Journal of Korean Society of Water and Wastewater
Vol.24, No.6, pp.661-674, December, 2010

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

# 정수장 활성탄 여과지의 생물막과 그 활성도

# Biofilms and their Activity in Granular Activated Carbons Established in a Drinking Water Treatment Plant

이지영[1] · 김세준[1] · 정익상[2] · 조경제[1]*

Jiyoung Lee[1] · Se Jun Kim[1] · Ik Sang Chung[2] · Gyeongje Joh[1]*

1 인제대학교 환경공학부, 2 김해시 정수과

(2010년 11월 3일 접수 ; 2010년 11월 25일 수정 ; 2010년 11월 19일 채택)

## Abstract

Bacterial biomass and its activity were measured in two kinds of granular activated carbon (GAC), the experimental and existing biofiltration system in a drinking water plant. The bacterial biomass was around 210 to 250 nmol P/g WW with phospholipid concentration at acclimation of ozonation treatment. The phospholipid biomass shows more or less a declining gradient along filter depth and no clear seasonality in its values. On the other hand, the microbial activity of [$^3$H]-thymidine and [$^{14}$C]-acetate incorporation within cells increased significantly along the filter depth, showing the difference of three fold between the upper and bottom layer. These factors support the different microbial composition or metabolic activity along the depth of GAC column. Turnover rates, the rate of bacterial biomass and production of biofilm, ranged from 0.26 /hr to 0.37 /hr, indicating a highly rapid recovery itself at amature state. In the non-ozonation treatment, the bacterial biomass was lower than in the ozonation and biological activity also declined towards the filter depth. The biomass levels during cessation of ozonation in the existing GAC filters were 68% of the actively ozonated state.

Key words : GAC filter, biofilm, biomass, phospholipid, [$^3$H]-thymidine, [$^{14}$C]-acetate

주제어 : GAC 여과지, 생물막, 생물량, 인지질, [$^3$H]-thymidine, [$^{14}$C]-acetate

## 1. INTRODUCTION

A biofilter is defined as any type of filter with an attached biofilm on the filter media. Biofiltration facilities have been successfullyused for water and wastewater treatment, even air clarification (Chaudhary *et al.* 2003b). In water treatment, granular activated carbon (GAC) or sand filters are usually common in water treatment plants (WTPs). With the threat to the safety of drinking water, the GAC filtration systems are set up in 21 WTPs in South Korea that are mostly concentrated in the Nakdong River channel, accounting for 10% of the national capacity of drinking water. In our study locality, river water is polluted or contaminated in the lower reaches of the Nakdong River where the advanced treatment facilities including the GAC should be constructed to produce safe drinking water. Regardless of the wide utilities of biofilters and their major applications, not so many studies on

* Corresponding author Tel:+82-55-320-3216, Fax:+82-55-334-7092, E-mail: kjcho@inje.ac.kr(Joh, G.)

biofiltration systems have been conducted on water treatment.

The observation that GAC filters were able to remove dissolved organic matter (DOM) far beyond the point at which the adsorption capacity is exhausted has led to the development of biofiltration processes for drinking water treatment (Bouwer and Crowe 1988). DOMin drinking water promotes the regrowth of microorganisms in the distribution systems, produces disinfection by-products and causes many problems to be hardly overcome in the drinking water. Biofiltration is effective to decrease or remove DOM substance. In fact, the synchronized treatment of biofiltration and ozonation are more effective to confront the DOM problem.

Microbial biomass attached to the filter media has been determined by various methodologies (Chaudhary *et al.* 2003b, Simpson 2008): total dry mass (Chaudhary *et al.* 2003a) or volatile solids (VS) (Rittmann *et al.* 2002), total organic carbon or chemical oxygen demand (Lazarova and Manem 1995), and direct counts of bacteria using heterotrophic plate count (HPC) (Stewart *et al.* 1990, Park *et al.* 2001), phospholipid analysis (Servais *et al.* 1991, Wang *et al.* 1995, Fonseca *et al.* 2001, Seredyńska-Sobecka *et al.* 2006), physical parameters such as biofilm thickness (Lazarova and Manem 1995). The activity of microbial biofilm can be achieved by biochemical parameters such as adenosine triphosphate (ATP), INT-dehydrogenase activity, oxygen uptake rate (OUR) (Simpson 2008), radioactive tracers such as [$^{3}$H]-thymidine (Park *et al.* 2001), [$^{14}$C]-acetate (and [$^{14}$C]-glucose (Servais *et al.* 1991, Servais *et al.* 1994). Radioactive incorporation of thymidine and acetate into cells are measured to clarify the bacterial metabolism (McKinley *et al.* 1982, Freeman *et al.* 1993, Sinsabaugh and Findlay 1995).

Especially, phospholipids only occur in the membranes of living cells and are not used as storage products in cells (Vestal and White 1989). This has a relatively rapid turnover rate in microbial systems to accurately estimate microbial biomass as a highly reproducible method (Wang *et al.* 1995). Over a short period of some minutes or hours after the cell death, the cell enzymes hydrolyze the phospholipids to release phosphate groups. From this behavior, the phospholipids can be an indicator of the total amount of cells or a quantity of biomass.

We focus the studies on the bacterial activity of GAC filters to clarify the effect of ozone application and empty bed contact time (EBCT) of filters. Bacterial activity was centered to phospholipid and two radioactive tracers, thymidine and acetate.

## 2. MATERIALS AND METHODS

### 2.1 GAC filters of experimental pilot

The pilot experiments were conducted at a WTP located in the lower reaches of the Nakdong River. Five laboratory-scale GAC columns were set up in parallel with clear acrylic cylinders and their dimensions were 100 cm in working depth and 6 cm in inner diameter. The columns of the filters were filled with activated carbons (coconut-shell based). The GAC media were operating in the downflow mode of the influent with discharge from 300 mL/min to 800 mL/min. The filters were operated at four different flow from 300 $m^3$/min to 800 $m^3$/min. Hydraulic loading rates (HLR) of GAC filters ranged from 6.4 m/hr (GAC 4) to 17.0 m/hr(GAC 2). A constant HLR was maintained throughout the experiment for all filters. As EBCT is defined as the empty bed volume of the column divided by the volumetric flow rate of the feed water, the system yields 7.0 min contact time from bed depths of GAC media with effective size. The biofilters were backwashed once two week to a bed expansion of 50% for 5 min with freshly collected filter effluent water. The system of GAC filters are shown in **Fig. 1.**

The feed water, flowing from sand filtration basin in the WTP, was ozonated in a contactor column with an ozone dose from 0.5 mg $O_3$ to 1.0 mg $O_3$ per mg TOC. The entering water was ozonated continuously with 2- mg $O_3$/L concentration of ozone. Ozone was produced from its generator (Ozonia Ltd., model LAB2B) and allowed to dissipate from the bottom in

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

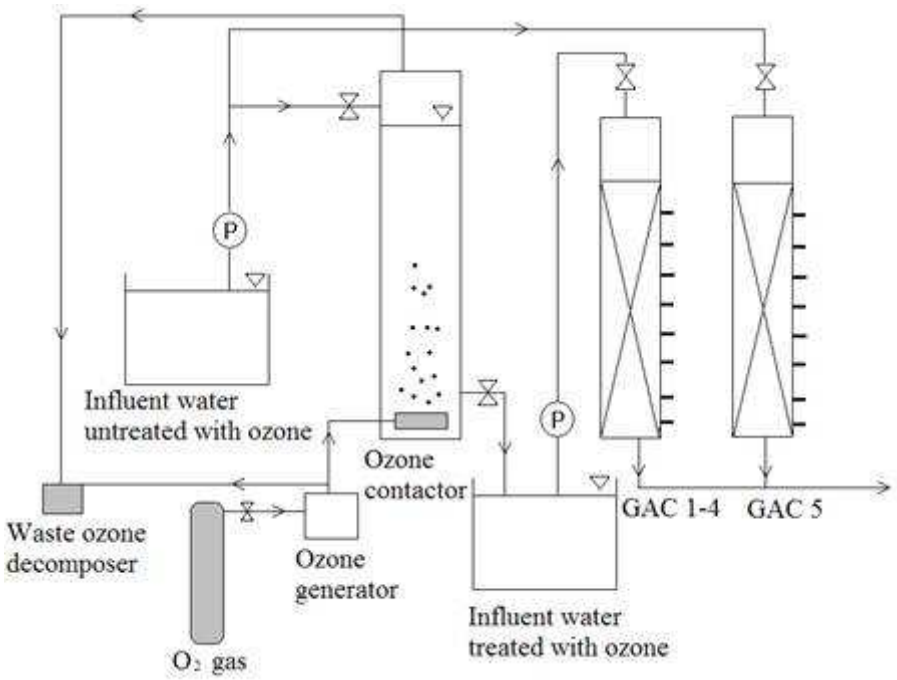


**Fig. 1.** A diagram showing GAC pilots (experimental GAC) for the study of filter biofilm in a water treatment system. Ozone was applied to feed water of four columns (GAC 1−), which have different hydraulic loading rates and was not applied to one column (GAC 5). P: pump.

an acryl cylinder (12 cm in inner diameter and 150 cm height) with a hydraulic retention time of approximately 2 hours before the water was pumped to four parallel GAC filters. Ozone concentration of feed water treated with ozone was checked with KI titration method (APHA 1992).

Eight sampling ports were installed along a vertical filter bed. Media were removed from the sampling portsbelow the surface and approximately 5 g of media were collected to routinely analyze the phospholipids once or twice a week throughout the study. To monitor the depth profile of the column, media were sampled from the top (10 cm), middle (50 cm) and bottom layer (100 cm). The samples were analyzed to measure the biomass parameters − dry weight (DW), volatile solids (VS), lipid phosphate. The activated carbon granules were incubated to determine the microbial activity or productivity in the controlled conditions using radioactive [$^{3}$H]−thymidine and [$^{14}$C]−acetate. In addition, filter media were prepared to be examined with scanning electron microscopy (SEM).

### 2.2 Existing GAC filters of the WTP

Materials for the GAC biofilms were additionally collected from the water treatment process of a WTP. Raw water is withdrawn from the lower parts of the Nakdong River. The water treatment processes have a classical and advanced type: pre−chlorination, pre−ozonation, coagulation and flocculation, sedimentation, sand filtration, post−ozonation, GAC filtration and post−chlorination (Gimhae 2007). Chlorine treatment before primary sand settling process was the level of 2.0 to 2.5 mg/L to protect pathogenic microorganisms. As an advanced treatment line, the GAC filtrations are equipped between the post−ozonation and post−chlorination stage, and this is the final process of water clarification. Six filters filled with coconut−based activated carbon in the plant are 6.0m in inner diameter, 15.6m in height and2.7m in bed depth. Three Hydraulic loading rate (HLR) was designed with 8.7 m/hr (208 m/day) and the EBCT was 10min

for all six filters. Ozone doses were applied from 0.21 to 2.05 g/m$^3$ (mean 0.98). The backwashing of GAC filters was repeated in intervals from 4 days to 6 days using high pressure air and water. The filter media were collected from the three layers of upper (10 cm depth), middle (50 cm depth) and lower depths (100 cm depth) in columns, using a granule sampler.In the WTP, six regenerated and six new GAC filters operate alternately every six months.

### 2.3 Determination of microbial biomass

Phospholipid analysis by the malachite green method was used to determine the microbial biomass from the GACbiofilm, especially the viable biomass. The concentration of phospholipid (nmol P/g wet weight (WW)) was converted to lipid and bacterial biomass. In addition to the two parameters, dry mass and VS of the biofilm were measured to estimate the bacterial biomass.

Phospholipid analysis was conducted with some modification to the process of Findlay *et al.* (1989). The 0.5 g wet GAC was transferred into a 10 mL vial containing the filtered raw water and the lipids were extracted with a mixture ratio (1:2:0.8) of chloroform-methanol mixtures by Bligh and Dyer (1959). The chloroform mixture was 7 or more times the wet weight of the materials. The mixtures were allowed to stand for 2 to 24 hours. Further addition of chloroform and DI water changed the ratio to 1:1:0.9 and supported the easy separation of the lipid containing chloroform. The extraction mixture was separated into a lower lipid and upper aqueous layer and was allowed to stand for 24 hours. The methanol-water layer was removed with a Pasteuer pipette and the residual chloroform phases were filtered with GF/C to over 5 mL of volume before being transferred to vials.

Lipid fractions in the vial were completely evaporated by the stream of nitrogen gas. In addition, a saturated solution of 0.90 mL potassiumpersulfate (5 g added to 100 mL of 0.36 N $H_2SO_4$)was added and the vial was heated at 95 °C for 2 hours. The next step wasa colorimetric measurement. Phosphate was determined from the digested samples by adding ammonium molybdate and malachite green. The 0.2 mL of 2.5% ammonium molybdate solution was added to the vials and the mixture was allowed to stand for 10 minutes. Then 0.90 mLof malachite green solution was added and stood for 30 minutes. A complex between phosphomolybdate and malachite green formed to display a strong blue color. Absorbance was read at 610 nm wavelength and a standard curve was calibrated with $K_2HPO_4$ solutions. The malachite green method is more sensitive than other methods when measuring the low concentration of phosphate (Van Veldhoven and Mannaerts 1987).

The simplified and sensitive method of phospholipidextraction has been applied to determine the microbial biomass in sediment, benthic organic matter, agricultural soil, biofilter, granular activated carbon, and activated sludge (Findlay *et al.* 1989, Frostegård *et al.* 1991, Fonseca *et al.* 2001, Christian and Smith, 2004, Seredyńska-Sobecka *et al.* 2006).

### 2.4 $^3$H-thymidine incorporation

Bacterial productions were tracked along two routes, $^3$H-thymidine and $^{14}$C-acetate incorporation into DNA and lipid, respectively. The 15 mL centrifuge tubes made with polypropylene were used as the incubation vessels. The sample materials were incubated under a constant temperature (20°C) for 2 hours in continuously shaking conditions (approximately 140 rpm) and in darkness. Five replicates per each sample and three blanks were prepared for the isotopic assays.

A 5.0 mL sample of water containing 1.0 g wet GAC was incubated at afinal concentration of 10 nM of tritiated methyl-[$^3$H] thymidine (Cat. No. NET-027E-1) from Perkin Elmer, its specific activity 6.7 Ci/mmol. The 33.6 μL of 100-fold diluted stock-solution of [$^3$H]-thymidine was added to the raw water. Prior to the isotope inoculation, the filtered raw water was enriched with a final concentration of 2 mg $NaNO_3$-N/L, 0.4 mg $K_2HPO_4$-P/L and 6 mg acetic acid-C/L having 15C : 5N : 1P ratios (weight bases) (Urfer and Huck 2001). This was an enriched nutrient medium (ENM) to

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

promote the bacterial growth in the incubation. The enrichment would preclude the limitation of the major nutrients for the bacterial growth. After 2 hour incubation, the radioactivity synthesized into the bacterial DNA was measured with a scintillation counter (Packard Instruments, model Tri-Carb 2000) after the addition of 10 mL of scintillation cocktail (Ultima Gold, Perkin Elmer).

### 2.5 $^{14}$C-acetate incorporation

To assess lipid biosynthesis of bacterial cells, the methods of McKinley *et al.* (1982) and Freeman *et al.* (1993) were followed. The 10.0 μL of [1-$^{14}$C] acetic acid, sodium salt (specific activity 45-60 mCi/mmol, Perkin Elmer) was added to the 5.0 mL of enriched raw water containing 1.0 g wet GAC. The final concentration of [$^{14}$C]-acetate was 100 nmol/L. When measuring acetic acid, carbon nutrients were not used to enrich the raw water. After incubation, the sample materials were fixed with 5% formaldehyde,and lipid extractions were conducted using the process of Bligh and Dyer (1959). The 5.0 mL methanol and 2.5 mL chloroform were added and shaken, and then additional 2.5 mL chloroform and 2.5 mL water were secondarily added, shaken and allowed to stand overnight to extract the cellular lipids. The aqueous layer was removed by aspiration and the chloroform phase containing the $^{14}$C-labelled lipid was evaporated with a stream of nitrogen gas. Radioactivity measurements in the liquid fraction were the same with the treatment of the [$^{3}$H]-thymidine.

The microbial uptake of radiolabeled acetate were applied to sediment, GAC, complex activated sludges (Lee *et al.* 1999) and the natural water - river biofilm on PVC pipe (Freeman *et al.* 1993), plant litter or detritus in the lake (White *et al.* 1977, McKinley *et al.* 1982).

### 2.6 Oxygen uptake rate

Oxygen uptake rates (OUR) of GAC filters and backwashing water were measured with 300 mL BOD bottles according to the process by the Urfer and Huck (2001). The suspensions containing 8 g wet activated carbon were incubated in effluent water through GAC filter to dechlorinate the sand-filtered raw water. Prior to the incubation, the ENM nutrient was added to the incubated water to protect possible nutrient limitations. The bottles were incubated for 5 to 7 hours in a shaking water bath in which water temperature was controlled to 20°C. Dissolved oxygen (DO) concentrations were measured with a BOD probe (YSI incorporated, model YSI 5905) and OURs were determined from the initial and final DO concentration. The oxygen uptake rates would be the potential values for the microbial respiration.

### 2.7 Analyses of water

Some inorganic nutrients such as $NH_4^+$, $NO_3^-$, $PO_4^{3-}$ and COD in water were analyzed by colorimetric methods according to the Standard Methods (APHA 1992). $UV_{254}$ of water collected from the GAC filters was measured with a UV/VIS spectrophotometer (Kontron instrument, model Uvikon 930). Sample water was filtered with GF/C, and dissolved organic carbon (DOC) was determined using a total organic carbon analyzer (Shimadzu Corporation, model TOC-V CPN).

## 3. RESULTS

### 3.1 Bacterial biomass and productionof experimental BAC

The basic nutrients for bacterial growth in biofilters were measured for the flowing water from sand filtration basin in the WTP. The concentration of inorganicelements in feed water of biofilters, $NH_4^+$, $NO_3^-$ and $PO_4^{3-}$ were 6 to 88 μg N/L with a mean of 74 μg/L, 1,956 to 2,802 μg N/L with a mean of 2,307 μg/L and 7 to 25 μg P/L with a mean of 16 μg/L. Organic elements, COD and DOM , were 3.1 to 7.2 mg $O_2$/L with a mean of 4.4 mg/L and 0.71 to 3.75 mg C/L with a mean of 2.10 mg/L.

The concentrations of phospholipid as bacterial biomass attached to the activated carbon granules were monitored throughout for over 70 days of operations (**Fig. 2**). The phospholipid began to rapidlyaccumulate for the early times and reached a

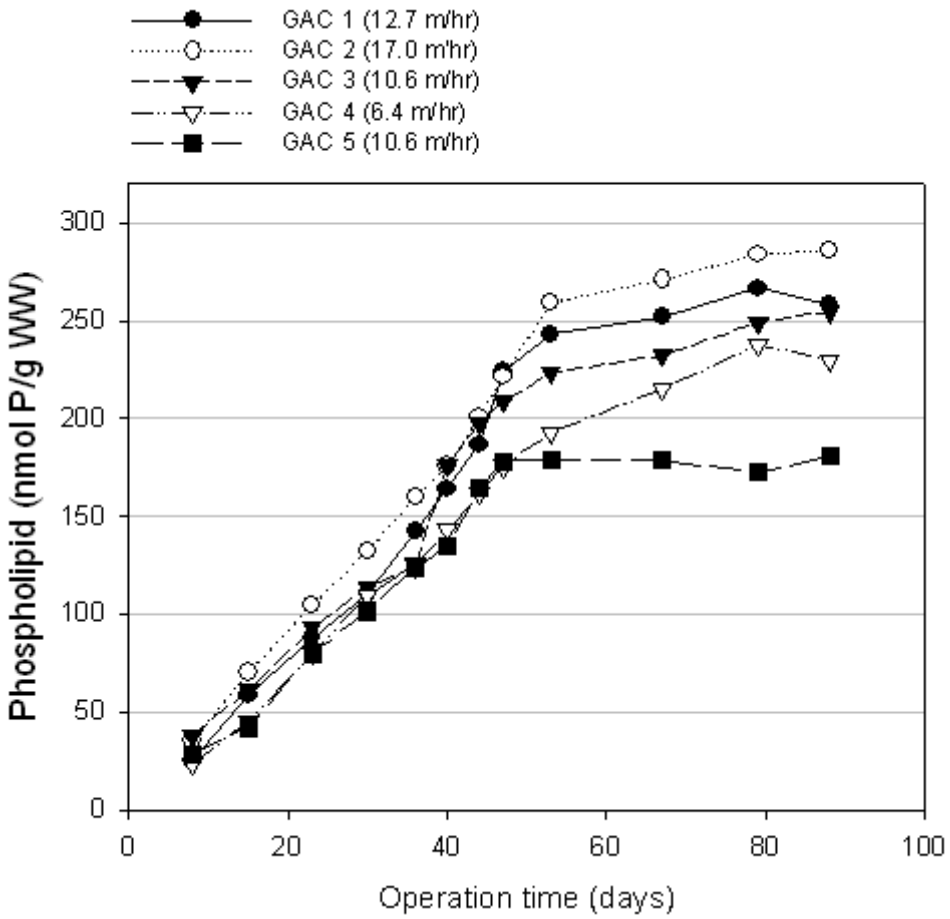


**Fig. 2.** Bacterialbiomass of GAC filters with ozonated water (from GAC 1 to GAC 4) and non-ozonated water (GAC 5), having different EBCT times among ozone treatment.

value of about 170 nmol P/g WW over 50 days. After that, the concentration further increased up to approximately 250 nmol P/g WW. However, the biomass of the fifth column injected with non-ozonated influent was much lower than those of the ozonated columns. Significant differences in bacterial biomass were observed in different HLRs from 6.4 m/hr to 17.0 m/hr in the four filters. Though another reference column or GAC was not adopted in our experiment, the existing GAC facilities set up in the WTP were considered as a referencefor the microbial biomass.

The bacterial biomass and its activity were determined along the depth in the experimental GAC columns. Each profile shown in this figure depicts the average of several determinations at the mature condition (**Fig. 3**). Phospholipid concentrations are 179 nmol P/g WW at upper parts of the filter, 151 nmol P/g WW at middle parts and 130 nmol P/g WW at lower parts of the column. Bacterial productionsvaried from 769 nmol H/g to 885 nmol H/g to show weak vertical gradients of bacterial activity from upper media to lower media. The bacterial biomass and thymidine incorporation were greatest over the top layer of the columns and gradually decreased towards the bottom of the filters. The VSs of the activated carbons were 0.063 mg/g WW at upper, 0.045 mg/g at middle and 0.031 mg/g at bottom layers to have significant differences along the vertical depth. The microbial biomass was

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

greatest over the top layer of the columns.

GAC filters were washed routinely every week. During the back-washing, the water level is drained to the column surface, followed by 2 min of air scouring at 30 m/hr, air scouring and backwashing at

experiment.

### 3.2 Microbial biomass and production of existing GAC in the WTP

Bacterial biomass of the filters set up in the WTP

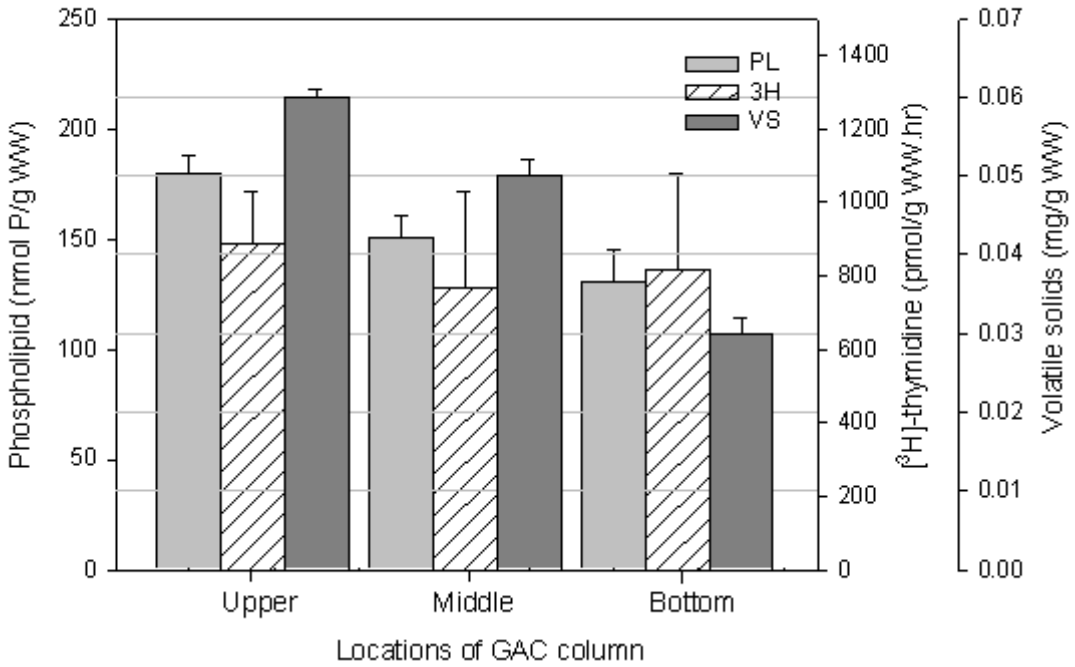


**Fig. 3.** Biomass parameters (VS and phospholipid concentration) and thymidine incorporation into cells at the three layers of biofilters treating non-ozonated water in an experimental GAC pilot. PL: phospholipid, 3H: [3H]-thymidine, VS: volatile solids.

10 m/hr until the water level reaches the washing troughs, and water backwashingat 25 m/hr for 20 min. In order to test the effect of filter washing on microbial biomass, OURs were determined with respirometry. OURs of backwashing water gradually decreased as the GAC columns were stabilized in its microbial biomass.

The microbial activity corresponded to the removal of biodegradable DOM (BDOM) in the GAC filters. $UV_{254}$ of influents and effluents was monitored through our experiments. As the influent water of the GAC filters was the effluent of sand filtrations in the WTP, the $UV_{254}$ of the influent ranged from 0.016 /cm to 0.040 /cm (average 0.029 /cm) and their removal efficiency was from 35% to 70% during the

was determined five times, and thebiomass and production along the 1.0m depth profile were estimated at three depths (**Fig. 4**). The concentrations of phospholipid were 213 nmol P/g inthe upper, 193 nmol P/g in middle and 184 nmol P/g in the bottom layer. The VS and oxygen consumption rates of GAC granules were slightly higher in the upper layer than in the two lower layers. The VS concentration and OUR were 57.2 mg/g WW, 53.7 mg/g and 54.0 mg/g, and 0.0157 mg $O_2$/g WW·hr, 0.0145 mg $O_2$/g·hr and 0.0147 mg $O_2$/g·hr, respectively. The biomass shows more or less a declining gradient along the profile and shows no clear seasonality in its values. During these experiments, the temperature of feed water in GAC

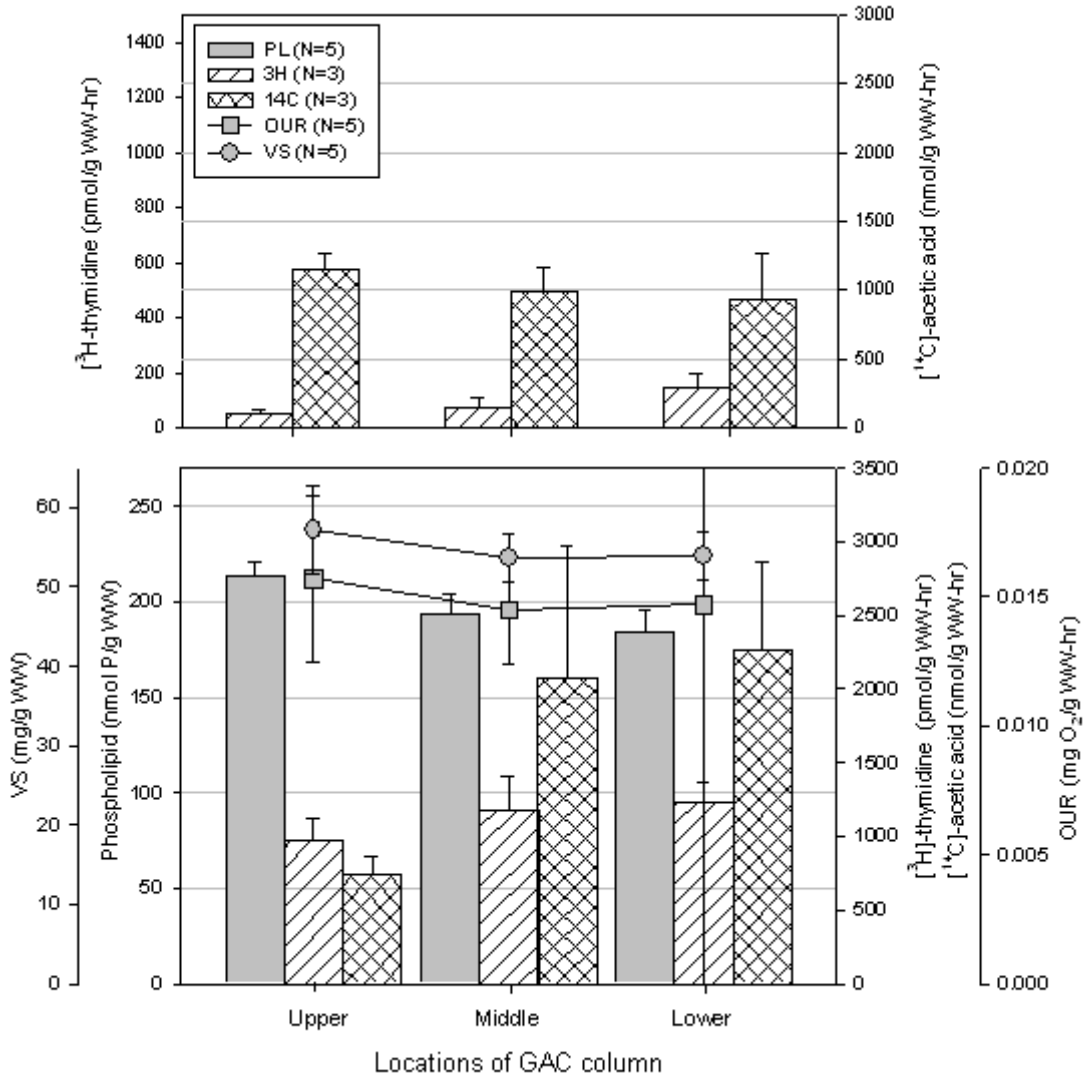


**Fig. 4.** Phospholipid biomass and thymidine activity under initial stages of a new GAC filter in the existing GAC plant (upper). Biomass parameters (VS, OUR and phospholipid) and specific activity (thymidine and acetate activity) at the three layers of the biofilters treating ozonated water in the existing GAC plant (lower). 14C: [14C]-acetate, OUR: oxygen uptake rate.

filters ranged from 5°C (in January) to 26 °C (in August) over a year.

On the other hand, the microbial activity of thymidine and acetate incorporation into cells increased significantly towards the filter depth: from 832 pmol/g to 1,054 pmol/g of thymidine and from 737 nmol/g to 2,264 nmol/g of acetate. These support better metabolic activity along the depth profile.

During our experiments, the post-ozonation for the GAC filters stopped due to the high level of manganese in the influent and the cessation continued from March 25 to June 7 in 2009. In this periods, the lipid biomass of the filters began to decline rapidly to 68% of the previous mature state and showed no clear differences along the depth profile (**Fig. 4**). In the operating system of GAC in the WTP, two

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

alternating columns were used; regenerated GAC filters were used for six months and new GAC filters were subsequently used for another six months in the WTP. GAC columns were alternated in six month intervals. The bacterial biomass and activity at the initial colonization stages for the biofilms were presented in **Fig. 4** as new GAC.

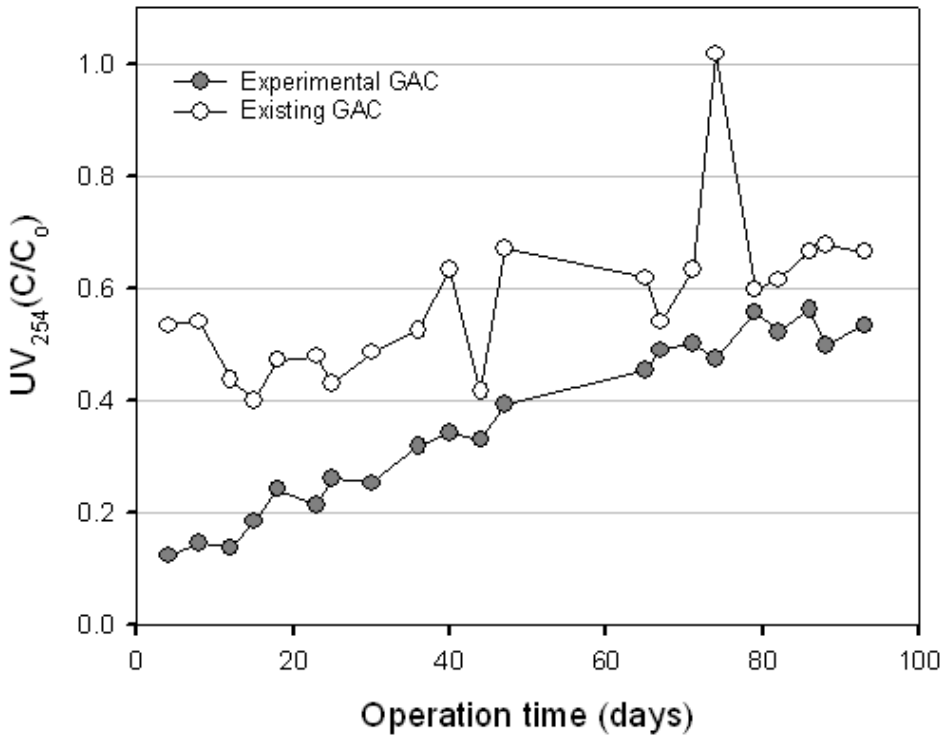


**Fig. 5.** Breakthrough curves of DOC parameter in experimental (GAC 3) and existing GAC filters.

### 3.3 OUR of the backwashing water from GAC filters

The backwashing water of experimental and existing GAC were analyzed to determine biological activity of the filter granules. The VS and OUR of the experimental GAC ranged from 0.08 g/L (0.63 g/g DW) to 0.18 g/L (0.89 g/g DW), respectively. The backwashing water of the existing filters was collected consecutively through four stages during operation. The VS of the existing filters decreased from 0.164 g/L at first washing to 0.005 g/L at last washing, while VS concentration slightly increased from 0.70 g/g DW to 0.77 g/g DW. The OUR decreased from 1.74 mg $O_2$/L·hr (0.011 mg $O_2$/g VS·hr) at first washing to 0.54 $O_2$/L·hr (0.103 mg $O_2$/g VS·hr) at fourth washing .

### 3.4 DOC removal efficiency

If necessary, DOC was determined in each stage of the WTP. Final removal of DOC and $UV_{254}$ were 59% and 83% in the process of water treatment andsignificant reductions by ozonation and GAC filtration was observed: 25% reduction of DOC and 62% reduction of $UV_{254}$. Removal efficiency of $UV_{254}$ in experimental GAC filter (No. 3) and extisting GAC were shown in **Fig. 5**.

### 3.5 Bacteria on GAC media

Though direct enumerations of bacteria attached to activated carbons are not possible, comprehensive observations were conducted using SEM in the experimental and existing plant (**Fig. 6**). Many

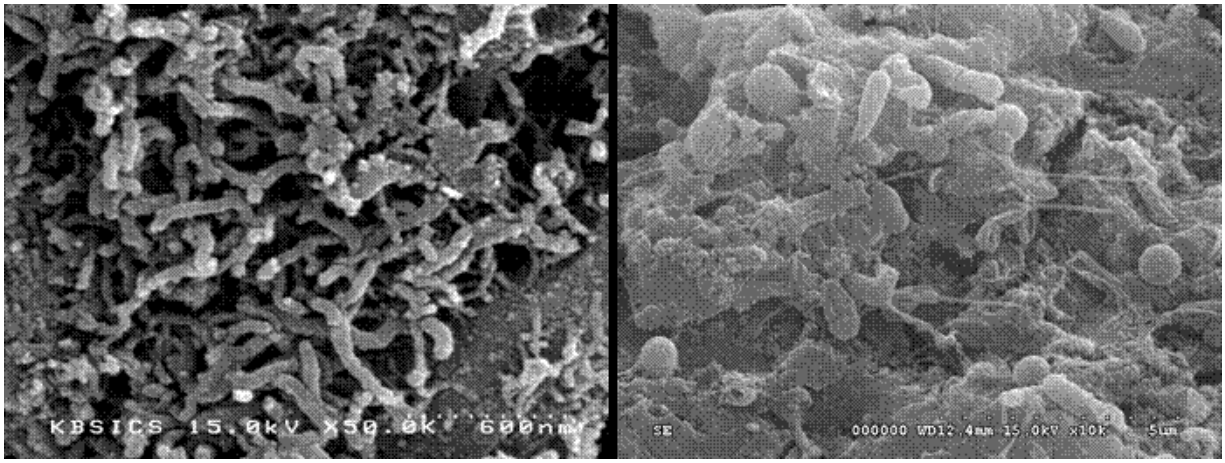


**Fig. 6.** SEM micrographs of biofilms that developed on granules of activated carbon in July 14, 2008 (left) and May 5, 2009 (right).

bacillus-typed bacteria were seen on the surface of GAC and they are assumed to be *Pseudomonas* genera. Bacterial cells were seen on the surface of the BAC, but extracellular polymer matrix was not frequently observed under electron microscopy.

## 4. DISCUSSION

### 4.1 The microbial biomass and activity of GAC filters

Microbial biomass of GAC biofilters have been reported by some authors and three months are required for accumulation of biomass before the ceilings are reached (Servais *et al.* 1994, Wang *et al.* 1995). Bacterial biomass usually exceeds 300 nmol P/g DW of phospholipid concentrations in activated carbons, and, sometimes, from 20 nmol P/g DW to below 100 nmol P/g DW (**Table 1**). The microbial biomass is endogeneously produced in the biofilters and fixed on the media. In the study of biofilters, the age of the top layer and biomassaccumulation showed strong positive correlations. In addition, the biomass in the biofilters likely resulted from microbial growths in the filters rather than from the filtration of bacteria in the source water (Collins *et al.* 1992). The difference in bacterial biomass was negligible as filter depth increased. The determination of the phospholipid ester-linked fatty acids (PLFA) provides a quantitative measure of a viable or potentially viable biomass of bacteria as evidenced by the enzyme hydrolysis that occurs to the phosphate group within minutes to hours after cells die (White *et al.* 1993). The phospholipid extractions have been widely used in various types of biofilters, GAC filters (Wang *et al.* 1995, Seredyńska-Sobecka *et al.* 2006), anthracite filters (Carlson and Amy 1998) and sand filters (Fonseca *et al.* 2001), to sensitively determine the bacterial biomass. Bacterial biomass based on phospholipid is varied according to filter media and hydrological factors (Table 1). GAC media are most effective for the bacterial accumulation or biofilm formation.

Thymidine incorporation estimates DNA synthesis and, consequently, the cellular division rate of bacteria is an indicator of productivity. The bacterial productions ranged from 23.3 μg C/g WWhrto 29.5 μg C/ghr in the existing GAC filters. In contrast, [$^{14}$C]-acetate incorporation into lipid biosynthesis denotes metabolic or respiratory activity and this activity of the existing GAC filters is similar to those of thymidine (**Fig. 4**). The phospholipid contents as viable biomass are converted to carbon biomass using a well-established conversion factor ($1.1\times10^7$ cells/nmol P, 0.625 μg C/nmol P) suggested by Findlay *et al.* (1989). Approximately $2.0\times10^9$cells or 28 μg C are produced for each nmol of thymidine incorporation based on empirical measurements (Wetzel and Likens 2000). Bacterial biomass and secondary production of existing GAC ranged from

상하수도학회지, 논문
24권 6호, pp.661-674, 12월, 2010

**Table 1.** Bacterial biomass of the top layer of biofilters, which were supplied with ozonated influents, in pilot plants. Bulk density of GAC is 0.48

| Filter media | HLR (m/hr) | EBCT (min) | Operation days | Biomass | References |
|---|---|---|---|---|---|
| GAC | 6.4 - 17.0 | 7.0 | 70 | 127−41 nmol P/g WW | This study |
| GAC | - | 9.2 | 95 | 305 ± 9 nmol P/g DW | Wang *et al.* (1995) |
| GAC | - | 9.2 | 95 | 465 ± 9 nmol P/g DW | Wang *et al.* (1995) |
| GAC | - | 9.2 | 95 | 382 ± 11 nmol P/g DW | Wang *et al.* (1995) |
| GAC | 0.75 - 1.5 | 0.8 | 42 | 38 nmol P/g carbon | Seredyńska-Sobecka *et al.* (2006) |
| Sand | - | - | - | 90.6 ± 1.3 nmol P/g DW | Wang *et al.* (1995) |
| Sand | 4.2 | 13 | - | 23−38 nmol P/g DW | Fonseca *et al.* (2001) |
| Anthracite | 5.0 | 2−0 | At least 60 | 22.8 nmol P/g DW | Carlson and Amy (1998) |
| Anthracite | 9.7 | 2−0 | At least 60 | 47.3 nmol P/g DW | Carlson and Amy (1998) |
| Anthracite | - | - | - | 2.0 ± 0.5 nmol P/g DW | Wang *et al.* (1995) |

79.7 μg C/g WW to 88.7 μg C/g and 23.3 μg C/g WWhr to 29.5 μg C/ghr, respectively, using these conversion factors. Turnover times of filter biofilm were from 2.7 hr to 3.8 hr at mature state. Turnover times for the biofilmwere calculated by dividing bacterial biomass by production. This indicates the rate of supply of bacteria by growth relative to standing biomass or the time required for the attached bacteria to recover themselves. Turnover times for GAC biofilm were relatively short, compared with natural microbial assemblages. Turnover rates of the biofilms were in the ranges of 0.26 /hr to 0.37 /hr, more rapid rate than those reported by Servais *et al.* (1991).

The microbial biomass of the filters significantly decreased from the top to the bottom of ozonated and non−ozonated filter (**Fig. 3** and **4**) and the same patterns were reported from some results of other GAC experiments (Servais *et al.* 1991, Servais *et al.* 1994). The trend that the biomass decreases progressively towards the bottom is a general pattern as DOM declines as filter depth increases. Through the direct HPC test, bacteria was significantly more rich on the top layer than in the bottom layers (Park *et al.* 2001). Such a distinct stratification of bacterial biomass or activity was observed at ambient temperatures above 15°C and BDOC compounds were more easily eliminated at higher temperatures (Servais *et al.* 1991). The trend of depth profile in GAC filters is more clear in biofilters treating sewages (Yu *et al.* 2005). However, the radioactivity of two isotopes was different between ozonation and non−ozonation. The isotopic activity in non−ozonation treatment decreased as function of filter depth, compared to the great increase under the ozonation. A decrease of biomass can be seen as function of filter depth due to a decrease in labile biodegradable DOM through filter depth and even a difference in microbial community structure under ozonation (Moll *et al.* 1998, Fonseca *et al.* 2001).

Besides of ozonation, the important variables influencing biofiltrations in water treatment were contact times (EBCT) or HLR in this pilot(**Fig. 2**). Important factors for the biofilters were reported by many authors: HLR (Huck *et al.* 1994, Urfer *et al.* 1997, Carlson and Amy 1998, Fonseca *et al.* 2001), temperature (Fonseca *et al.* 2001, Hozalski and Bouwer 2001), DOC loadings (Collins *et al.* 1992, Chaudhary *et al.* 2003b), and backwashing. The biological removal of organic matter in GAC filters having a given EBCT is independent of a HLR from

6 m/hr to 18 m/hr (Servais *et al*. 1994). However, the biomass accumulation was dependent upon HLR and the organic concentration in the GAC treatment of low strength synthetic wastewater (Chaudhary *et al*. 2003a). A strong positive correlation between biomass and DOM loadings wassuggested to indicate the importance of organic matter for microbialgrowth in the biofilters (Collins *et al*. 1992). DOC loading rate of GAC filters in this experiment was estimated to be 17.7 g C/$m^2$hr on the average.

In this experiment, even the air scouring and subsequent backwashing with water of the filters had below 1% loss of VS contents in a GAC filter to show no significant effect on the microbial biomass. Backwashings at weekly intervals would seldom impair the biofilm growth. Through the direct enumeration of bacteria in the backwashing waters, only a small limited fraction of the biomass on the GAC column was eliminated during backwashing (Servais *et al*. 1991, Ahmad *et al*. 1998).

In the WTP, the ozone treatment before GAC filters has occasionally increased when toxic substances such as dioxane have been added from the upper regions of the river. On the other habd, it has occasionally stopped when manganese has been produced from the process of retreatment of solid residuals settled in sedimentation basin. Ozone is effective preventing the destructive oxidation of dioxane, while colloidal Mn is immediately formed to break out color problem after ozone treatment when chlorine diminished (Kohl and Medlar 2007). Excessive preozonation was applied before the coagulation process to cope with high Mn situations. In 2009, four accidents to stop the post-ozonation occurred in the local plant.

### 4.2 Bacteria on the GAC filter

Bacterial abundance in the experimental filters was estimated to be approximately $2\times10^{10}$ cells/g WW at mature state or conditions using the conversion factor, $10^8$ bacteria of the size of *Escherichia coli*/ nmol lipid-P (Vestal and White 1989). Rod-shaped bacteria predominantly occupied the surface of activated carbons and coccus-shaped bacteria were occasionally observed (**Fig. 6**). The dominant bacteria colonizing theactivated carbons in the water treatment process were reported to be rod shaped bacteria, *Pseudomonas* and *Flavobacterium* (Stewart *et al*. 1990, Park *et al*. 2001). Biological filtration can be a source of microorganisms in the treatment lines through the sloughing or detaching of filter biomass. As nonpathogenic bacteria, most bacteria identified in GAC media and effluents are typical soil and water bacteria such as *Pseudomonas*, *Acinetobactor*, *Bacillus* and *Corynebacterium* and cell counts ranged to 600 colonies/ml in effluent from GAC columns (Bouwer and Crowe 1988).

In this experiment, microbial biomass of GAC filters is an important parameter to remove effectively DOC from water and its profile along the filter depth is another factor for the normal function. The use of GAC may increase in South Korea to acquire drinking water safety as water utilities face regulations governing the types and concentrations of disinfection by-products released as well as micro-contaminants in the treated water.

## ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD)" (KRF-2006-511-D00226). Great thanks goto Pyung-Jong Yu, Director of the Water Quality Institute of the Busan Water Authority in Busan Metropolitan City, and Jong-Moon Jung, a doctoral scientist of the Institute for providing a lot of assistance.

## REFERENCES

Ahmad. R. and Amirtharajah, A. (1998) Detachmnet of particles during biofilter backwashing. J. AWWA, 90(12), 74-85.

APHA. (1992) Standard methods for the examination of water and wastewater. 18th edition. APHA, AWWA and WEF.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem.*

**상하수도학회지**, 논문
24권 6호, pp.661-674, 12월, 2010

*Physiol.*, 37(8), pp.911－917.

Bouwer, E.J. and Crowe, P.B. (1988)Biological processes in drinking water treatment. *J. AWWA* , 80(9), pp.82－3.

Carlson, K.H. and Amy, G.L. (1998) BOM removal during biofiltration. *J. AWWA*, 90(12), 42－52.

Chaudhary, D.S., Vigneswaran, S., Ngo, H.H., Shim, W.G. and Moon, H. (2003a)Granular activated carbon (GAC) biofilter for low strength wastewater treatment. *Environ. Eng. Res.*, 8(4), pp.184－92.

Chaudhary, D.S., Vigneswaran, S., Ngo, H.H., Shim, W.G. and Moon, H. (2003b) Biofilter in water and wastewater treatment. *Korean. J. Chem. Eng.*, 20(6), pp.1054－065.

Christian, A.D. and Smith, B.N. (2004)Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca:Unionidae) in 2 small Ohio streams. *J. North Am. Benthol. Soc.*, 23(1), pp.101－13.

Collins, M.R., Eighmy, T.T., Fenstermacher Jr., J.M.. and Spanos, S.K. (1992) Removing natural organic matter by conventional slow sand filtration. *J. AWWA*, 84(5), pp.80－0.

Findlay, R.H., King, G.M. and Watling, L. (1989) Efficacy of phospholipids analysis in determining microbial biomass in sediments. *Appl. Environ. Microbiol.*, 55(11), pp.2888－2893.

Fonseca, A.C., Summers, R.C. and Hernandez, M.T. (2001) Comparative measurements of microbial activity in drinking water biofilters. *Wat. Res.*, 35(16), pp.3817－824.

Freeman, C., Fiebig, D.M. and Lock, M.A. (1993) [$^{14}$C] acetate incorporation, an indicator of lipid biosynthesis within intact river biofilms. *J. Aquat. Ecosys. Health*, 2(4), pp.329－34.

Frostegård, A., Tunlid, A. and Bååth, E. (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol Methods*, 14(3), pp.151－163.

Gimhae. (2007) *Report of Technological Diagnosis on Facilities for Drinking Water Treatment*. Gimhae City, South Korea. (in Korean)

Huck, P.M., Zang, S. and Price, M.L. (1994) BOM removal during biological treatment: a first-order model. *J. AWWA*, 86(6), pp.61－1.

Kohl, P. and Medlar, S. (2007) *Occurrence of Manganese in Drinking Water and Manganese Control*. AWWARF Research Foundation. 460 pp.

Lazarova, V. and Manem, J. (1995)Biofilm characterization and activity analysis in water and wastewater treatment. *Wat. Res.*, 29(10), pp.2227－245.

Lee, N., Nielsen, P.H., Andreasen, K.H., Juretschko, S., Nielsen, J.L., Schleifer, K.H. and Wagner, M. (1999) Combination of fluorescent *in situ*hybridization and structure-function analyses in microbial ecology. *Appl. Environ. Microbiol.*, 65(3), pp.1289－297.

McKinley, V.L., Federle, T.W. and Vestal, J.R. (1982) Effects of petroleum hydrocarbons on plant litter microbiota in an arctic lake. *Appl. Environ. Microbiol.*, 43(1), pp.129－35.

Moll, D.M., Summers, R.S. and Breen, A. (1998) Microbial characterization of biological filters used for drinking water treatment. *Appl. Environ. Micro*biol.,64(7), pp.2755－2759.

Park, H.K., Na, Y.S., Chung, C.M., Ryu, D.C., Lee, S.J. and Hong, Y.K. (2001)Improving water quality and bacterial characteristics during water treatment process using biological activated carbons on downstream of the Nakdong River. *J. Korean Environ. Sci. Soc.*, 10(2), pp.105－11.

Rittmann, B.E., Stilwell, D., Garside, J.C., Amy, G.L., Spangenberg, C., Kalinsky, A. and Akiyoshi, E. (2002) Treatment of a colored groundwater by ozone-biofiltration: pilot studies and modeling interpretation. *Wat. Res.*, 36(13), pp.3387－397.

Seredyńska-Sobecka, B., Tomaszewska, M., Janus, M. and Morawski, A.W. (2006) Biological activation of carbon filters. *Wat. Res.*, 40(2), pp.355－363.

Servais, P., Billen, G. and Bouillot, P. (1994) Biological colonization of granular activated carbon filters in drinking-water treatment. *J. Environ. Eng.*, 120(4), pp.888－899.

Servais, P., Billen, G., Ventresque, C. and Bablon, G.P. (1991) Microbial activity in GAC filters at the Choisy-le-Roi Treatment Plant. *J. AWWA*, 83(2), pp.62－8.

Simpson, D.R. (2008) Biofilm processes in biologically active carbon water. *Wat. Res.*, 42(12), pp.2839－848.

Sinsabaugh, R.L. and Findlay, S. (1995) Microbial production, enzyme activity and carbon turnover in

surface sediments of the Hudson River Estuary. *Microb. Ecol.*, 30(2), pp.127－141.

Stewart, M.H., Wolfe, R.L. and Means, E.G. (1990) Assessment of the bacteriological activity associated with granular activated carbon treatment of drinking water. *Appl. Environ. Microbiol.*, 56(12), pp.3822－3829

Urfer, D. and Huck, P.M. (2001) Measurement of biomass activity in drinking water biofilters using a respirometric method. *Wat. Res.*, 35(6), pp.1469－1477.

Urfer, D., Huck, P.M., Booth, S.D.J. and Coffey, B.M. (1997) Biological filtration for BOM and particle removal: a critical review. *J. AWWA*, 89(12), pp.83－8.

Vahala, R. (2002) *Two-step Granular Activated Carbon Filtration in Drinking Water Treatment*. Ph. D. thesis of Helsinki University of Technology, Finland. 81 pp.

Van Veldhoven, P.P. and Mannaerts, G.P. (1987)Inorganic and organic phosphate measurements in the nanomolar range. *Anal. Biochem.*, 161(1), pp.45－8.

Vestal, J.R. and White, D.C. (1989) Lipid analysis in microbial ecology: Quantitative approaches to the study of microbial communities. *Bioscience*, 39(8), pp.535-541

Wang, J.Z., Summers, R.S. and Mittner, R.J. (1995)Biofiltration performance: Part 1, Relationship to biomass. *J. AWWA*,87(12), pp.55－3.

Wetzel, R.G. and Likens, G..E. (2000) Bacterial growth and production. In: *Limnological Analyses*. 3rd edition. Springer Science + Business Media, Inc., New York. pp. 271－87.

White, D.C., Bobbie, R.J., Morrison, S.J., Oosterhof, D.K., Taylor, C.W. and Meeter, D.A. (1977) Determination of microbial activity of estuarine detritus by relative rates of lipid biosynthesis. *Limnol. Oceanogr.*, 22(6), pp.1089－099.

White, D.C., Meadows, P., Eglington, G. and Coleman, M.L. (1993) *In situ*measurement of microbial biomass, community structure and nutritional status. *Phil. Trans. R. Soc. Lond. A.*, 344(1670), pp.59－7.

Yu, B., Jie, Z., Yi-fan, L., Yu-nan, G. and Yong, L. (2005) Biomass and microbial activity in a biofilter during backwashing. *J. Zhejiang Univ. SCI.*, 6B(5), pp.427－32.