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Understanding biotoxicity for reusability of municipal solid waste incinerator (MSWI) ash

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Abstract

This feasibility study using *Escherichia coli* DH5α as a reporter microorganism tended to disclose toxicity ranking of various ashes of municipal solid waste incinerator (MSWI) in comparison with typical toxic chemicals for reusability in further applications. Previous study indicated that growth inhibition to bacterial cells occurred at concentrations above 0.156, 0.625 and 0.0195 g/L for bottom ash (BA), cyclone ash (CA), scrubber ash (SA), respectively, suggesting the toxicity ranking of SA > BA > CA. This follow-up study clearly stated that compared to cadmium(II) and chromium(II) SA seemed to be the most toxic species to DH5α. Large amounts of supplemented lime (CaO) were used for neutralization of acid gas in incinerator, SA was thus contained high-levels of sulfate, chloride and nitrate salts. Therefore, compared to other ashes a marked increase in toxicity was observed in SA. Regarding soluble cations and anions in ashes, nitrite ion seemed to stimulate instead of repress cell growth. In contrast, nitrate ion showed so-called "sufficient challenge" characteristics for growth enhancement and inhibition at low and high concentration, respectively. Low solubility of metallic ions (e.g., Pb(II) and Cu(II)) in ashes likely resulted in low mobility in the environment and low risk to humans. The findings showed that toxicity attenuation of SA will be inevitably required as SA is even more toxic than Cr(II) and Cd(II).

Keywords: MSWI ash; Toxicity; Dose–response curve

1. Introduction

According to Environmental Protection Administration (EPA) in Taiwan, approximately 28% of municipal refuse collected in Taiwan was treated by incineration in 1999. It was also estimated that 90% of municipal refuse will be treated via incinerated by 2011 [1]. Thus, incineration will become a major refuse disposal method in Taiwan. The municipal solid waste incinerator (MSWI) residues were reduced by 80–85% (w/w) and contained about 3–7% fly ash as well as heavy metals. Municipal solid waste (MSW) basically composed of an organic fraction, an inorganic fraction and moisture. The organic fraction is primarily lignocellulosic material, a potential source for energy recovery. Approximately, 85% of the moisture-free MSW is combustible or convertible to liquids [2]. However, to a highly populous and small island country (e.g., Taiwan), the worst is that the volume of MSW generated increases as a result of an increase in population and a marked rise in living standards. Thus, MSWI is usually one of most viable alternatives in place of landfill disposal, since MSWI considerably reduced the volume and weight of solid waste by 90% and 70%, respectively [3]. Additional benefit is that the waste-to-energy systems have been incorporated into MSWI management programs for energy recovery. In the populous Taiwan, finding appropriate sites for landfill is extremely not easy, as the population size significantly augmented for decades [4]. Furthermore, the MSWI residues in reuse may still contain high-level toxic substances (e.g., heavy metals and augmented chemicals), leading to a persistent threat to the environment. Apparently, post-treatment of MSWI residues must be carried out to ensure safety to humans. In Taiwan, landfill is still the most frequently used method to deal with solid waste up to now. However, in the populous Taiwan the persistent need to construct incinerators will significantly increase due to difficulties to obtain appropriate sites for landfill [4]. Thus, how to seek economically feasible ways to recycle

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and/or reuse the remaining waste is evidently the first priority issue prior to further treatment of MSWI residues.

As MSWI residues (e.g., fly ash, scrubber ash) often contain high levels of leachable heavy metals and salts, they are usually classified as hazardous wastes worldwide. Hence, ashes must be appropriately treated by means of wastes intermediate treatment process (e.g., melting [5,6] and sintering [7]) prior to recycle and reuse. Basically, typical MSWI residues contain chemical compositions of Si, Al, K, Ca, Fe, Mg and Na. For safe reuse and/or disposal, all compositions of MSWI residues must achieve the criteria of EPA's regulation to be termed as environmentally friendly and ecologically sound materials to be reused. Thus, owing to the specific characteristics of MSWI residues, recycle and reuse of residues as the construction materials should be more promising to further treatment. For instance, as a result of the similar physical and chemical characteristics between the bottom ash and the nature gravel, reuse of the bottom ash from MSWI residues has become very popular [8–11]. For example, much attention on reuse of MSWI fly ash has been paid for production of cement mortars [12,13], concrete mixtures [14] and fine aggregate in mortars [15].

In the view of human risk and toxicity, the leaching of heavy metal and/or other toxic chemicals from residues will become a rank one problem prior to applications. In Taiwan, up to now toxic characteristic leaching procedures (TCLP) has been adopted as a typical analytical method to inspect the concentration of leaching heavy metals. However, due to a lack of assessment guideline (e.g., risk and toxicity evaluation) to guarantee the long-term safety of remaining leachable heavy metals and slats, whether MSWI residues are feasible for safe reuse is still remained uncertain. To reveal the feasibility of utilizing MSWI ash residues for reuse as construction material (as mentioned before), the previous first attempt in biotoxicity assessment on model ashes was introduced to present a quantitative measure for the practicability of ash reuse in practice. This model MSWI ash used was collected from the cyclone of a mass-burning incinerator located in Taipei county, northern Taiwan.

Obviously, without adequate toxicity figures of MSWI residues, reusability of ashes *is* unsafe and unreliable for further applications. Previous study [16] provided a first attempt to show the toxicity rankings of various ashes of MSWI. This follow-up study tended to evidently uncover the ranking of quantitative toxicity of various ashes compared to essential toxic chemicals as a guideline criterion to show the feasibility of ashes for reuse and recycling afterwards. As a matter of fact, Chen et al. [17] and Chen [18] provided a model attempt from a toxicological perspective to put forward the toxicity ranking of metallic ions to *Pseudomonas aerugina* PU21 (Rip64) and toxicity series of aromatic amines to *Pseudomonas luteola* for bioremediation. Similar perspective [19] was adopted to reveal to explore whether there exists a noteworthy change in combined biotoxicity of phenol to *Ralstonia taiwanesis* in the presence of other carbon sources during biostimulation for bioremediation. However, whether this assessment is viable to be used in practice on site still remained open to be discussed. From practicality perspectives, this study tended to employ such aspects in pursuit of *toxicity series* of *various* MSWI ashes as a guideline for toxicity attenuation prior to on-site and in situ applications. Thus, how to significantly attenuate toxicity from safety perspectives in reuse will be the first priority issue in the follow-up study. Apparently, our toxicity assessment is more economically viable compared to Microtox®, since DH5α cells were much easier to be cultivated on agar-plates to determine percentage cellular viability for toxicity in the tested population.

2. Materials and methods

2.1. Materials

The municipal solid waste incinerator ash used was collected from the mass-burning incinerator located in Taipei County, Taiwan. The incinerator, capable of processing approximately 1350 metric tonnes of local municipal solid waste per day, is equipped with air pollution control devices (APCD) consisting of a cyclone, an adsorption reactor and a fabric baghouse filter. The tested materials (e.g., three residual MSWI solid ashes and chemicals) were obtained as follows:

(1) Cyclone ash: The incinerator systems were equipped with cyclone separators that employed inertial forces to separate particles (i.e., cyclone ash) down to approximately 5 μm in size.
(2) Scrubber ash: The semi-dry systems have introduced into lime slurry, activated carbon and diatomaceous earth, and removed acid gas from the gas stream. The scrubber ash was collected from the baghouse filter systems.
(3) Bottom ash: The procedure was performed with water-quenched bottom ash taken from a MSWI. The ash was screened and magnetically separated to remove its coarse non-ferrous impurities and ferrous substances. The cyclone ash, scrubber ash and bottom ash were individually dried at 105 °C for 24 h until a constant weight (ASTM D 2216) was reached (i.e., almost moisture-free), and then the chemical compositions were characterized.
(4) Chemicals $CdCl_2$ (>99%, A.C.S. reagent), $CrCl_2$ (anhydrous powder, 99.99%), $NaNO_3$ (>99.0%, A.C.S. regent) and $NaNO_2$ (>97%, A.C.S. reagent) were all purchased from Aldrich.

2.2. Analytical methods

2.2.1. Chemical analysis

MSWI cyclone ash, scrubber ash and bottom ash were used for analysis by means of ion chromatograph (IC) and chemical composition determination as follows: chemical composition determination was conducted by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Kontron, S-35). Two-gram predried samples were digested with ultra pure grade reagents through a three-step procedure to release all soluble chemicals: a concentrated hydrofluoric/nitric acid mixed at a ratio of 5 mL/5 mL was added to the sample; after evaporation, a 3 mL/9 mL mixture of concentrated nitric/hydrochloric acid was

added; after another evaporation, the samples were dissolved in a 5% nitric acid solution.

IC was employed to analyze anions via Metrohm 761 Compact IC. In addition, Metrosep SUPP3 column was used in IC with a flow rate of 1.0 mL min^{-1} and suppressed conductivity mode detection settings at a system pressure of 12.9 MPa.

2.3. Microorganism and culture condition

Escherichia coli DH5α (generously provided by Professor Jo-Shu Chang, NCKU, Taiwan) was used as a probing strain for biotoxicity assessment. A loopful of the indicator strain seed taken from an isolated colony in LB-streak plate was precultured in 50 mL Luria–Bertani medium (LB broth, Miller, Difco) for 12 h at 37 °C, pH 7.0, 200 rpm. To ensure the synchronous growth activity and maximum metabolic functioning in the same growth phase for bioassay, 5% (v/v) cultured broth was then inoculated to fresh sterile LB medium and a cell culture was harvested at approximately mid-exponential growth phase (ca. 4 h) for further toxicity assessment. The 1.0 mL cell culture was then serially diluted with 9.0 mL sterile saline solution (SSS; NaCl 10.0 g L^{-1}) and only the diluent with appropriate cell concentrations (ca. 1500–15,000 cells/mL) was chosen as the test seed (TS) for later uses.

2.4. Biotoxicity assessment

Biotoxicity assessment was specially designated through a modification of dose–response analysis [17–19] as follows: the sampled chemicals (i.e., $NaNO_3$, $NaNO_3$, $CrCl_2$, $CdCl_2$) were first sterilized via moist-heat method (121 °C at 15 psi for 20 min) to exclude the presence of unwanted bacterial contaminants. The concentration of samples defined herein was the concentration of tested chemicals and their serial diluents well mixed with sterile saline solution (SSS; $NaCl_{(aq)}$ 10.0 g L^{-1}). It was postulated that the sodium chloride solution used for serial dilutions of various anions and cations was the toxicity-free control. It was also assumed that no marked interactive relationship between sodium cation and chloride anion, both ionic species were assumed as non-toxic background ions. For example, the toxic effects of nitrate and cadmium ion were studied in the presence of sodium ion (e.g., $NaNO_3$) and chloride ion (e.g., $CdCl_2$), respectively. Note that to exclude confounding interferences phosphate buffered saline (PBS) solution, which is regularly used for biological assay, was not used here, since metallic phosphate precipitates might be formed in serial dilution. The initial concentration C_0 for toxicity tests of all chemicals was chosen at ca. 20.0 g L^{-1}. Serial-half dilution of initial concentration C_0 (i.e., $1/2C_0$, $1/4C_0$, $1/8C_0$, $1/16C_0$, $1/32C_0$, ..., $1/2^nC_0$) was carried out by using 50 mL ash solution or its derived diluents mixed with 50 mL SSS. The 9.0 mL resulted serial diluents (RSD) were all placed in sterile test tubes for use in quantification of viable cells afterwards. The 1.0 mL freshly harvested TS was then well shaked with RSD ca. 20 times through a 35-cm arc elbow motion to form serial plate count diluents (SPCD). Meanwhile, 1.0 mL fresh TS mixed with 9.0 mL pure SSS was used as the toxicity-free control. The numbers of survival bacterium (i.e., *E. coli* DH5α) in SPCD or the control were estimated by the standard plate count method [20]. Standard plate count in LB medium was carried out as follows: SPCD were serially diluted with SSS immediately after sampling, and then appropriate volumes (ca. 0.20 mL) of SPCD were spread onto agar *Petri* plates. Note that all cells in SPCD would be assumed metabolically viable and culturable on LB-medium plates [21] due to fresh preparation of fast-growing *E. coli* cells in all steps. The LB-medium plates were then incubated at 37 °C for ca. 16–24 h to form observable colonies for enumeration. Plates with between 30 and 300 colonies are statistically appropriate for counting. Serial dilution-agar plating procedures were carried out at least in duplicate for quality assurance and control (QA/QC). The microbial population in the original RSD can then be calculated using the following formula (CC: cell count):

$$\text{cells per liter of broth (CC)} = \frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution factor}}$$

To have quantitative toxicity for comparison, CC_0 was chosen as the CC at zero-toxicity control. The ratio CC/CC_0 of 0 and 1 directly indicated complete inhibition and no inhibitory toxicity to bacterial cell, respectively. The unity of this ratio simply suggests that the present toxicity of this diluent at this concentration is nearly equal to the toxicity of SSS (i.e., "zero" toxicity). The concentration range for the ratio jumped from 1.0 to 0.0 in dose–response curves [16] is defined here as the "threshold toxicity" (TT) range. The comparison on TT range could provide an obvious diagram of toxicity ranking for various chemicals. For example, if the TT range for chemical A is much less than that for chemical B, chemical A is inevitable much more toxic than chemical B and this indicated that much higher dilution factor must be carried out for chemical A in order to have "zero" toxicity as the same as control (SSS).

3. Results and discussion

3.1. Characterization of MSWI cyclone, scrubber and bottom ash

The chemical composition of the MSWI cyclone ash is shown in Fig. 1. SiO_2, CaO and Al_2O_3 comprised 28.3%, 22.8% and

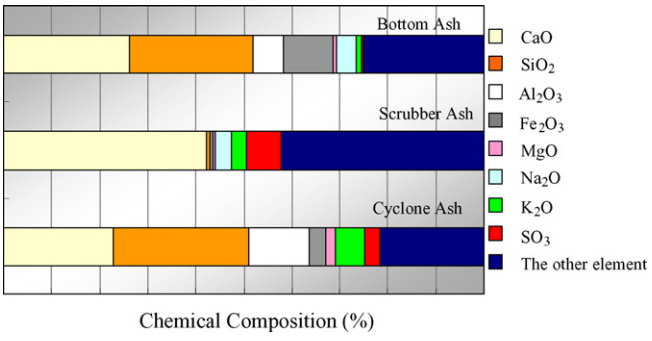

Fig. 1. Chemical composition of MSWI cyclone ash, scrubber ash and bottom ash (analyzed by ICP-AES after $HF/HClO_4/HNO_3$ digestion).

Table 1
Chloride ions and anions of MSWI cyclone ash, scrubber ash and bottom ash

	Cyclone ash	Scrubber ash	Bottom ash
Pb (mg kg^{-1})[a]	650 ± 14.8	1229 ± 84.1	1230 ± 33.2
Cd (mg kg^{-1})[a]	53 ± 1.7	130 ± 5.9	93 ± 0.6
Cr (mg kg^{-1})[a]	274 ± 24.6	26 ± 1.7	248 ± 12.4
Cu (mg kg^{-1})[a]	850 ± 89.1	740 ± 13.8	1130 ± 47.9
Zn (mg kg^{-1})[a]	5540 ± 163.0	7780 ± 256.0	8210 ± 184.0
Nitrite (mg L^{-1})[b]	542.6 ± 3.7	1563.7 ± 23.9	1041.3 ± 4.9
Nitrate (mg L^{-1})[b]	1168.1 ± 26.1	24.5 ± 1.0	210.5 ± 2.6
Chloride (mg L^{-1})[b]	222.7 ± 6.4	21050 ± 62.9	32000 ± 1000

Mean ± standard deviation (n = 5).
[a] Analyzed by ICP-AES after HF/$HClO_4$/HNO_3 digestion.
[b] Analyzed by IC.

12.6%, respectively. The next most abundant components were K_2O and Fe_2O_3, contributing ca. 6.1% and 3.1%, respectively. As indicated in Table 1, the fingerprint speciation of the cyclone ash, identified by IC techniques, revealing that the major anions were nitrite, nitrate and chloride comprised 542.6, 1168.1 and 222.7 mg L^{-1}, respectively.

Since a lime slurry was introduced into the semi-dry system to removal of both HCl and SO_2, the most abundant component in the MSWI scrubber ash (Fig. 1) was CaO in 42.3%. The next plentiful components are SO_3, Na_2O and K_2O contributing about 7.3%, 3.0% and 3.5% (w/w), respectively. The main anions were nitrite, nitrate and chloride comprised 1563.7, 24.5 and 21,050 mg L^{-1}, respectively.

As known, MSWI bottom ash was composed of equal amounts of fine ash materials and melted components in which half has crystallized, small quantities of metallic components, ceramics and stones. In addition, the major components of the MSWI bottom ash (Fig. 1) are comparable in concentration with igneous rocks and the predominant compositions SiO_2, CaO, Fe_2O_3 and Al_2O_3 comprised 25.8%, 26.1%, 10.3% and 6.3%, respectively. The next most abundant components are Na_2O, K_2O and MgO at about 3.9%, 1.1% and 0.8%, respectively. The major anions were nitrite, nitrate and chloride comprised 1041.3, 210.5 and 32,000 mg L^{-1}, respectively.

Table 1 listed top five most abundant metal species in various ashes (i.e., Zn, Cu, Pb, Cr, Cd). It indicates that metals with higher vapor pressures (e.g., Pb, Zn) have a tendency towards penetration into the fly ash. On the other hand, metals with higher boiling points (e.g., Cu) still primarily remained in the bottom ash. In addition, heavy metals could react with chlorine, sulfur and oxygen during incineration and subsequently generate diverse compounds. Apparently, chlorine had a significant effect on the characteristics of these metals [22]. Assuming the most stable forms of metallic ions were present in ashes, for risk assessment on various ashes prior to practical reuse the solubility products of these metallic hydroxides were firstly to be considered (i.e., K_{sp} of $Zn(OH)_2$, $Cu(OH)_2$, $Cr(OH)_3$ and $Cd(OH)_3$ are 3.0×10^{-16}, 4.8×10^{-20}, 1.6×10^{-30} and 4.5×10^{-45}, respectively; [23]). Note that it was assumed that all the solubility products of the compounds were reasonably held under the conditions for inspection. For ash reuse at neutral pH, the solubilities of Zn(II), Cu(II), Cr(III) and Cd(II) were 0.03, 4.6×10^{-6}, 1.6×10^{-14} and 0.45 M, respectively, indicating the harmless characteristics of Cu(II) and Cr(III) in environment due to their low solubility. In contrast, the most common state of lead (i.e., plumbous ion, Pb^{2+}) in aqueous solution is a weak acid according to the reaction

$$Pb^{2+} + H_2O = PbOH^+ + H^+, \qquad K \cong 10^{-8}.$$

That is, when an alkali is added to the Pb(II) solution for neutralization, $Pb(OH)_2$ precipitates and thus Pb(II) is termed insoluble as well. Although zinc was the most abundant species (e.g., 8210 mg/kg in bottom ash), zinc is usually harmless due to significant bioaccumulation and tolerance to organisms [17,24]. According to these evaluations, we intentionally selected chromium and cadmium as *typical predominant toxic* species for assessment.

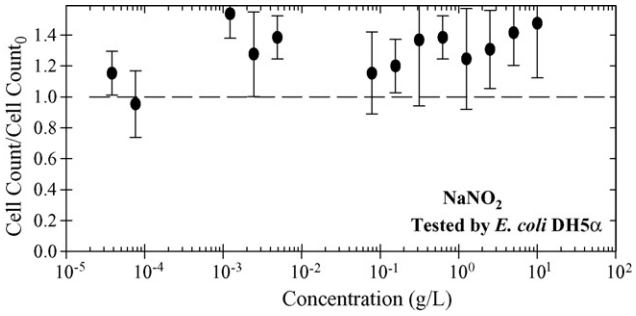

Fig. 2. Dose–response curve of sodium nitrite using *Escherichia coli* DH5α as the indicator microorganism.

3.2. Toxic effects of nitrite ion

As shown in Fig. 2, compared to the control $NaCl_{(aq)}$ there was a marked rise (ca. 20–30%) in cell viability in the presence of nitrite ion. It is noted that large variations in data were very likely due to serial diluents in non-buffered saline ($NaCl_{(aq)}$) used as described before (M&M). As nitrite is a conjugate base to weak acid HNO_2, the characteristics of nitrite ion might play a crucial role to enhancement of cell growth. During cellular growth, *E. coli* would metabolically produce some weak acids ΣHA_i (e.g., by-products acetic acid and/or citrate, oxaloacetate in tricarboxylic acid cycle; [25]) as shown in the following proposed reaction:

$$X + S \xrightarrow{\text{cells}} \sum_i HA_i + nX \leftrightarrow H^+ + \sum_i A_i^- + nX, \qquad (1)$$

where X, S, HA_j and A_j^- denoted *E. coli* cells, substrate, produced acid species j and its conjugate base during cell growth, respectively. This side reaction for acid formation during cell growth led to gradual decreases in pH values. According to LeChatelier's principle, if a system at equilibrium is subjected to a disturbance or stress that changes any of the factors that determine the state of equilibrium, the system will reach in such a way as to eliminate the effect of this introduced disturbance. This implied that supplementation of nitrite ion as a base is favorable to enforce removal of proton H^+ and generation of conjugate bases A_j^- and cells (i.e., growth enhancement). For example, if

a side-product acetic acid is generated during cell propagation as follows [23]:

$$CH_3COOH \rightarrow H^+ + CH_3COO^-, \qquad K_a^{HOAc} = 1.8 \times 10^{-5}. \tag{2}$$

Moreover, the acid dissociation constant of nitrous acid is

$$HNO_2 \rightarrow H^+ + NO_2{}^-, \qquad K_a = 4.5 \times 10^{-4}. \tag{3}$$

Thus, the resulted equilibrium constant of the reaction apparently confirms this possibility as follows:

$$CH_3COOH + NO_2{}^- \rightarrow HNO_2 + CH_3COO^-,$$
$$K = K_a^{HOAc}/K_a = 0.0396. \tag{4}$$

As cell growth is autocatalytic in that the more cells you have, the greater the growth rate (i.e., Eq. (1)). Thus, any extracellular driving force (e.g., an addition of a weak base) to create an environment of neutral and/or slightly basic pH (i.e., optimal growth pH to cells) would be spontaneous and thermodynamically favorable. These all pointed out why nitrite ion simply showed the stimulating rather than inhibitory characteristics to cell growth; of course nitrite ion was thus not the possible source of toxicity present in ashes [16].

3.3. Toxic effects of nitrate ion

As revealed in Fig. 3, the toxicity potency of nitrate ion seemed to express in a complex manner. Low level nitrate ion (ca. <0.01 g L^{-1}) assisted significant increase (ca. 20–40%) in cell growth. Approximately, ranged from 0.01 to 0.1 g L^{-1}, there was no significant difference in cellular growth from the control. When the nitrate ion exceeded 0.1 g L^{-1}, significant decreases of cellular viability were gradually disclosed very likely due to inhibitory effect of higher-level nitrate ion to cell growth. As known, nitric acid is a very strong acid, thus nitrate ion is a very weak conjugate base and characterized a neutral ion. This phenomenon of beneficial effects from exposures to trace amounts of "toxic chemicals" (e.g., nitrate ion) are termed "sufficient challenge" (first proposed by the late Henry F. Smyth, Jr., a noted toxicologist; [18,26]). With small concentrations of nitrate ion, no effect on cell growth was observed (ca. <0.1 g L^{-1}). On the contrary, DH5α receiving slightly lower concentrations (ca. <0.01 g L^{-1}; the range of sufficient challenge or reverse effect) had better cellular viability (i.e., beneficial effects) than that in the absence of this "toxic" source. Approximately, at the concentration 0.01 g L^{-1}, the response curve passed back through normal growth (i.e., toxic stress-free growth or control growth) and at the concentration above 0.1 g L^{-1} deleterious effects of toxicity responses started to take place. These points also suggested non-toxic nature of nitrate ion at least at levels less than 1 g L^{-1}.

3.4. Toxic effects of chromium ion

Chromium is an important component of many industrial and consumer products [27]. Its specific chemical property is significant to control its environmental fate and biotoxicity. Although the most stable form of chromium is the trivalent oxide, this form has very low solubility [23] and low reactivity resulting in low mobility in the environment and low toxicity to living organisms as receptors. Thus, this study intentionally selected soluble species Cr(II) as the most toxic form of chromium for assessment. As indicated in Fig. 4, chromium(II) ion was evidently highly toxic and apparently DH5α was less tolerant to this cation. The dose–response curve showed three parts [17,26]. The first part (i.e., no-effect range; ca. <0.021 g L^{-1}) of the curve showed the range of doses that produced no detectable effect (i.e., almost identical response as zero-toxicity control) since cells had a defense mechanism to handle this toxic chemical in a manner that prevented an effect from occurring. The second segment (i.e., the range of decreasing percentage viability with increasing concentration) of the curve began at a threshold (i.e., EC_0) and decreased with increasing concentration until the complete cell death response occurred. At the concentration above the threshold (i.e., $EC_0 = 0.021$ g L^{-1}), the defense mechanism was saturated and the degree of loss of cell viability increased with increasing concentration. Relatively higher concentrations (i.e., >EC_0) of toxic chemical might be easier transported across the membrane from the region of high concentration to low concentration via passive diffusion [18] due to a relatively higher concentration gradient as a driving force. According to thermodynamics (i.e., the free energy change $\Delta G^0 = RT \times \ln(c_{in}/c_{ex})$), passive diffusion is spontaneous (i.e., $\Delta G^0 < 0$), where c_{in} and c_{ex} denoted intracellular and extracellular toxic chemical concentration, respectively. Moreover, the difference of these two terms of free energy $\Delta G^0|_{HC} = \Delta G^0|_{LC} - RT\ln(HC/LC)$ simply suggested the larger equilibrium constant at higher concentra-

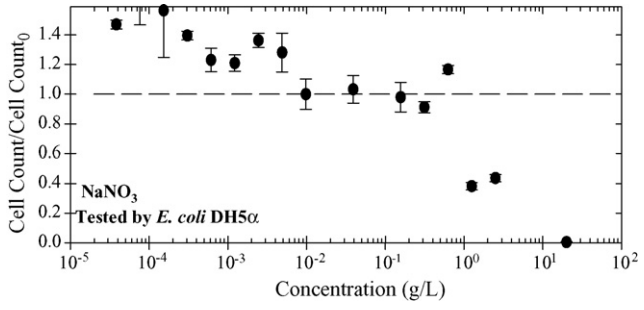

Fig. 3. Dose–response curve of sodium nitrate using *Escherichia coli* DH5α as the indicator microorganism.

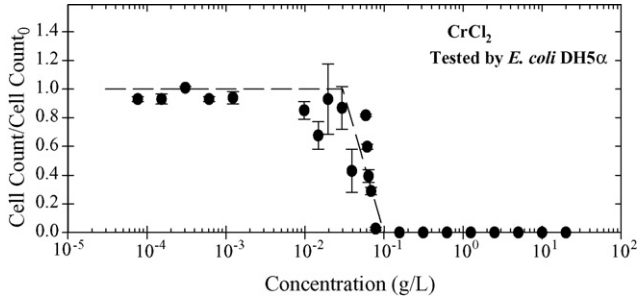

Fig. 4. Dose–response curve of chromium(II) chloride using *Escherichia coli* DH5α as the indicator microorganism.

tion (HC) due to the more negative ΔG^0 at HC than that at lower concentration (LC). This point implied that toxic chemical at HC are more favored to move across the membrane than that at LC. Owing to this reason, chromium might still appreciably penetrate into the plasma membrane to establish significant toxicity to DH5α cells. Once the maximal response (i.e., complete loss of viable cells as a 100% mortality response) was achieved, no greater response could occur with increasing concentration. This is so-called the maximum effect range (ca. $>EC_{100} = 0.1\ g\ L^{-1}$) and obviously the whole cell population can no longer tolerate and death supervenes [26]. Although recent biochemical findings indicated that chromium may be necessary for glucose metabolism [28], the narrow range (ca. 0.021–0.1 $g\ L^{-1}$) in the second portion of the response curve of chromium ion still strongly revealed that DH5α cells might be significantly susceptible to this toxic metal. Apparently, toxicity attenuation and/or species removal of chromium(II) in ashes was inevitably requisite prior to reuse of MSWI ash for on-site and in situ application.

3.5. Toxic effects of cadmium ion

Compared to chromium, cadmium was also in a highly toxic potency and DH5α was highly sensitive to this cation. However, it was relatively less toxic than chromium(II), as lower levels of threshold EC_0 and the maximum effective concentration EC_{100} for cadmium was obtained at ca. 0.069 and 0.2 $g\ L^{-1}$, respectively. The reasons to cause such significant toxicity of Cr(II) and Cd(II) are straightforward [29]. Bacterial cells have a remarkable capacity to repair damage caused by extracellular chemicals (e.g., metals). In addition, a minimum threshold concentration of a toxic metal must reach a cellular target (e.g., cell wall) before any biological response take place. Polysaccharides of bacterial extracellular polymeric substances (EPS) as cellular target are often responsible for metal binding through glucuronic acid subunits [30,31]. This mechanism is so-called metal tolerance or resistance [32]. In addition, many toxicants bind with varying affinity to plasma proteins [33,34]. Binding to toxicants (e.g., Cr(II) and Cd(II)) to high-molecular-weight (HMW) proteins might be an important factor in controlling subsequent distribution to critical compartments within cells, reversibly creating a HMW polar complex incapable of crossing cell membranes. Apparently, when this reversible binding is saturated at the concentration exceeded the threshold, the remaining fraction of free, unbound metals is thus available for active transport and facilitated diffusion across membrane to trigger significant toxicity due to a failure of an effective defense mechanism. As a matter of fact, intracellular protein binding via metallothionein is primarily a significant detoxification mechanism against toxic metals (e.g., cadmium and mercury; [35]). Certainly, cadmium must be removed if MSWI residues are considered for reuse (Fig. 5).

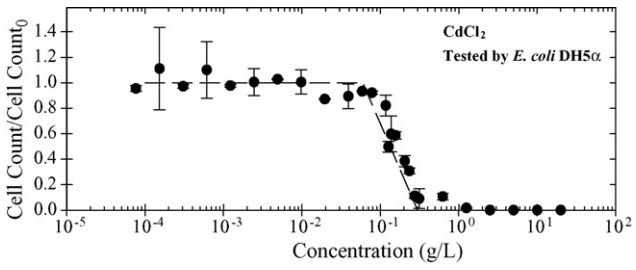

Fig. 5. Dose–response curve of cadmium(II) chloride using *Escherichia coli* DH5α as the indicator microorganism.

4. Conclusions

Compared to prior study [16], the approximated effective concentrations of ashes and metal ions were listed as follows:

bottom ash: $EC_0 = 0.044\ g\ L^{-1}$, $EC_{100} = 0.56\ g\ L^{-1}$;
cyclone ash: $EC_0 = 0.21\ g\ L^{-1}$, $EC_{100} = 0.63\ g\ L^{-1}$;
scrubber ash: $EC_0 = 0.0098\ g\ L^{-1}$, $EC_{100} = 0.0195\ g\ L^{-1}$;
Cr(II): $EC_0 = 0.021\ g\ L^{-1}$, $EC_{100} = 0.1\ g\ L^{-1}$;
Cd(II): $EC_0 = 0.069\ g\ L^{-1}$, $EC_{100} = 0.2\ g\ L^{-1}$.

Thus, according to fingerprint toxicity profiles of some essential toxic chemicals, relative toxicity rankings of ashes and metal ions (i.e., EC_0: SA > Cr(II) > CA > BA > Cd(II) and EC_{100}: SA > Cr(II) > BA > Cd(II) > CA) *clearly* indicated that synergistic interactions among cations and anions might still exist in ashes. Significant toxicity in the SA might be due to synergistic interactions among its toxic species. In addition, the species of great toxicity might still considerably remain in unknown portions of ashes (Fig. 1). This analysis also suggested that any means (e.g., acid wash) to remove metallic ion(s) for toxicity attenuation should be feasible prior to reuse of MSWI ashes for on-site and in situ applications. Follow-up study will be focused on the investigation of residual toxic species (e.g., cations and anions) present in MSWI ashes after and before toxicity attenuation strategy to be applied and may suggest cost-effective means to reduce toxicity for safe reuse of MSWI residues. Moreover, although TCLP may be not appropriate enough to assess reusability of ashes, our toxicity assessment seemed to be more viable to indicate whether ashes were feasible to be reused in further application.

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References

[1] http://www.epa.gov.tw/english/.
[2] P. Sivakumar, H. Jung, J.W. Tierney, I. Wender, Coprocessing of lignocellulosic wastes and coal to liquid fuels, in: M.R., Khan (Ed.), Conversion,

Utilization of Waste, Materials, Appl. Energy Technol. Series, 1996, pp. 199–219.
[3] P. Jean, W. Assefa, C. Michel, Hydraulic activity of slags obtained by vitrification of wastes, ACI Mater. J. 93 (1996) 112–131.
[4] C.C. Tu, Production of microstructure materials from incinerator ashes using thermal plasma technology, National Taipei University of Technology, Institute of Materials and Mineral Resources Engineering, Master thesis, 2004 (in Chinese).
[5] K.L. Lin, The influence of municipal solid waste incinerator fly ash slag blended in cement pastes, Cem. Concr. Res. 35 (4) (2005) 979–986.
[6] K.L. Lin, Effects of SiO_2 and Al_2O_3 on the hydration characteristics of MSWI fly ash slag, J. Ind. Eng. Chem. 11 (6) (2005) 834–840.
[7] S. Bethanis, C.R. Cheeseman, C.J. Sollars, Properties and microstructure of sintered incinerator bottom ash, Cer. Int. 28 (8) (2002) 881–886.
[8] D.J. Rivard-Lentz, Incinerator bottom ash as a soil substitute: physical and chemical behavior, Fuel Energy 39 (1) (1998) 27.
[9] A. Polettini, R. Pomi, G. Carcani, The effect of Na and Ca salts on MSWI bottom ash activation for reuse as a pozzolanic admixture, Res. Conserv. Rec. 43 (4) (2005) 403–418.
[10] R. Forteza, M. Far, Characterization of bottom ash in municipal solid waste incinerators for its use in road base, Waste Manage. 24 (9) (2004) 899–909.
[11] C.R. Cheeseman, A. Makinde, S. Bethanis, Properties of lightweight aggregate produced by rapid sintering of incinerator bottom ash, Res. Conserv. Rec. 43 (2) (2005) 147–162.
[12] S. Rémond, D.P. Bentz, P. Pimienta, Effects of the incorporation of municipal solid waste incineration fly ash in cement pastes and mortars, Cem. Concr. Res. 32 (2002) 565–576.
[13] T. Mangialardi, A.E. Paolini, A. Polettini, P. Sirini, Optimization of the solidification/stabilization process of MSW fly ash in cementitious matrices, J. Hazard. Mater. 70 (1–2) (1999) 53–70.
[14] C. Collivignarelli, S. Sorlini, Reuse of municipal solid waste incineration fly ash in concrete mixture, Waste Manage. 22 (2002) 909–912.
[15] T. Mangialardi, Sintering of MSW fly ash for reuse as a concrete aggregate, J. Hazard. Mater. 87 (1–3) (2001) 225–239.
[16] B.Y. Chen, K.L. Lin, Biotoxicity assessment on reusability of municipal solid waste incinerator (MSWI) ash, J. Hazard. Mater., 2006, doi:10.1016/j.hazmat.2006.01.009.
[17] B.Y. Chen, C.H. Wu, J.S. Chang, An assessment of the toxicity of metals to *Pseudomonas aeruginosa* PU21 (Rip64), Bioresour. Technol., 2006, in press.
[18] B.Y. Chen, Toxicity assessment of aromatic amines to *Pseudomonas luteola*: Chemostat pulse technique and dose–response analysis, Proc. Bioochem. 41 (2006) 1529–1538.
[19] B.Y. Chen, J.S. Chang, Phenol degradation and toxicity assessment upon biostimulation to an indigenous rhizobium *Ralstonia taiwanensis*, Biotechnol. Prog. 21 (2005) 1085–1092.
[20] J.M. Becker, G.A. Caldwell, E.A. Zachgo, Biotechnology: A Laboratory Course, second ed., Academic Press, San Diego, 1996.
[21] C. Ewards, Environmental Monitoring of Bacteria. Methods in Biotechnology, vol. 12, Chapter 1, Some Problems Posed by Natural Environments for Monitoring Microorganisms, Human Press, Totowa, New Jersey, U.S.A, 1999, p. 1.
[22] M.C. Wei, M.Y. Wey, J.H. Hwang, J.C. Chen, Stability of heavy metals in bottom ash and fly ash under various incinerating conditions, J. Hazard. Mater. 57 (1998) 145–154.
[23] D.C. Harris, Quantitative Chemical Analysis, sixth ed., W.H. Freeman and Company, New York, 2005, pp. AP10–AP11 (Appendix A).
[24] R.C. Waritz, Chapter 3 biological markers of chemical exposure, dosage, and burden, in: L.J. Cralley, L.V. Cralley, J.S. Bus (Eds.), Patty's Industrial Hygiene and Toxicology, vol. 3, Part B, third ed., John Wiley & Sons, Inc., 1995, pp. 159–191.
[25] G. Gottschalk, Chapter 2 how *Escherichia coli* synthesizes ATP during aerobic growth on glucose, in: Bacterial Metabolism, second ed., Springer-Verlag, New York, 1988, pp. 13–35.
[26] M.A. Ottoboni, Chapter 8 chronic toxicity, in: The Dose makes the Poison, second ed., ITP Company, New York, 1997, pp. 79–90.
[27] J. Barnhart, Chromium chemistry and implications for environmental fate and toxicity, J. Soil Contam. 6 (6) (1997) 561–568.
[28] M.K. Campbell, S.O. Farrell, Chapter 24 Integration of Metabolism: Cellular Signaling. Biochemistry, fifth ed., Thomson Brooks/Cole, Thomson Learning, Inc., Belmont, CA, 2006, pp. 662–689.
[29] J.V. Rodricks, Chapter 9 dose and response, in: Calculated Risks, Univ. Press Cambridge, 1992, pp. 158–179.
[30] B.Y. Chen, V.P. Utgikar, S.M. Harmon, H.H. Tabak, D.F. Bishop, R. Govind, Studies on biosorption of zinc(II) and copper(II) on Desulfovibrio desulfuricans, Int. Biodeterior. Biodegrad. 46 (1) (2000) 11–18.
[31] G.G. Geesey, L. Jang, T.J. Beveridge, R.J. Doyle, Metals Ions and Bacteria, Wiley, New York, 1989.
[32] B.Y. Chen, J.S. Chang, Characterization and theoretical analysis on toxicological threshold of mercuric ions to *Pseudomonas aeruginosa* PU21 (Rip64), Bioprocess. Eng. 23 (6) (2000) 675–680.
[33] F.E. Guthrie, Absorption and distribution, in: E. Hodgson, F.E. Guthrie (Eds.), Introduction to Biochemical Toxicology, Elsevier, New York, 1980, p. 10.
[34] J.S. Bus, J.E. Gibson, Chapter 4 body defense mchanisms to toxicant exposure, in: L.J. Cralley, L.V. Cralley, J.S. Bus (Eds.), Patty's Industrial Hygiene and Toxicology, vol. 3, Part B, third ed., John Wiley & Sons, Inc., 1995, pp. 159–191.
[35] R.A. Goyer, Toxic Effects of metals, in: M.O. Amdur, J. DOull, C.D. Klaassen (Eds.), Toxicology: The Basic Science of Poisons, fourth ed., Pergamon Press, New York, 1991, p. 257.