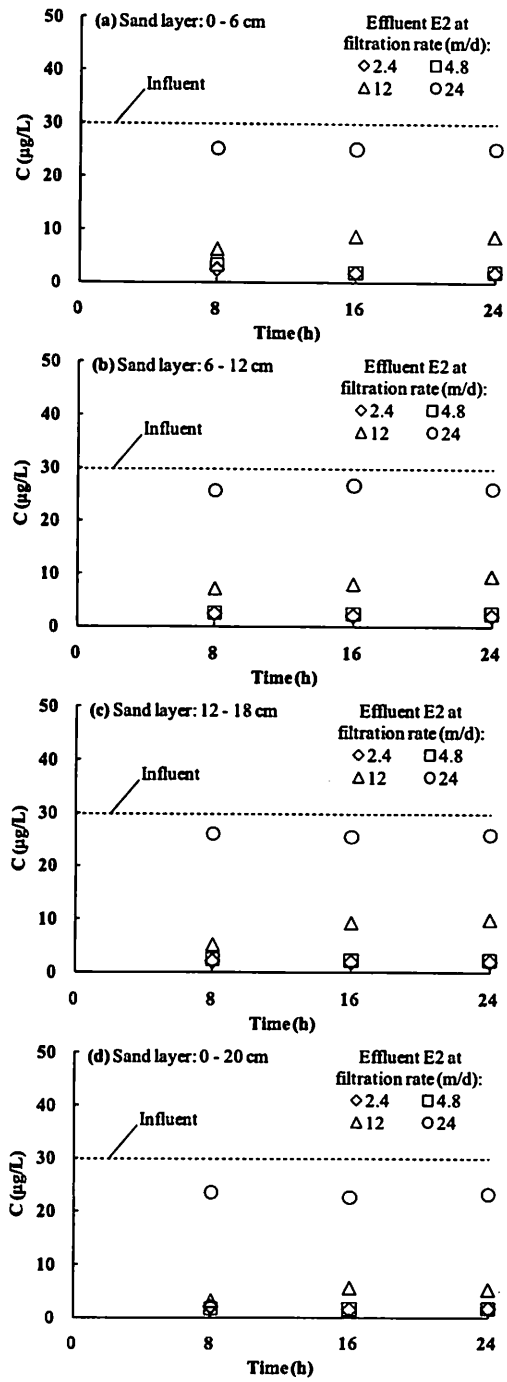

Fig.2 Concentration profiles of E2 with time at different filtration rates for Run 1-4 (at tempt. 20 °C).

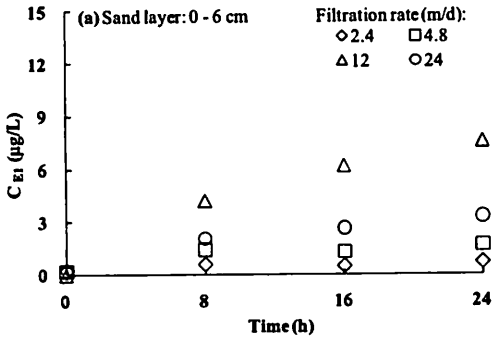

Fig.3 Concentration profiles of E1 (by-product of E2) with time at different filtration rates for Run 1 (at temp. 20 °C).

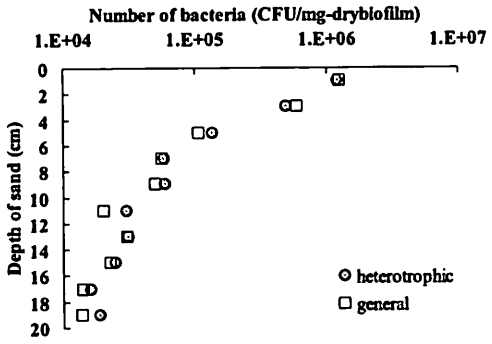

Fig.4 Distribution of microbial density along the vertical direction of the sand layer.

an estrogenic potency weaker than E2 but much stronger than nonyphenol and its precussors, and (3) some conjugated species of E1 may get disassociated after reaching receiving water bodies[16], the emerged concentration of E1 in the effluent should be concerned. However, Li *et al*[16] observed the apparent conversion ratios of E1 from E2. By increasing the glucose concentration, the conversion ratio decreased, thus suggesting that the enhanced presence of easily-biodegradable substrates in the influent wastewater be probably capable of hindering the conversion of E1 from E2. Since slow sand filtration is used for treating river water containing low content of turbidity and organic matters, the 'hindering effect' could be ignored.

(2) Behavior of E2 under different temperatures

The concentration profiles of E2 for filtration rate of 2.4 m/d under temperatures of 5, 16 and 20 °C (run 1, 5 and 9, respectively) are displayed in **Fig.5**. At all temperature levels, the residual E2 concentration decreased with increasing of temperatures. For the sand layer 0-6 cm (**Fig.5a**), the average concentrations of E2 in the effluent were 3.88, 4.94 and 8.41 μg/L, representing the residual of E2 by 12.96, 16.48 and 28.05% for temperature of 20, 16 and 5 °C, respectively. Similar results were also obtained for runs conducted in parallel using different sand layer as shown in **Fig.5b-d** for sand layer 6-12, 12-18 and 0-20 cm, respectively, and for all run series (data not shown).

For all sand layers and four variable filtration rates, the higher the temperature was the lower the residual E2 concentration. These results supported the results obtained by Li *et al.*[23] and Layton *et al.*[24]. Using a sludge sample from a sewage treatment plant, Li *et al.*[23] studied the degradation behavior of E2 under aerobic batch process at three different temperatures (5, 20 and 35 °C) and found the higher the temperature was the higher the rate constant. On the other hand, Layton *et al.*[24] compared E2's batch degradation behavior under two different temperature ranges (22-25 and 5-10 °C). A faster rate of mineralization was found for the run conducted at the former temperature range.

(3) Half-life of E2

To further examine the effect of filtration rates and temperatures on the filtration performance, the half-life of E2 in the continuous flow experiments under all experiment conditions were estimated from the mass balance equation as described below by fitting experiments data with the calculations.

$$V\frac{dC_{(eff)}}{dt} = QC_{(inf)} - QC_{(eff)} + V\left[-kC_{(eff)}\right] \quad (1)$$

Where V is volume of sand media (L), $C_{(inf)}$ and $C_{(eff)}$ is the concentration (μg/L) in the influent and effluent, respectively, t is time (h) and k is the first order rate constant (h^{-1}). k was approximated when the flux dC/dt was zero, i.e., using the average effluent concentration after reaching the steady state. The half-life for E2 was found by using the equation:

$$t_{1/2} = \ln 2/k \quad (2)$$

As shown in **Eq.1**, the decreasing of E2 in the sand filtration was assumed to follow the first-order rate constants. To support this assumption, preliminary batch degradation experiments for E2 spiked to the same water source were conducted using the same biofilms detached from 5 sand layers of the sand core collected.

The batch degradation experiments were performed using glass reactors (500 mL flask glass) placed on a shaker at 120 rpm and 20 °C. The detached biofilm solutions were related to the sand

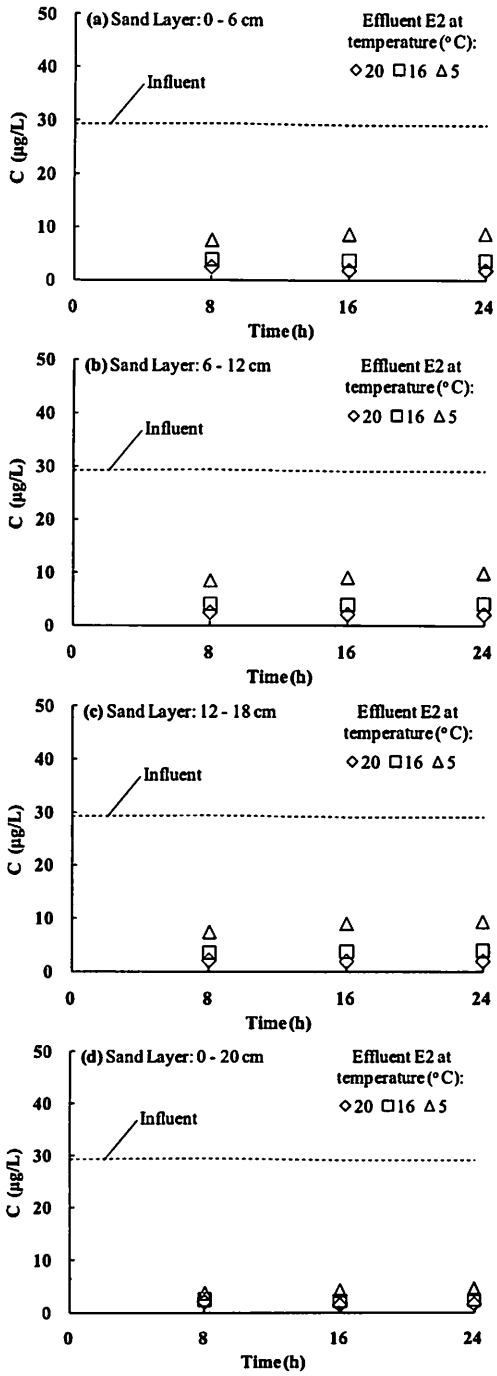

Fig.5 Concentration profiles of E2 with time at different temperatures for Run 1, 5 and 9 (at filtration rate: 2.4 m/d).

layers of 0-2, 2-4, 6-8, 14-16 and 18-20 cm, respectively. The concentration of the biofilm related SS in each reactor was adjusted to 500 mg/L as MLSS, and the initial concentration of E2 was 30 μg/L. Calculation based on first-order rate constants fitted data with high correlation coefficient, as shown in **Fig. 6**, thus suggesting that the assumption of the first-order reaction was reasonable.

a) Effect of filtration rates

The estimated $t_{1/2}$ value under different filtration rates for running series 1 (Run 1-4) are displayed in **Fig.7**. A trend of increasing of $t_{1/2}$ with increasing of filtration rate was revealed. The $t_{1/2}$ values fell in the ranges of 0.78-3.26, 1.56-5.55, 1.29-14.59 and 0.47-1.72 h for sand layers of 0-6, 6-12, 12-18 and 0-20 cm with filtration rates of 2.4, 4.8, 12 and 24 m/d, respectively. For all filtration rates, $t_{1/2}$ values followed the order of sand layers of 0-20<0-6<6-12< 12-18 cm. Similar results were also obtained for running series 2 and 3 (data not shown).

These results supported previous results obtained by Campos et al.[12] that a lower filtration rate allows an increasing of contact time between microorganism and substrate and allows biofilms to become better developed.

b) Effect of temperatures

Fig.8 shows the estimated $t_{1/2}$ values under different temperatures for Run 1, 5 and 9 (filtration rate: 2.4 m/d). The $t_{1/2}$ fell in the range of 0.78-2.05, 1.56-2.10, 1.29-2.30, and 0.47-0.86 h for sand layers of 0-6, 6-12, 12-18, and 0-20 cm with different temperatures of 20, 16 and 5 °C, respectively. As shown, for nearly all sand layers, the higher the temperature was the lower the $t_{1/2}$. Based on the estimated k values and corresponding MLVSS levels, Li *et al.*[22] confirmed an obvious increasing trend of k

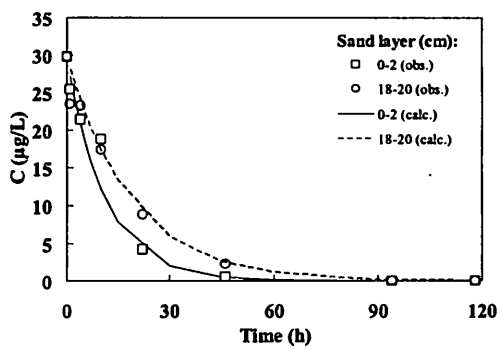

Fig.6 Description of batch degradation of E2 by the first-order reaction for biofilms of different sand layers.

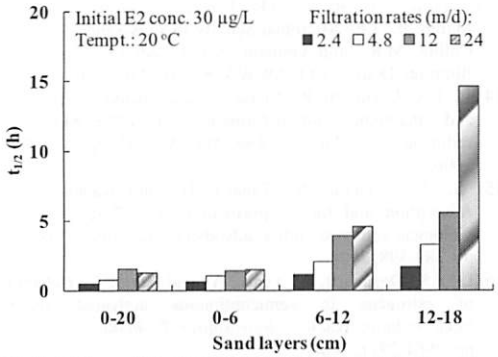

Fig.7 Values of half-life of E2 at different filtration rates for Runs 1-4 (at tempt. 20 °C).

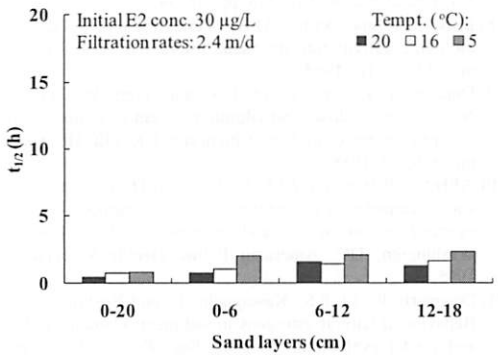

Fig.8 Values of half-life of E2 at different temperatures for Runs 1, 5 and 9 (at filtration rate: 2.4 m/d).

value with the increase of temperature. Since the k value has inversely related to $t_{1/2}$, see **Eq. (2)**, these results supported their finding.

To observe whether the finding results can contribute to operate a practical slow sand filtration or not, the evaluation performance between data from the experiment and from full scale practical slow sand filtration was made as shown in **Table 4**. The removal efficiency of dissolved organic carbon (DOC) from the experiment (for Run no. 2: filtration rate 4.8 m/d and tempt. 20 °C) are 16.02%, 12.84%, 9.08% and 17.69% for the sand layer of 0-6, 6-12, 12-18 and 0-20 cm, respectively, compared with 40.84% for practical slow sand filtration at the similar filtration rate. Based on the evaluation, the higher removal efficiency of DOC in practical slow sand filtration was caused by the deeper of sand layer. The evaluation suggested that the practical slow sand filters probably have good performance in E2 removal by controlling the filtration rate. The efficiency of slow sand filtration may also be seriously reduced by low temperatures, owing to the influence of temperatures on the rate of metabolism of bacteria and other microorganisms[11]. Therefore, to achieve optimum conditions for E2 removals at lower temperatures, decreasing the filtration rate is probably an effective way.

4. CONCLUSIONS

Continuous flow experiments were carried out to investigate the fate of 17β-estradiol (E2) in biological sand filtration under different filtration rates and temperatures. For each run, apparent changes in the effluent concentration of E2 were not appeared after initial period, indicating that the degradation had reached the steady state. With the decrease of E2, the E1, by-product of E2, was detected for all runs indicating that degradation by microorganism took place. The results clearly indicated that the apparent disappearance of E2 depended on the filtration rates and temperatures.

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Table 4 Evaluation of natural organic carbon (NOM) removals between filtration experiment and practical slow sand filtration.

Parameter	Influent	Filtration Experiment (Run No. 2) (Filtration rate: 4.8 m/d) Effluent (at sand layer: 0-20 cm):	Influent	Practical Slow Sand Filtration (Filtration rate: 4.0 m/d) Effluent (at sand layer: 70 cm):
DOC (mg/L)	1.86	1.53	0.95±0.21	0.56±0.13
UV_{260} (m^{-1})	2.35	2.10	3.38±1.34	1.78±0.71

Note: Sand depth of full scale practical sand filters is 70 cm and the data for measurement related to data from September 2010 to Februari 2011.

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