Bull. Environ. Contam. Toxicol. (2003) 71:204–211

DOI: 10.1007/s00128-003-0150-x

Bulletin of Environmental Contamination and Toxicology

Mutagenicity of Polluted Reservoir Water and Its Reduction by a Pilot-Scale Integrated Biological Treatment Process

J. Y. Hu,[1] S. L. Ong,[1] W. J. Ng,[1] B. P. He,[2] W. J. Liu,[2] Z. D. Fang,[2] X. H. Zhang,[2] Z. S. Wang[2]

[1] Center for Water Research, Civil Engineering Department, The National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260
[2] Water Pollution Control Group, Environmental Science and Engineering Department, Tsinghua University, Beijing 100084, People's Republic of China

Received: 2 October 2001/Accepted: 11 April 2003

The presence of organic compounds in drinking water may pose potential public health concern to consumers. It has been known that organics even at concentration of ppb levels could impose potential health risks to human through a lifetime consumption of affected water. A review of literature revealed that biological treatment could be an effective technology for removing organics present in drinking water (Takasaki et al. 1988; Shibata et al. 1987; Arvin et al. 1991; Hu et al. 1999b). For example, biological treatment has been commonly used in Western Europe to produce chemically and biologically safe as well as aesthetically pleasing water. Biological treatment methods have also been investigated in Japan as a pre-treatment process for removing aquatic pollutants such as odour, NH_4-N and BOD_5 from raw water (Takasaki et al. 1992). Similarly, submerged biofilm processes have been installed in Northern China since early 1990s for pre-treating polluted raw waters (Li 1992). In view of the potential usefulness of pre-treating contaminated freshwater supplies with bio-treatment processes, the objective of this study was to assess the performance of an integrated bio-treatment process for reducing the mutagenicity associated with reservoir water containing oily contaminants. The reservoir, located in Northern China, has been used as one of the major water sources for the surrounding communities. It was reported that the reservoir water has been heavily polluted with oily organic matters which were suspected to come from an upstream petrochemical industry as well as from fishing activity taking place within the reservoir. The innovative treatment system used in this study is consisting of a bio-ceramic pre-treatment unit along with a conventional water treatment system. The performance characteristics of the innovative treatment system were meticulously evaluated in this study.

MATERIALS AND METHODS

The pilot plant studies were conducted over a period of nearly one year. This pilot plant consisted of a 4 m high packed bed column of 25 cm diameter (Fig. 1). The column was packed with granular ceramic support media of 2-5 mm diameter (media depth = 1.8 m).

Correspondence to: J. Y. Hu

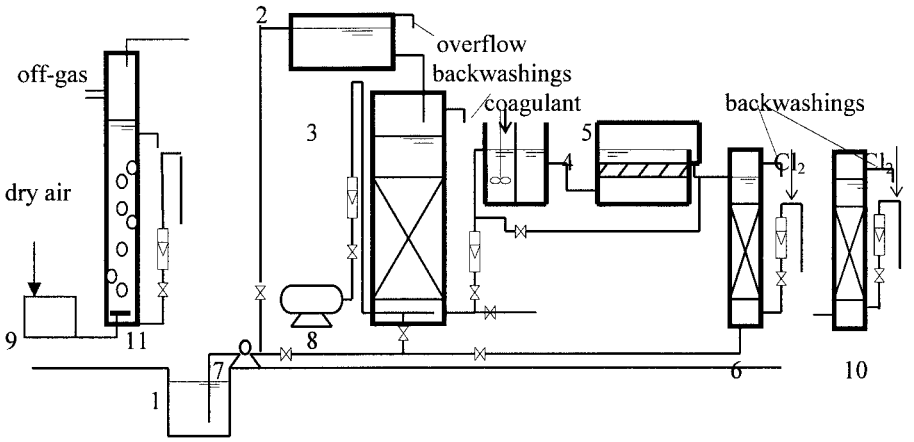

Figure 1. Pilot-scale experimental set-up.
Note: 1. Water storage tank 2. High water tank 3. Bio-ceramic packed column 4. Coagulation tank 5. Sedimentation tank 6. Sand filter 7. Pump 8. Blower 9. Ozone contactor 10. GAC column 11. Ozonator

Influent water was pumped to a high level feed tank above the column and it was then percolated through the filter by gravitation. Compressed air was injected from the bottom of the column to maintain an effluent dissolved oxygen concentration of greater than 2 mg/L. The hydraulic loading rate through the column was controlled from 2 to 5 $m^3/m^2 \cdot h$, corresponding to empty bed retention time of 20 to 140 min. Backwashing was performed fortnightly. Raw water was fed to the reactor system at a temperature of around 10°C for about one and a half months prior to the start of the experiment so that natural microbial film could be developed onto the ceramic media. As shown in Fig. 1, coagulation, sedimentation, filtration and chlorination were also incorporated into the pilot-scale plant. 0.1% ferrous sulfate solution was used as coagulant with a dosage of 15 mg/L. Coagulation and sedimentation was conducted with a retention time of 15 and 30 min, respectively. Raw water was then filtered through a downflow filter (packed with 0.5-1.0 mm diameter media) with an effective depth of 0.7 m. The flowrate of filtration was designed at 10-12 m/h. To simulate the effect of chlorination, 1.5 mg/L NaOCl was added into the product water samples to achieve a residual free chlorine concentration of 0.40 mg/L after 0.5-hr reaction. The entire chlorination process lasted for 24 hrs. A separate conventional treatment system (consisted of only coagulation, filtration, and chlorination) was also operated to serve as a basis for comparison. In addition, pre-ozonation and GAC post-treatment were assessed together with the integrated biological treatment system. For GAC post-treatment, treated effluent was filtered through a downflow GAC column (filled with ϕ1.5×4 mm GAC media) with an effective depth of 0.9 m. The filtration rate through the GAC column was maintained at 8 $m^3/m^2.h$. For pre-ozonation treatment, a 4 m high glass column

was used as the ozone contactor which has a contact time of 20 min. A counter-current flow pattern was used where ozone gas was introduced from the bottom while raw water percolated from the top of the glass column.

Reservoir water and biofilter effluent were taken for Gas Chromatographic/Mass Spectrometric (GC/MS) scan and the Ames bioassay. GC/MS scan (HP5890) was used to identify the suspected mutagens present in treated effluent. The GC/MS operation conditions were as follows: Silica capillary column (SE-54): 25m*0.32mm; Temperature program: 40°C for 2 min, rising 3-5°C/min to 250°C, holding 3 min; Injection: 0.1 μL; Mass spectrometer: 70eV electron energy. The effluents of the conventional and the integrated bio-treatment systems with or without chlorination were taken respectively for Ames bioassay. *Salmonella microsomal* mutagenicity test was also conducted for biologically pre-treated water samples. Around 150 L water samples were acidified by H_2SO_4 and then pre-treated by XAD-2 resin adsorption, diethyl ether elution, and K-D concentrator evaporation. The solvent of the samples was exchanged into 2.5 mL dimethyl-sulfoxide (DMSO) to prepare concentrates used for the Ames test. The most commonly used tester strains for environmental samples testing, namely TA98 and TA100, were adopted in the Ames bioassay. Standard procedure without metabolic activation (-S_9 mix) using pour-plate method was adopted in this study. This is because the amounts of indirect mutagens, which can only be detected through metabolic activation, were found to be insignificant in the reservoir water (Hu et al. 1999a, 2000). The Ames bioassay was conducted in accordance with Standard Methods (APHA, 1999). Samples were assayed at doses equivalent to L of water per plate, using duplicate plates per dose. To avoid inhibition by high dosage of water sample, varying doses were applied to different samples. Mutagenic activity was expressed as a mutagenicty ratio (MR). Samples were considered to be mutagenic when MR values were two folds of spontaneous revertants and that a linear relationship between dosage and MR was found. MImax, a maximum mutagenic index (mutagenic ratio over water volume equivalent) was thus applied for comparison of mutagenicity of different water samples. Reservoir water and treated effluent were characterized by using Oxygen Consumed by permanganate (OC), Total Organic Carbon (TOC), phenols, and Oil & Grease (O & G) in accordance with Standard Methods (APHA, 1999).

RESULTS AND DISCUSSION

The characteristics of raw water are summarized in Table 1. It is noted that the highest O & G detected was 1.8 mg/L which occurred in the spring season when reservoir turnover would likely happen. The high O&G value suggested that there was a significant amount of oily pollutants present in the raw water during the study period. The relatively high concentrations of other parameters such as TOC, ammonia-nitrogen and phenol also implied the possibility of pollution in raw water.

Typical petroleum compounds present in the raw water were identified by GC/MS scan (data not shown). These compounds were mainly from the PAHs, phenols, alkanes and benzenes families. Among the identified compounds, PAHs and benzenes, which are typical petroleum hydrocarbons, represent the major contributors to health effect concern (Hu et al. 2000).

Table 1. Characteristics of reservoir water.

Parameters	T (°C)	Color (TCU)	NH_4^+-N (mg/l)	Phenol (mg/l)	O & G (mg/l)	TOC (mg/l)	Suspended solids (mg/l)
Results	0.5-19	7-15	0.007-1.71	<0.002-0.0163	0.3-1.8	11.5-13.5	2.0-10.0

The results of the Ames tests on reservoir water are summarized in Table 2. The results obtained in the summer season suggested that water samples contained substance(s) that would cause both frame-shift and base-pair substitution mutations (represented by TA98 and TA100) in testing bacteria DNA. It was noted that the reservoir water posed the strong mutagenic activity, with a MR value of 7.97 at a dosage of 2 L/plate, with respect to strain TA98. The results obtained also suggested that only frame-shift mutagens were detected in the spring water sample. This observation suggested that mutagenicity in reservoir water was probably due to the presence of polycyclic aromatic compounds as reported by Hu et al. (1999a). The lower mutagenic activity detected in spring season implied a seasonal variation of raw water quality.

Table 2. Ames test results of reservoir water.

	MR for TA98		MR for TA100	
Dosage (L/plate)	Spring	Summer	Spring	Summer
2.0	4.76	7.97	1.94	2.16
1.2	4.06	6.53	1.60	1.95
0.4	2.33	2.79	1.31	1.28
0.2	2.06	2.49	1.02	1.33
Correlation coefficient, R	0.982	0.980	-	0.984

The performance of the bio-ceramic pre-treatment for removing O&G in the raw water was meticulously observed in this pilot-scale study. The organic compounds possibly present in the effluent of biological pre-treatment unit were identified by GC/MS. The disappearance of some peaks in the chromatographic diagram implied that biological pre-treatment was able to degrade a wide range of organic compounds and this would help in the reduction of trace organic pollutants. The results also indicated that bio-treatment had converted some high molecular compounds (with high retention time) to lower molecular compounds (with lower retention time) which in turn suggested that some of the PAHs and phenols were reduced by bio-ceramic pre-treatment.

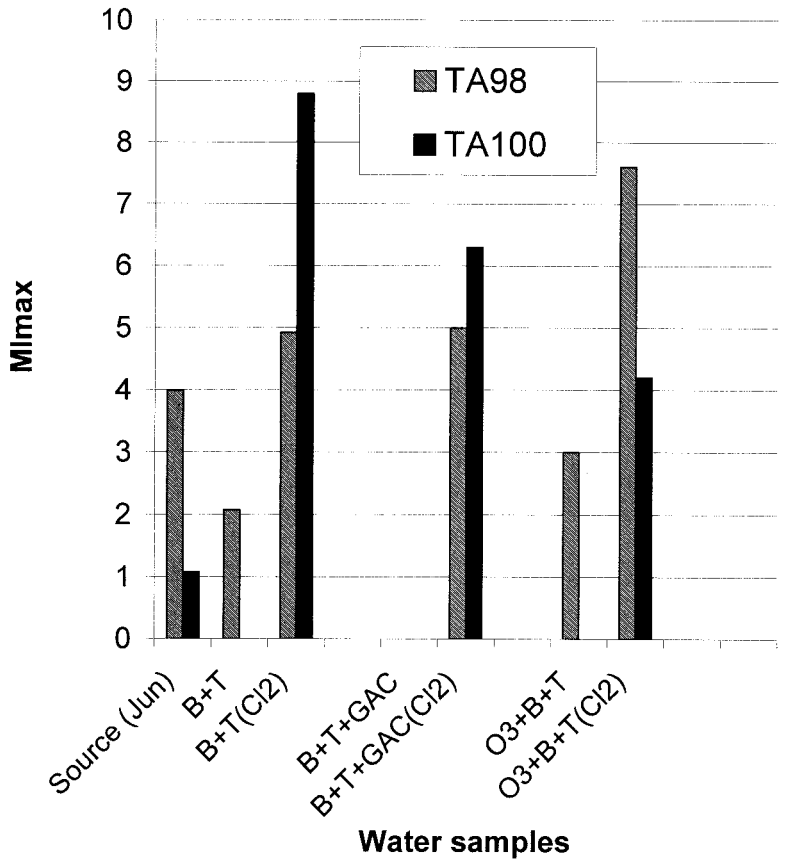

Figure 2. Comparison of mutagenicity of source water and the treated effluent in integrated bio-treatment system.

Note: B+T (Cl_2)—integrated bio-treatment with chlorination; B+T+GAC (Cl_2)—integrated bio-treatment with post GAC treatment with chlorination; O3+B+T (Cl_2)—integrated bio-treatment with pre-ozonation with chlorination.

These observations agreed with the findings reported by Voice et al. (1992) and Bouwer et al. (1992). They noted that alkylbenzenes (benzene, toluene, xylene) and polynuclear aromatic hydrocarbons (PAHs) were easily degraded by biological fluidized bed or biofilm column. In addition, Hu et al. (2000) also reported about 40% of oily contaminants could be reduced by bio-treatment when the influent oil concentration fluctuated from 0.2 to 2.2 mg/L. In an effort to assess the contribution of the bio-ceramic pre-treatment with respect to the quality of the final product water, the integrated biological treatment process was assessed. The effluent quality of the system was fairly good, with an average O & G of 0.12 mg/L. It was noted that the removal efficiency for O & G could be as high as almost 60% (Hu et al. 2000).

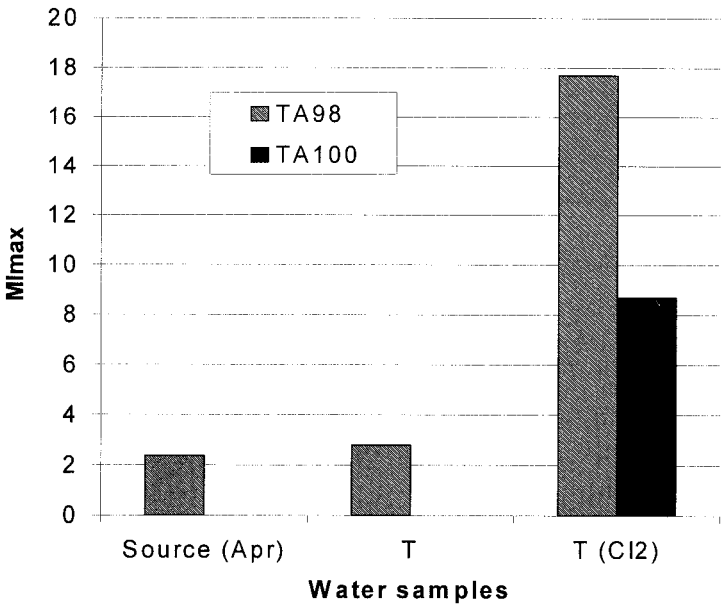

Figure 3. Comparison of mutagenicity of source water and the treated effluent in conventional treatment system.
Note: T (Cl_2)—conventional treatment with chlorination.

The mutagenicity of the effluent with and without chlorination was assessed by using the Ames bioassay, and compared with the corresponding values of the influent samples. The effect on mutagenicity reduction was also compared for both treatment trains used in this study. The integrated biological treatment processes either with pre-ozonation or post GAC treatment were also assessed by the Ames test in terms of their ability in mutagenicity reduction. The results shown in Fig. 2 suggested that only frame-shift mutagens were detected in the effluent water of the integrated bio-treatment system. The highest MR was 2.08 at a dosage of 1 L/plate. No base-pair mutagens were detected. This treatment system could reduce frame-shift mutagens in raw water by 47.9%. In contrast, the conventional treatment system could generate frame-shift mutagens with a MR of 2.81 at a dosage of 1 L/plate (Fig. 3). That is, the conventional treatment system alone could only reduce the mutagenicity by 18.1% which is much lower than that achieved by the integrated bio-treatment system. The latter observation obtained in this study was in agreement with findings noted in other pilot-scale applications of integrated bio-treatment process. However, the range of removal efficiencies on mutagenicity varied considerably from 18% to 62% (TA98) and 10% to 20% (TA100), dependent on the organics composition present in the raw water (Li 1992; Dai 1994).

Mutagens shown by TA98 and TA100 strains were found in both chlorinated effluents. As shown in Fig. 2, chlorinated effluent of integrated bio-treatment process posed two types of mutagens at a dosage of 0.5 L/plate; namely MRs of 2.46 for TA98 and 4.40 for TA100. The corresponding MR values for chlorinated effluent of conventional treatment process were 3.59 for TA98 and 4.35 for TA100 (Fig. 3). Thus, chlorinating effluent from integrated biological treatment system increased mutagenicity by 23.5% for TA98 and 714.8% for TA100. The residual precursors in the effluent could have attributed to the increased mutagenicity after chlorination. This findings contradicted to the claims reported in other studies that bio-ceramic pre-treatment had the advantage of eliminating both mutagenic matters and mutagenic precursors, and also prevent further mutagen production in the coagulation and chlorination processes (Takasaki et al. 1988). It was also noted in this study that the mutagenicity of chlorinated effluent of the conventional treatment system were increased by 517.6% for TA98 and 705.6% for TA100. The increased in mutagenicity associated with TA98 obtained from the latter treatment system was much higher than the corresponding value obtained from the former treatment system. This observation suggested that there was less frame-shift mutagenic precursors present in the integrated bio-treatment processes (i.e. former treatment system). The results obtained were in agreement with the findings noted in the Turin Water Treatment Plant studied by Carraro et al. (2000).

The large increase in mutagenicity after chlorination suggested that further polishing treatment such as GAC might be necessary for removing mutagen precursors prior to chlorination. In view of this, the pilot-plant study also explored the feasibility to reduce mutagenicity by using integrated biological treatment with either post GAC treatment or pre-ozonation (Fig. 2). Compared to integrated biological treatment alone, post GAC treatment train could generate effluent which showed no sign of mutagenicity. However, its chlorinated effluent still showed mutagenicity with respect to both strains although with a lower MR value for TA100. In contrast, the effluent without chlorination from integrated biological treatment with pre-ozonation would show more mutagenicity with respect to TA98. However, the chlorinated effluent showed some fluctuation with higher TA98 but lower TA100 mutagenicity. The reason for the inconsistency is not understood. The findings obtained from this study indicated that pre-ozonation or post GAC treatment could not fully eliminate the mutagenic precursors that in turn contributed to the mutagenicity once chlorinated.

The full-scale integrated bio-treatment system has been used to handle up to 4,000,000 m^3 water per day in China (Xu et al. 2001). It could significantly remove compounds such ammonia, COD, turbidity, precursors and phenol which could not be achieved by conventional treatment process alone. Dai (1994) noted that the operating cost of the integrated biological treatment process was 20-30% higher than the conventional treatment process alone. The magnitude of increased should be acceptable in view that the effluent quality will be significantly

improved when integrated bio-treatment process is adopted. In addition, it could greatly reduce the public health risk to the consumers.

REFERENCES

APHA, Standard Methods for the Examination of Water and Wastewater (20th Edition) (1999) American Public Health Association Washington D. C.

Arvin E, Jensen BK, Gundersen AT (1991) Biodegradation kinetics of phenols in an aerobic biofilm at low concentrations. Wat Sci Technol Kyoto 23: 1375-1384

Bouwer EJ, Chen CT, Li YH (1992) Transformation of a petroleum mixture in biofilms. Wat Sci Technol 26: 637-646

Carraro E, Bugliosi EH, Meuci L, Baiocchi C, Gilli G (2000) Biological drinking water treatment processes, with special reference to mutagenicity. Wat Res 34: 3042-3054

Dai ZC (1994) Study on the biological pretreatment in two typical micropolluted source water. Dissertation for PhD degree Tsinghua University Beijing China

Hu JY, Wang ZS, Ng WJ, Ong SL (1999a) Disinfection by-products in water produced by ozonation and chlorination. Environ Monit Assess 59: 81-93

Hu JY, Wang ZS, Ng WJ, Ong SL (1999b) The effects of water treatment processes on the biological stability of potable water. Wat Res 33: 2587-2592

Hu JY, Wang GF, Ong SL, Ng WJ (2000) Mutagenicity reduction by combined bio-ceramic process for polluted reservoir water treatment. Proc 10th Kyoto-KAIST-NTU-NUS symposium in environmental engineering pp.49-62 Sokcho Korea

Li YQ (1992) Study on biological pretreatment in drinking water treatment. PhD Dissertation Tsinghua University Beijing China

Shibata M, Nakamura K, Miyaji Y (1987) Biological decomposition of trace organic compounds. Wat Sci Technol 19: 417-427

Takasaki M, Sudo R, Nishimura O, Kim HY (1992) Simultaneous removal of nitrogen and THM precursor by developed submerged biofilm process for drinking water. Wat Sci Technol 26: 2021-2024

Takasaki M, Harada M, Sato A (1988) Evaluation of the NH_4-N and DOC removal efficiency by submerged biofilm process in tap water supply pretreatment system. Wat Sci Technol 20: 517-519

Voice TC, Pak D, Zhao X, Shi J, Hichey RF (1992) Biological activated carbon in fluidized bed reactors for the treatment of groundwater contaminated with volatile aromatic hydrocarbons. Wat Res 26: 1389-1401

Xu YT, Shi JL and Zhang M (2001) (eds.) Microbiological engineering for pollution control. Chemical Engineering Publisher Beijing