Environmental impacts of wastewater from low-rank coal handling processes in terms of mutagenicity and acute toxicity

T. Nakajima, K. Kuzumaki, H. Hasegawa, H. Takanashi & A. Ohki
Department of Bioengineering, Faculty of Engineering,
Kagoshima University, Kagoshima, Japan

Abstract

Two low-rank coals, a sub-bituminous coal (BA) and a lignite (LY), were treated by hot water extraction (HWE) and hydrothermal treatment (HTT), and the environmental impacts of water-soluble matters eluted from coal by the HWE and HTT processes were evaluated in terms of TOC, mutagenicity, and the acute toxicity against freshwater organisms. When HWE was performed at 80°C, the degree of TOC for LY was much higher than that for BA. However, for HTT, the two coals gave comparable TOC values in L/S ratio of both 100 and 3. The HWE and HTT eluents of two coals were assessed by the Ames mutagenicity assay with *Salmonella typhimurium* TA100 and TA98 strains, and no notable mutagenicity was observed in the presence or absence of metabolic activation. When the mutagenicity of the 300ºC-HTT eluent of LY was analyzed, no notable mutagenicity was also observed. For the HWE and HTT eluents of LY, the acute toxicity test was carried out by use of *Daphnia magna* and *Oryzias latipes*. When the 80°C-HWE eluent was tested, almost no toxicity was observed for *D. magna* and *O. latipes*. However, when the HTT eluents were examined, the toxicity increased as the elevation of the HTT temperature, and the toxicity of organic matters dissolved in the 350°C-HTT eluent was comparable to that of reference phenolic compounds. From the FTIR analysis of organic matters eluted in the HTT eluents, it is found that the toxicity is caused by the presence of aromatic compounds with hydrophilic substituents, such as carboxyl and hydroxyl groups.
Keywords: low-rank coal, wastewater, water-soluble organic matters, mutagenicity, ames assay, acute toxicity.

WIT Transactions on Biomedicine and Health, Vol 11, © 2007 WIT Press
www.witpress.com, ISSN 1743-3525 (on-line)
doi:10.2495/EHR070211

1 Introduction

In coal handling processes, there are many cases in which coal contacts with water. Various water-soluble hazardous matters may be eluted from coal into effluent in coal washing processes, storage pile exposed to rain and/or water spray, and transport in coal-water slurry. In previous studies, many organic compounds, such as phenols, polycyclic aromatic hydrocarbons (PAHs), and humic substances, have been detected in coal washing water [1], natural coal storage pile runoff [2, 3], simulated coal storage pile runoff [4-6], coal slurry transport wastewater [7-9], and wastewaters from hydrothermal treatment (HTT) of low-lank coals [10]. These wastewaters, if released into natural water, may have deleterious damage to aquatic ecosystem and human life. There have been only a few studies about the evaluation of environmental impacts, particularly mutagenicity for water-soluble organic matters eluted from coal [7, 11, 12].

In this study, an Indonesian sub-bituminous coal and an Australian lignite were subjected to HWE and HTT, the leaching of organic matters was measured by total organic carbon (TOC), and the environmental impacts of these water-soluble organic matters were evaluated in terms of mutagenicity and acute toxicity against some freshwater organisms.

2 Materials and methods

2.1 Coals

An Indonesian subbituminous coal (BA), and an Australian lignites (LY) were used in this study. Table 1 presents the analyses of these coals. The particle sizes of coals were under 149 μm (100 mesh).

Table 1: Analytical data of coal.

Coal	Proximate (wt%, dry base)			Ultimate (wt%, dry ash free)				
	I.M.	Ash	V.M.	C	H	N	O	O/C
BA	10.70	2.30	44.80	71.20	5.20	1.10	22.10	0.23
LY	24.90	0.60	39.80	69.57	5.00	0.60	26.20	0.28

I.M., inherent moisture; V.M., volatile matter.

2.2 Hot water extraction (HWE) procedure

Powdery coal (1.0 g) was placed in a filter paper thimble (ADVANTEC No. 84, i.d. 28mm x 100 mm), and the thimble and 100 ml of deionized water were set in a Soxhlet extractor (Buchi B-811). The procedure of HWE was the same as that described in our previous paper [11].

2.3 Hydrothermal treatment (HTT) procedure

The powdery coal was mixed with 200 ml water and the mixture was placed in an autoclave (Suzuki Rika Seisakusho 200H). The liquid per solid ratio (L/S) was 3, 20, and 100 in this study. The atmosphere in the autoclave was purged by N_2. The autoclave was heated at a rate of 3-4ºC/min to a desired temperature and the temperature was kept for 1 h. After cooling, the eluent was separated from coal by filtration, and analyzed.

2.4 Measurement of TOC

The determination of total organic carbon (TOC) in the eluents was conducted by catalytic oxidation followed by IR spectrometry for CO_2 measurement using an automatic TOC analyzer (Shimadzu TOC-V CSH).

2.5 FTIR analysis

The organic matters dissolved in the HWE and HTT eluents were freeze-dried into powdery form. The powdery organic matters (0.01 g) were mixed with KBr (1.0 g) in a motor with pestle and a portion of the resulting mixture was molded into a disc. The disc was analyzed by a FTIR spectrophotometer (JASCO FT/IR-420).

2.6 Mutagenicity assay

The organic matters dissolved in the HWE and HTT eluents were 100 times concentrated into a dimethyl sulfoxide (DMSO) solution by a solid phase extraction (SPE) method using an adsorbent cartridge (Sep-Pak Plus CSP-800, Nihon Waters) according to a literature [13]. The mutagenicity of the eluent was evaluated by the Ames *Salmonella* mutagenicity assay according to the authorized method [14]. Two *Salmonella typhimurium* strains TA100 and TA98, which were obtained from the National Institute of Public Health, Japan, were used. The preincubation method using TA100 and TA98 strains with or without metabolic activation by a rat liver homogenate S9 mix was employed. S9 mix was purchased from Oriental Yeast Co., Ltd. Dose-response tests were carried out using three dose-steps and one negative control (DMSO). Positive control tests were concurrently performed with a DMSO solution of 4-nitroquinoline-1-oxide (4NQO) for the -S9 condition and that of 2-aminoanthracene for the +S9 condition to confirm the activity of the strain. After incubation at 37°C for 48 h, colonies on the plate were scored, and the numbers of net revertant colonies per plate (net rev./plate) were obtained as the difference with negative control.

2.7 Toxicity test for freshwater organisms

In order to evaluate the acute toxicity of HWE and HTT eluents against freshwater organisms, *Daphnia sp*. acute immobilization test and fish acute toxicity test were conducted in accordance with the OECD guideline for testing of chemicals using *Daphnia magna* (OECD TG202) [15] and *Oryzias latipes*

(OECD TG203) [16]. The HWE eluent was produced by the 80ºC-HWE (L/S=100), while the HTT eluents were produced by the 200-350ºC-HTT (L/S=20). In *Daphnia sp.* acute immobilisation test, the adequately diluted eluents were exposed to *D. magna* for 48 h, and the concentrations of organic matters which produced 50% immobilization of *D. magna* (EC_{50}; mg-C/l) was calculated by probit analysis. In fish acute toxicity test, the adequately diluted eluents were exposed to *O. latipes* for 96 h, and the concentrations of organic matters which produced 50% mortality of *O. latipes* (LC_{50}; mg-C/l) was calculated by the same method.

3 Results and discussion

3.1 TOC in eluent

Two coals BA and LY were subjected to the HWE procedure, and the TOC and pH in the eluents were measured. In Table 2, the TOC values obtained are listed. The eluent of LY gave a higher TOC value (42.8 mg-C/l) than that for BA (13.3 mg-C/l) in the 80ºC-HWE. For pH value, considerably low pH was observed in the LY eluents. It is evident that the organic matters eluted from LY contain more acidic components, compared to that from BA in the HWE eluent.

When the 350ºC-HTT was performed in L/S=100 and 3, the results are also recorded in Table 2. For comparison with 80ºC-HWE eluents in the same L/S (100) condition, the 350ºC-HTT eluents of BA and LY gave much higher TOC values than the 80ºC-HWE eluents. For the HTT eluents, there is not so big difference between BA and LY in the degree of TOC. Practical HTT process is usually carried out in L/S=3-4. When the 350ºC-HTT was performed in L/S=3, the degree of TOC in the BA and LY eluents were 4220 and 5910 mg-C/l, respectively. Racovalis et al. [10] reported that LY produced 6000-7000 mg-C/l of TOC when 350ºC-HTT was carried out in L/S=3. The TOC values obtained in this study (L/S=3) is reasonable compared with their results. For the pH value of 350ºC-HTT eluent, almost same pH was observed in both of the eluents.

Table 2: TOC and pH in the HWE and HTT eluents.

Coal	Procedure	L/S	TOC (mg-C/l)	pH
BA	80ºC-HWE	100	13.3	6.6
	350ºC-HTT [a)]	100	489	-
	350ºC-HTT	3	4220	4.8
LY	80ºC-HWE	100	42.8	4.7
	350ºC-HTT [a)]	100	535	-
	350ºC-HTT	3	5910	4.1

a) From [12].

3.2 Effect of HTT conditions upon leaching

The effect of HTT temperature upon the degree of TOC in the HTT eluent was investigated, and the results are shown in Figure 1. When the 200ºC-HTT (L/S=3) was performed for LY, the TOC in the eluent was 440 mg-C/l, and the degree of TOC was increased as the temperature was raised. In case of BA, the degree of TOC was about the same as that for LY in the 200ºC-HTT eluent, and that was greatly increased as the temperature was raised from 300 to 350ºC. In our previous paper [17], we reported that the oxygenic functional group, such as COOH and OH presented in coal was diminished as the elevation of HTT temperature. Also, the autoclave pressure was greatly increased between 300 and 350ºC (from 96 to 180 kg/cm^2). It is proposed that the decomposition of these functional groups is greatly enhanced when the 350ºC-HTT is performed, and therefore the leaching of organic matters from coal is greatly increased.

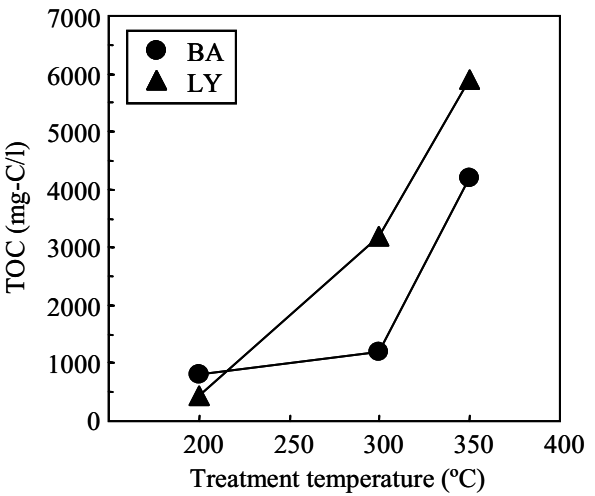

Figure 1: Effect of treatment temperature upon TOC in HTT eluent (L/S=3).

3.3 Ames *Salmonella* mutagenicity assay

The organic matters dissolved in the HWE eluent of coals were concentrated into DMSO by an SPE method [13]. The resulting DMSO solution was analyzed by the Ames *Salmonella* mutagenicity assay using TA100 and TA98 strains according to the authorized method [14]. To check the activity of the *Salmonella* strains used, positive controls using 4NQO for -S9 condition and 2-aminoanthracene for +S9 condition were performed, and the both TA100 and TA98 strains were adequately responsible to these mutagenic compounds.

In Figure 2, dose responses of the HWE eluents of BA and LY coals are indicated. For both of the eluents, there were almost no linear responses and no significant differences between the number of revertant colonies per plate for the maximum dose and that for the negative control, either in the presence or in the

absence of metabolic activation (S9). Therefore, it is evident that the HWE eluents of BA and LY coals do not give significant mutagenicity. When the mutagenicity of the 300ºC-HTT (L/S=100) eluent obtained from LY was analyzed, no notable mutagenicity was also observed.

Also, the effect of HTT temperature upon the mutagenicity of eluent was investigated under TA100-S9 conditions, when LY lignite was extracted by 200 to 350ºC-HTT (L/S=3). From the dose responses listed in Table 3, although a little larger number of revertant colonies was observed for 300 and 350ºC-HTT eluents compared to 200ºC-HTT eluent, no significant mutagenicity was detected. It is concluded that no notable mutagenicity was observed for either HWE or HTT eluents.

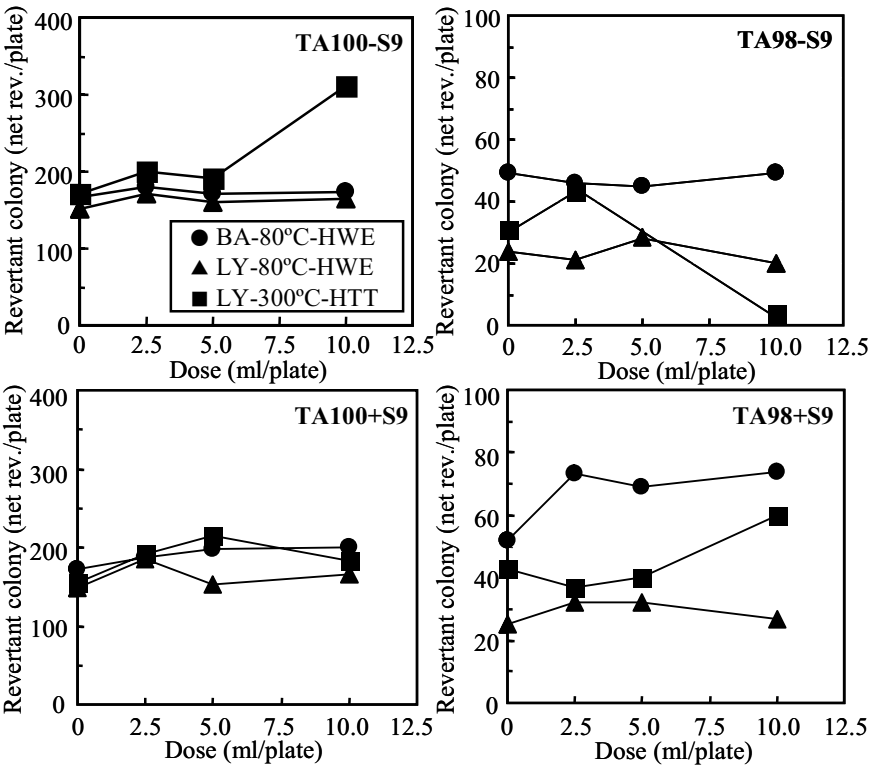

Figure 2: Ames mutagenicity assay of HWE and HTT eluents.

3.4 Toxicity test for fresh water organisms

When the HWE and HTT of coal were performed, not only organic matters but also inorganic matters will be leached out. When the concentrations of some toxic metals (As, B, Cd, Cr, Cu, Mn Pb, and Zn) in the HWE and HTT eluents obtained from LY lignite were measured, it was found that the concentrations of these hazardous metals were below the detection limits and the leaching of the

metals was negligible in the HWE and HTT eluents (data not shown). Hence, the acute toxicity of HWE and HTT eluent was evaluated only for the organic matters in the eluents.

Table 3: Effect of HTT temperature upon mutagenicity of HTT eluent (L/S=3)[a].

Coal	Dose (ml/plate)	Number of revertant colony (net rev./plate)		
		200ºC	300ºC	350ºC
LY	0 (Control)	149	149	149
	2.5	146	172	169
	5.0	165	194	167
	10.0	168	228	220

a) Mutagenicity was assayed under TA100-S9 conditions.

Table 4: Acute toxicity test of HWE and HTT eluent for *D. magna* and *O. latipes*.

Test sample	*D. magna*	*O. latipes*
	48 h-EC_{50} (mg-C/l)	96 h-LC_{50} (mg-C/l)
80ºC-HWE eluent	a)	a)
200ºC-HTT eluent	14.6	66.5
300ºC-HTT eluent	13.2	21.6
350ºC-HTT eluent	5.7	7.6
Phenol	15.8	25[b]
p-Cresol	8.5	14[b]
Catechol	1.2[b]	3.5[b]
4-nonyl phenol	0.059[b]	0.24[b]

a) Practically non-toxic.
b) From [18].

Toxicity tests were carried out using *D. magna* and *O. latipes* as test organisms for characterizing the HWE and HTT eluents of LY. In Table 4, the 48 h-EC_{50} value for *D. magna* and the 96 h-LC_{50} value for *O. latipes* were listed, when the 80ºC-HWE and 200 to 350ºC-HTT eluents are examined. It has been reported that wastewaters obtained from low-rank coal handling processes

contain various phenolic compounds [5, 6, 19]. Therefore, the EC_{50} and LC_{50} value of four reference phenolic compounds which may present in the HWE and HTT eluent were also listed in Table 4. The EC_{50} and LC_{50} value obtained from each assay were described as mg carbon per litter (mg-C/l).

When the HWE eluent was tested, almost no acute toxicities were observed for either *D. magna* or *O. latipes*. For the HTT eluents, significant acute toxicities which were comparable to those of the reference phenolic compound were detected for each organism. The 48 h-EC_{50} values for *D. magna* decreased in the order, Phenol ≈ 200ºC-HTT eluent > 300ºC-HTT eluent > *p*-Cresol ≈ 350ºC-HTT eluent > Catechol > 4-nonyl phenol. The 96 h-LC_{50} values for *O. latipes* were also decreased in about the same order.

The organic matters dissolved in the HWE and HTT eluents were freeze-dried, which were analyzed by FTIR, and aromatic ring peak (ca. 1610 cm^{-1}), aliphatic and aromatic carboxyl peak (ca. 1750 and 1710 cm^{-1}, respectively), and hydroxyl peak (ca. 3450 cm^{-1}) were observed in the FTIR spectrum. As shown in Figure 3, the organic matters dissolved in the HWE eluent gave aromatic ring peak and weak aliphatic carboxyl peak. For the organic matters form HTT eluents, aromatic ring peak and the aromatic carboxyl peak was found in their spectrum, and the intensity of the aromatic carboxyl peak was increased as the elevation of HTT temperature. These results suggest that the organic matters dissolved in the HTT eluents under higher temperature conditions possess more aromatic carboxyl groups. Consequently, the HTT eluents under higher temperature conditions give more toxicity provably due to the elution of toxic aromatic compounds with hydrophilic substituents, such as carboxyl and hydroxyl groups.

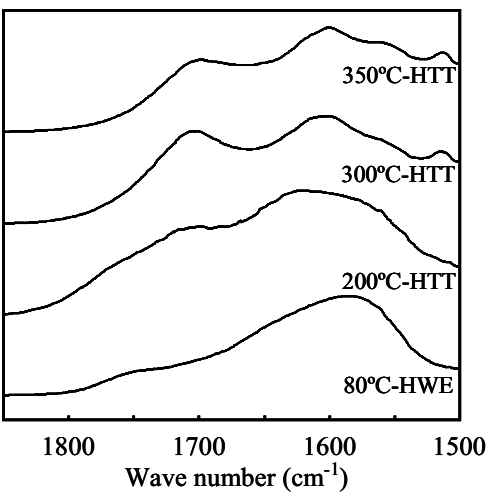

Figure 3: FTIR spectra of organic matters dissolved in HWE and HTT eluent of LY lignite.

4 Conclusions

A sub-bituminous coal BA and a lignite LY were subjected to the HWE and HTT processes, and the leaching of organic matters from coal was evaluated by TOC. When the HTT process was carried out, much higher TOC was obtained compared to HWE, and the degree of TOC increased as the increase of HTT temperature. When the HWE and HTT eluents of two coals were assessed by the Ames mutagenicity assay, no notable mutagenicity was observed in the presence or absence of metabolic activation. The 300ºC-HTT eluent of LY also gave no notable mutagenicity. For the HWE and HTT eluents of LY, the acute toxicity test was carried out. When the 80°C-HWE eluent was tested, almost no toxicity was observed. However, when the HTT eluents were examined, the toxicity increased as the elevation of the HTT temperature. From the FTIR analysis of organic matters eluted, it is found that the toxicity is caused by the presence of aromatic compounds with hydrophilic substituents, such as carboxyl and hydroxyl groups.

References

[1] Berrueta, A., Fernandez, L.A., Vicente, F., Fluorescence study of the solubilization of benzo [a] pyrene: application to its detection in coal washing waters. *Anal. Chem. Acta.*, 243 (1), 115-119 (1991).

[2] Atwater, J.W., Jasper, S.E., Parkinson, P.D., Mavinic, D.S., Organic contaminants in Canadian coal wastewaters and associated sediments. *Water Pollution Research Journal of Canada*, 25 (2), 187-200 (1990).

[3] Curran, K.J., Irvine, K.N., Droppo, I.G., Murphy, T.P., Suspended solids, trace metal and PAH concentrations and loadings from coal pile runoff to Hamilton Harbour, Ontario. *J. Great Lakes Res.*, 26 (1), 18-30 (2000).

[4] Stahl, R.G., Liehr, J.G., Davis, E.M., Characterization of organic compounds in simulated rainfall runoffs from model coal piles. *Arch. Environ. Contam. Toxicol.*, 13 (2), 179-90 (1984).

[5] Fendinger, N.J., Radway, J.C., Tuttle, J.H., Means, J.C., Characterization of organic material leached from coal by simulated rainfall. *Environ. Sci. Technol.*, 23 (2), 170-177 (1989).

[6] Fendinger, N.J., Radway, J.C., Tuttle, J.H., The effect of environmental storage conditions on the organic content of simulated coal leachates. *Arch. Environ. Contam. Toxicol.*, 19 (2), 249-256 (1990).

[7] Reid, M.C., Davis, J.W., Sayler, G.S., Minear, R.A., Dissolved organic carbon of coal slurry transport water. *Water Res.*, 19 (19), 1199-1203 (1985).

[8] Reid, M.C., Davis, J.W., Minear, R.A., Sayler, G.S., Fulvic acid constituents of coal slurry transport wastewater. *Water Res.*, 22 (1), 127-131 (1988).

[9] Sayler, G.S., Minear, R.A., Reid, M.C., Davis, J.W., Enhanced reuse potential of coal slurry transport water: Toxic organics assessment and removal. *PB Rep. PB-84-230432*, p.75 (1983).

[10] Racovalis, L., Hobday, M.D., Hodges, S., Effect of processing conditions on organics in wastewater from hydrothermal dewatering of low-rank coal. *Fuel*, 81 (10), 1369-1378 (2002).
[11] Nakajima, T., Kanda, T., Fukuda, T., Takanashi, H., Ohki A., Characterization of eluent by hot water extraction of coals in terms of total organic carbon and environmental impacts. *Fuel*, 84 (7-8), 783-789 (2005).
[12] Nakajima, T., Nakamata, S., Takanashi, H., Ohki, A., Bioassay for environmental impacts of water-soluble matters eluted from coal. Proceedings of the International Conference on Coal Science and Technology, Okinawa, Japan, paper No. 2P504 (2005).
[13] Takanashi, H., Urano, K., Hirata, M., Hano, T., Ohgaki, S., Method for measuring mutagen formation potential (MFP) on chlorination as a new water quality index. *Water Res.*, 35 (7), 1627-1634 (2001).
[14] Ministry of Labor Japan, Mutagenicity assay for the occupational safety and health act. Test guideline and GLP, Occupational Health Service Center, Tokyo, (1991), (in Japanese).
[15] OECD, Test guideline 202, *Daphnia sp.* Acute immobilisation test and reproduction test (adopted April 4, 1984), In section 2 Effects on biotic systems, OECD guidelines for the testing of chemicals, Paris, (1984).
[16] OECD, Test guideline 203, Fish acute toxicity test. In section 2 Effects on biotic systems, OECD guidelines for the testing of chemicals, Paris, (1992).
[17] Xie, X.-F., Ohki, A., Morimatsu, K., Nakajima, T., Oda, H., Maeda, S., Surface properties of coals upgraded by hydrothermal treatment. *J. Jpn. Inst. Energy*, 77 (6), 478-484 (1998).
[18] Chemicals Evaluation and Research Institute, Japan, Hazard data book of existing chemicals, available from web site, http://www.cerij.or.jp/db/sheet/hazard_databook.htm.
[19] Yukawa, K., Hirashima, T., Tsunekawa, M., Suyama, T., Sasaki, K., Fukushima, M., Kobayashi, H., Hirai, T., Characterization of liquid products obtained from hot water-drying treatment of low rank coal and their ability of Cr(VI) reduction. *Shigen to sozai*, 119 (4-5), 161-169 (2003), (in Japanese).