Aim and Scope

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CONTENTS

Research

Design of Post-Treatment Unit for Compost from a Composting Toilet with Microbial Risk Assessment

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ABSTRACT: The compost withdrawn from a composting toilet still contains pathogens and therefore requires a post-treatment unit to treat the compost prior to reuse. A quantitative microbial risk assessment Monte Carlo was conducted to evaluate the risk of infectious diseases and the length of time for the post-treatment. The accidental ingestion of compost (0.5–0.8 g) in a worst case scenario was evaluated. High temperature was efficient in reducing the risk of pathogens; however, the temperature distribution in the unit is not sufficient to reduce pathogens. Therefore, to efficiently reduce pathogens during the post-treatment, the unit requires an insulator to maintain the temperature.

INTRODUCTION

COMPOST of human faeces used as fertilizer can be harmless and useful because it becomes part of nutrient recovery. A pilot model of a composting toilet was installed in a rural region of Burkina Faso to perform a source recycling system which makes compost from human faeces. Initial experiments were performed on some samples taken from the composting toilet. Results showed that pathogens such as bacteria and parasites still remained in the compost after withdrawal from the rural model of composting toilet after three months of operation. Therefore, post-treatment of the collected compost is required to minimise the health risk when recycling the faeces as fertilizer on farmland. For the inactivation of pathogens, several methods of treatments are proposed, including heating, drying, chemical treatments, treatment by worms, long storages times, etc. In low income countries like Burkina Faso, people cannot pay consumptions for post-treatment, however, they have abundant solar energy. Therefore; this study proposes a solar disinfection unit to inactivate the pathogens. The operation conditions to inactivate pathogens should be designed based on the risk assessment by setting a safe level of pathogens concentration in the compost after post-treatment.

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Norovirus and *Ascaris* eggs were selected for the reference pathogens in this study. Because, noroviruses are a major cause of human gastroenteritis, and they are frequently associated with food, water contamination [1] and accidental ingestion. On the other hand, *Ascaris* infections are very common in developing countries. One fertile egg can cause infection of Ascarisis to humans.

These enteric infections can be transmitted through the compost from faeces to the human body with pathogenic species. Quantitative microbial risk assessment (QMRA) has been widely used to establish the health risks associated with wastewater reuse in both developed and developing regions under different scenarios. The QMRA-Monte Carlo techniques (QMRA-MC) based on the work of Haas *et al.* [2] was used to estimate risk in this study.

The objectives of this study are to perform risk assessment for the design of the post-treatment unit by using the QMRA-MC techniques and to determine the treatment time to reach the safe level of pathogens in the compost.

MATERIAL AND METHODS

Post-treatment Unit

People would collect the compost from the rural model of composting toilet with urine diversion (Fig-

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ure 1) in the pilot families and use in their gardens as fertilizer. Application of the post-treatment would be achieved by spreading the compost evenly on the steel box as shown in Figure 2. The box was fabricated with a length of 60 cm, a width of 40 cm, and a depth of 10 cm. The total volume of the box is 24L. The steel box has steel septa which facilitate deep penetration of heat to compost. The steel box is painted black in colour to aid in the absorption of heat. The steel box does not have a solar concentrator [3,4,5]. The temperature distribution of the compost in the box was measured at 3 positions which were 1 cm, 5 cm, and 10 cm from the surface.

Scenarios for Reuse of Compost

During the utilisation of the compost, people may accidentally ingest compost with the pathogens orally. The people exposed to the pathogens would have diseases with a probability estimated by risk assessment. We set 6 scenarios, the compost at constant temperatures (40°C, 50°C and 60°C, S-1 to S-3) and actual temperature at 3 positions (1 cm, 5 cm and 10 cm from the surface) in the steel box (S-4 to S-6) as a post-treatment for the assessment. For the calculation of concentration in the compost, the inactivation rate coefficient from our previous measurement was used [4,5]. The details of the ingestion model are as follows:

- To consider the worst case, 50,000 eggs/g in wet faeces is excreted from a heavily infested person [6]. The value of the initial concentration of *Ascaris* eggs was 336 eggs/g-dry compost. This number was estimated by multiplying the number of eggs excreted per gram (50, 000) by the 100g of compost dividing by the bulk density of the compost (14881 g/cm^3).
- Highly infested person of viral infection excretes a maximum of 10^{11} viral copies/g in faeces from highly infected person [1,7,8] was used for the risk assessment taking account of the highest risk. Assuming this concentration, the initial concentration was estimated at 6.72×10^8 viral copies/g-dry compost. This number was estimated by multiplying the number of norovirus excreted per gram (10^{11} viral copies/cm^3) by the 100 g of the compost and dividing by bulk density of the compost (14881 copies/cm^3).
- Ingestion rate of compost is 150–800 mg/event. This is used in the risk assessment of dioxin in soil ingestion rate [9].
- Post-treatment would be done every four months.
- The concentration of pathogens in the compost after the post-treatment was estimated using the first-order kinetic model from our earlier studies on *Ascaris* eggs and indicator MS2 bacteriophage inactivation. The estimated number of days was assumed to be 9 days, 6 days and 3 days for 40°C, 50°C and 60°C respectively. The estimated inactivation rate coefficient values for 40°C, 50°C and 60°C were 0.22 h^{-1}, 0.92 h^{-1} and 1.22 h^{-1} respectively, for *Ascaris* eggs and MS2 k values were 0.25 h^{-1}, 0.45 h^{-1} and 0.80 h^{-1}[4,5].
- The moisture content of all treatments was 50%.

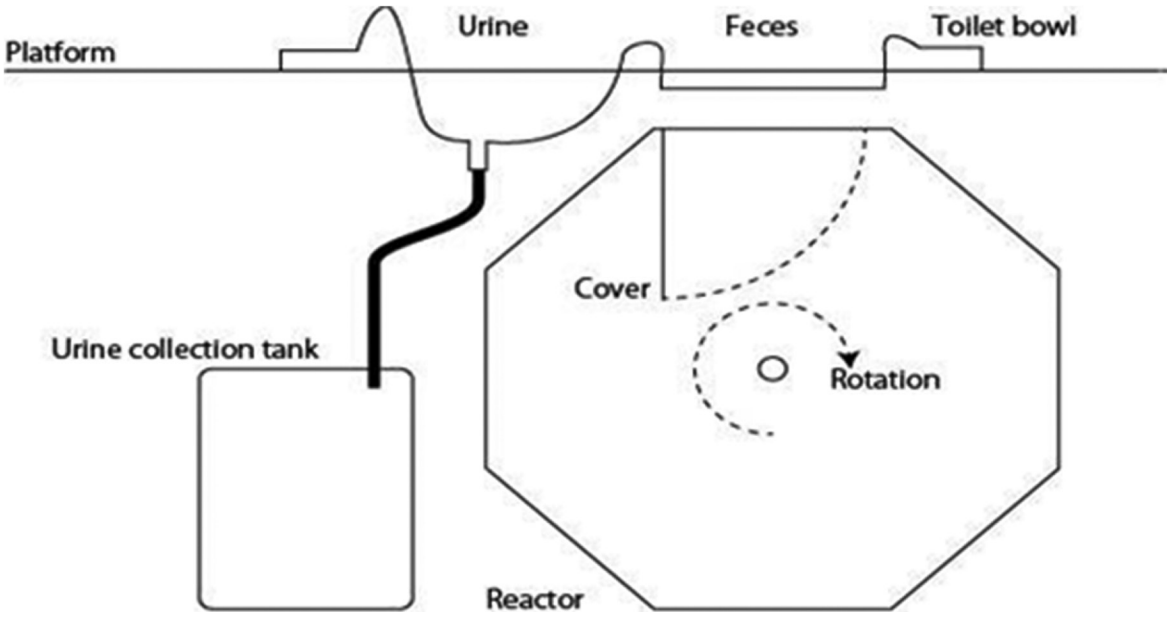

Figure 1. *Arrangement of composting toilet.*

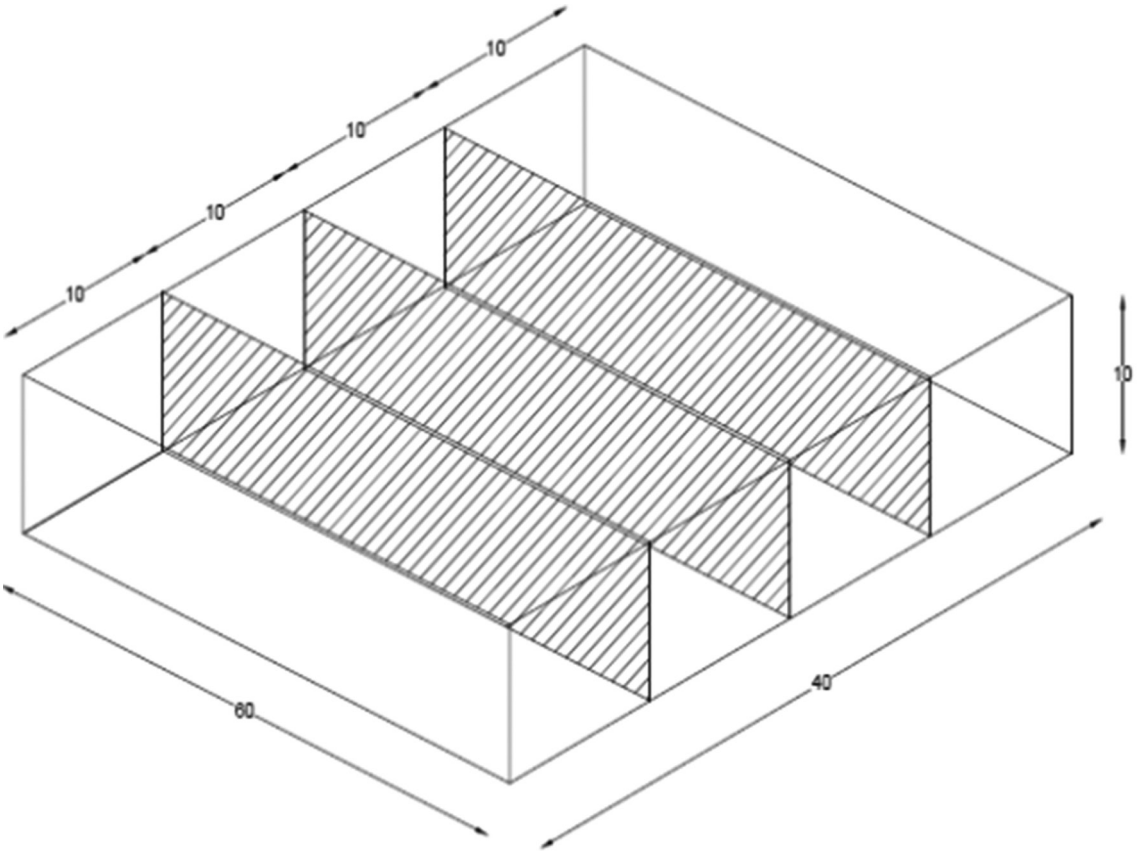

Figure 2. *A proposed compost solar sanitisation installation to reduce the heat loss (Andreev and Samoil, 2009).*

Hazard identification—Farmers performing post-treatment would be exposed to pathogens in the compost. There are several groups of pathogens, but the pathogens of considerable interest in the study area are *Ascaris* eggs and viral infections (norovirus) because *Ascaris* and norovirus are also known to be the most resistant to treatment processes [10,11]. Accidental ingestion of a small dose consequently implies a high risk of infection compared to many other pathogens [9].

Dose-response assessment—The QMRA-MC was used to estimate risks of *Ascaris* and norovirus infection. The study by Navarro *et al.* found that *Ascaris* infection data best fitted the β-Poisson dose-response equation [12]:

$$P_I(d) = 1 - [1 + (d / N_{50})(2^{1/\alpha} - 1)]^{-\alpha} \quad (1)$$

where $P_I(d)$ is the probability of infection in an individual (infection/event), d is the ingested number of *Ascaris* eggs on one occasion (eggs/event), N_{50} is the mean infective dose number of *Ascaris* eggs (eggs), I means considerable spice for calculation of probability (–) and α is an infectivity constant of *Ascaris* (–). They found the values of N_{50} and α to be 859 and 0.104, respectively. Since they were working with epidemiological data on *Ascaris* prevalence rather than conducting human *Ascaris* dose-challenge studies, the value found for N_{50} is not a measure of the actual median *Ascaris* infective dose, but rather an empirical value arising from their statistical analyses [13].

The annual probability of infection, $P_{I(A)}(d)$ (pppy), is given by:

$$P_{I(A)}(d) = 1 - [1 - P_I(d)]^n \quad (2)$$

Where n is number of events per year to the single *Ascaris* dose (–) [13]. For norovirus, the dose response data set of Teunis *et al.* [1] was used in place of the β-Poisson equation [13].

Exposure assessment—The human exposure assumed to take place is an event when farmers work on compost. Practically, one egg is enough to cause an infection. Norovirus has an extremely low infectious dose [8].

Risk characterisation—The Monte Carlo technique has been used to evaluate the infection risk. The random number is applied for estimation of variables with distributions for simulation of Equations (1) and (2).

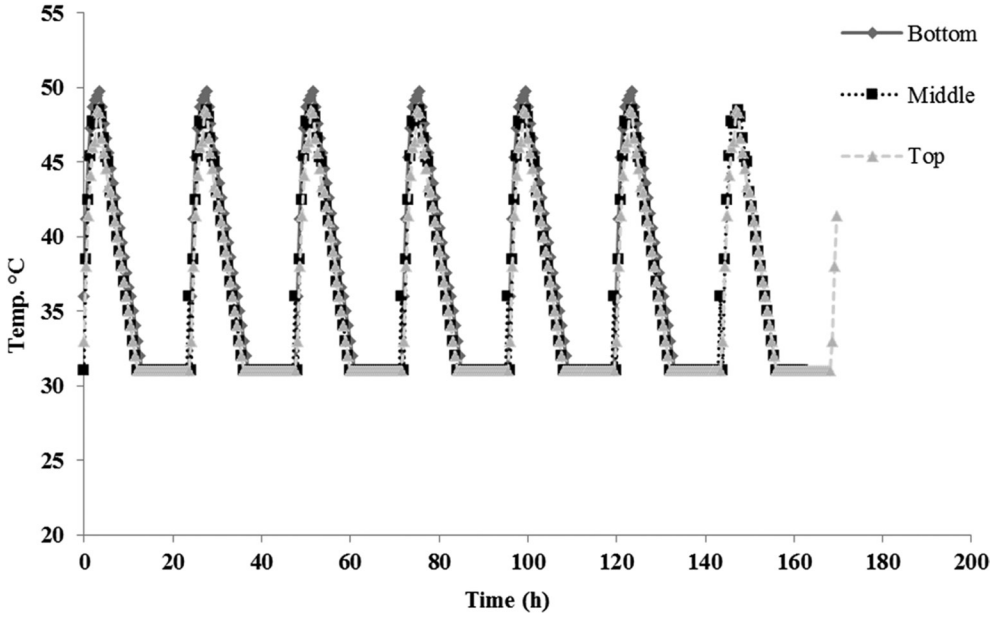

Figure 3. *Temperature distribution assumed in the estimation of risk.*

The simulation was repeated 10,000 times [13]. Then, 95 percentile of the probability was estimated as the infection risk.

Temperature Distribution

Considering actual practices, solarisation is one of the main processes for disinfection of enteric pathogens, because sunlight is available in the study region. The solarisation relates to the ambient temperature, while the temperature is not constant as shown in Figure 3. A two day diurnal average ambient temperature was measured during April 2014 in the post-treatment unit. April is one of the hottest months during the year in the study region. From the temperature profile, it was assumed that temperature remains constant during the night and continually increases during the day.

RESULTS AND DISCUSSION

The change in concentration of *Ascaris* under S1 to S3 is shown in Figure 4. The concentration declined

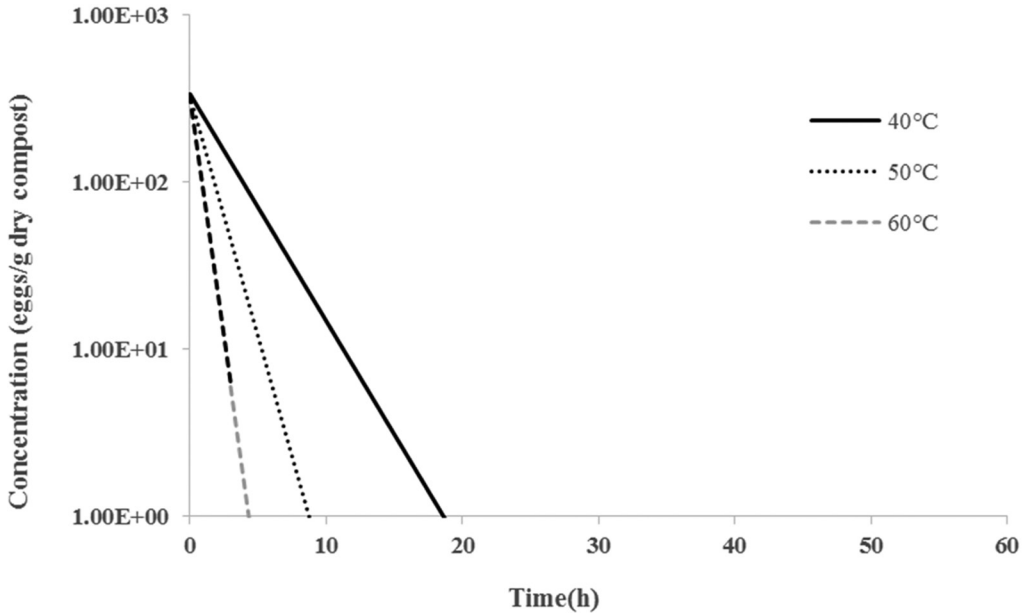

Figure 4. *Change in Ascaris eggs concentration.*

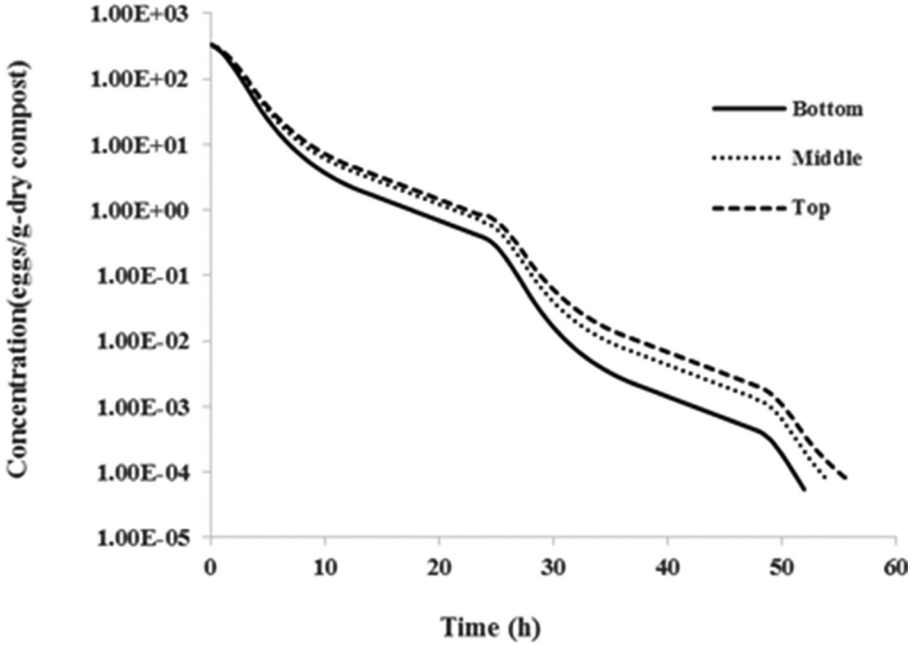

Figure 5. *Change in Ascaris concentration in the steel box.*

from the initial value of 336 eggs/g-dry compost. High temperature gives high decline rate of the concentration due to high inactivation rate coefficient. Figure 5 illustrates the decrease concentration of *Ascaris* eggs with time under S4 to S6. High and low reduction rates are found in the figures. This is because high temperature at day time and low temperature in night respectively give high and low reduction. All conditions obtained 6 log reduction of eggs in 60 hours and the difference of the position in the steel box gave slight difference of the concentrations. The change in concentration of norovirus with elapse of time under S1 to S3 are shown in Figure 6. The concentration declined from the initial of 6.72×10^8 copies/g-dry compost. Higher temperature condition also gives higher decline rate. The time course of concentrations under scenarios S4 to S6 is represented in Figure 7. The reduction rate of norovirus concentration had slight difference among three

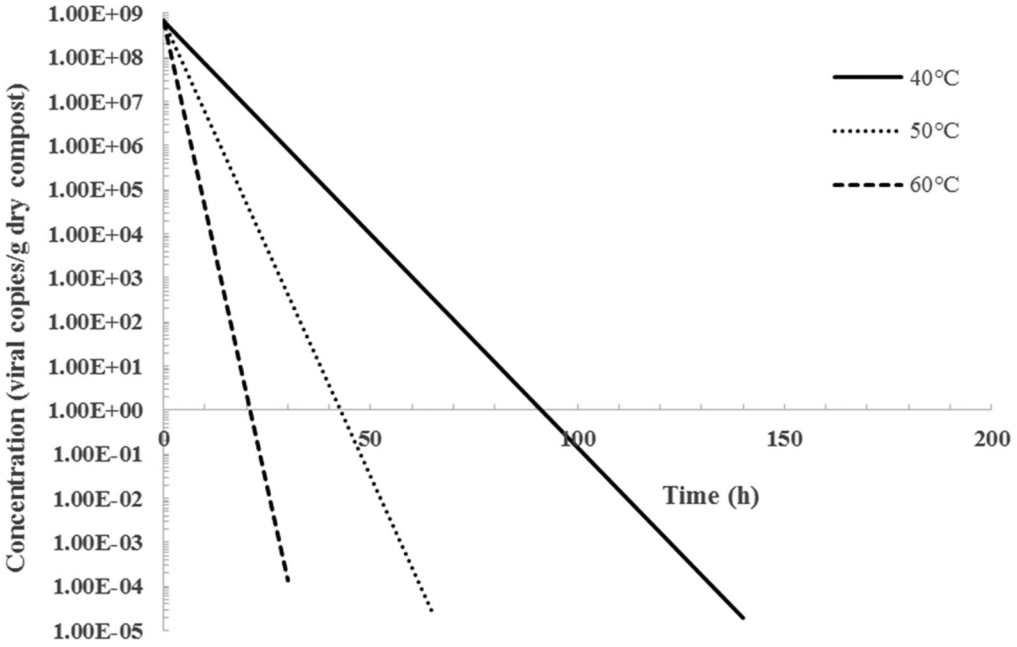

Figure 6. *Change in Norovirus concentration.*

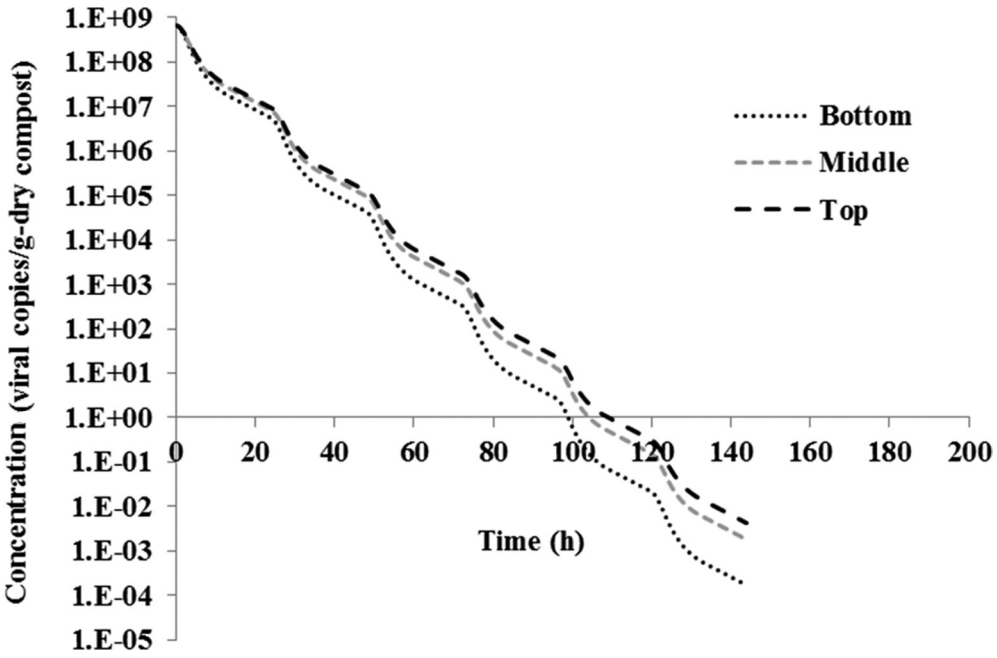

Figure 7. *Change in norovirus concentration in the steel box.*

scenarios like *Ascaris* case and was lower than 40°C, however, S4 to S6 have higher temperature period than 40°C. This might be result of much effect of low temperature less than 40°C, especially at night.

The 95-percentile annual risk of *Ascaris* and norovirus infections for the scenarios from the their concentration in the compost as shown in Figures 8–11. The risk of the both pathogens are almost 1 at the initial for all scenario. This means the people who uses the compost would be heavily polluted by the pathogens. They would be infected if the composting reactor fails to reduce the pathogen concentration and also if they do not apply the post-treatment. Schönning *et al.* [14] also reported a 95-percentile risk of rotavirus and *Ascaris* for 0 months' storage in a worst case as 1. After the post-treatment, the risks for the *Ascaris* under S1 to S3 were reduced and reached a safe level at 48 h, 21 h and 10 h for 40°C, 50°C and 60°C, respectively. Under the steel box, the required times to reach the safe level were respectively 51.5 h, 54 h and 54.5 h for S4 to S6. This was same level of S1 while the temperature distributions in the steel box would give longer time

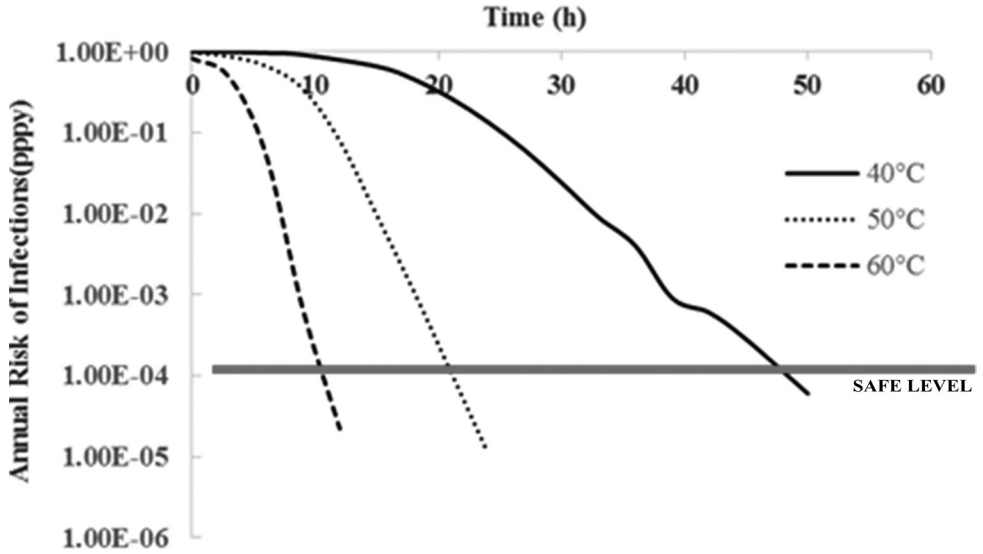

Figure 8. *Ascaris annual infection risk associated with post-treatment at: 40°C, 50°C and 60°C where the line indicates the safe level.*

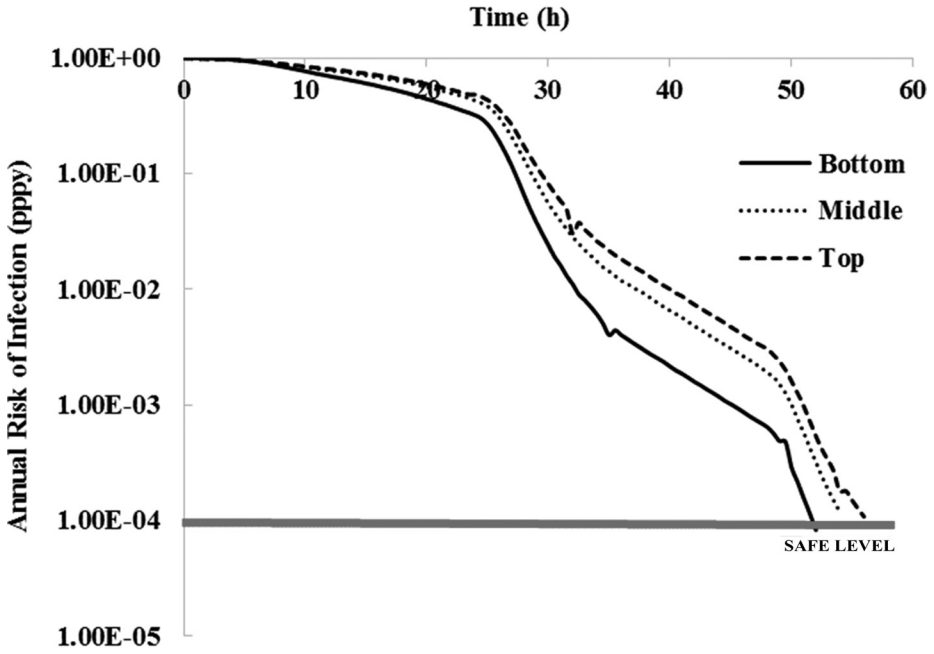

Figure 9. *Ascaris annual infection risk associated with post-treatment at: bottom, middle and top of the steel box, where the line indicates the safe level.*

to reduce the concentration of pathogens from estimation from maximum temperature. During the day, there is sufficient increase in temperature but it suddenly decreases towards the evening and in the nights. This phenomena causes sufficient inactivation by the balance of the high inactivation rate at high temperature and the low inactivation at low temperature. To reduce treatment time, we need to improve the post-treatment unit by increasing the maximum temperature and keeping temperature during the night. The required times to the safe level for norovirus under constant temperatures were 139 h, 62 h and 28 h for 40°C, 50°C and 60°C, respectively. The time required to reach the safe level in the steel box at the bottom, middle and

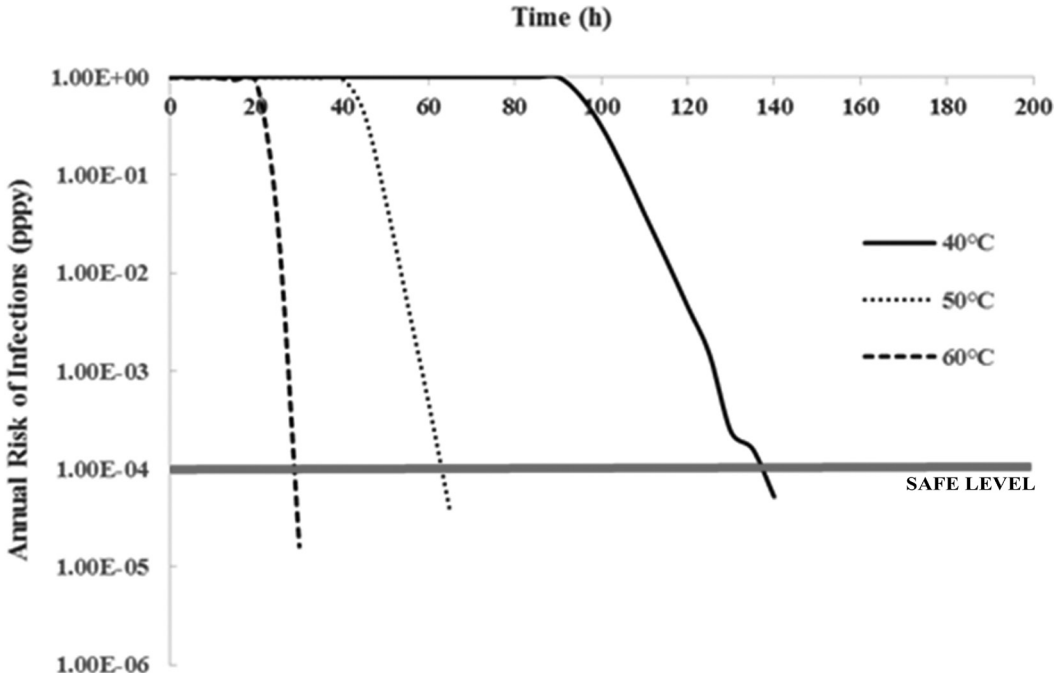

Figure 10. *Norovirus annual infection risk associated with post-treatment at: 40°C, 50°C and 60°C where the line indicates the safe level.*

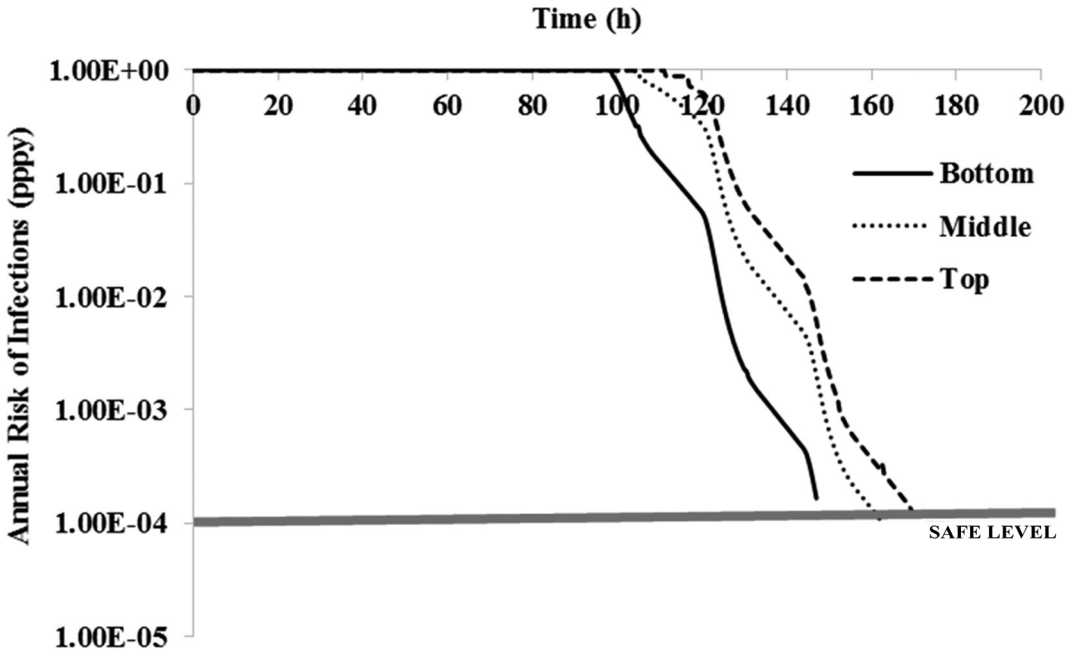

Figure 11. *Norovirus annual infection risk associated with post-treatment at: bottom, middle and top of the steel box, where the line indicates the safe level.*

top were respectively 147 h, 161.5 h and 170 h. These are longer than 40°C case, therefore, the treatment unit should be improved. Comparing *Ascaris* and norovirus, norovirus requires more time than *Ascaris* to reach safe level of 10^{-4} pppy [15]. Therefore, norovirus is more important indicator for the design of the unit, even *Ascaris* eggs have possibility to survive several months in a soil system [16].

Risk assessments for post-treatment of compost have received very little documentation. Seidu *et al.* [16] reported increased levels of *Ascaris* and rotavirus infection for farmers due to accidental ingestion of contaminated soils. The estimated median risk values for farmers were 0.99 and 7.2×10^{-2} pppy for Ascariasis and rotavirus. The study indicated that the elevated hazard posed by the soils on the farm could be attributed to the persistence of *Ascaris* in the soils. This implies that compost must be treated properly before reuse as fertilizer so as not to pose even greater risk in the soils. However, in semi-arid regions where the compost is expected to be used, inactivation of *Ascaris* occurs in soils rapidly [8] which indicates that post-treatment in these regions could be feasible. The results of our study indicate that high temperature with prolonged treatment time could reduce the hazard considerably.

Mara and Sleigh [13] reported risk of fieldworkers' involuntary ingestion of 1–10 mg of waste-water contaminated soils. The median of norovirus infection risk for an ingestion of 100–1000 mg, 10–100 mg, 1–10 mg of contaminated soil were 0.98, 0.32, and 3.7×10^{-2} pppy respectively. The study also reported the median *Ascaris* infection risk for ingestion of 100–1000 mg, 10–100 mg, 1–10 mg of contaminated soils as 0.14, 1.5 $\times 10^{-2}$, and 1.5×10^{-3} pppy respectively. In this study, the risk associated with the exposure of norovirus was estimated to be the highest, thus, this level of pathogen reduction will provide sufficient protection against bacterial and protozoa infections.

Conclusions

Higher temperature is efficient for reducing risk of pathogens. Temperature distribution in the steel box is not sufficient to reduce pathogens. Therefore, to efficiently reduce pathogens during post-treatment the steel box needs an insulator to maintain temperature. Guidelines for the design of the post-treatment facility are as follows:

- For norovirus, under S1 to S3, post-treatment requires approximately 140 h, 60 h, and 30 h for 40°C, 50°C, and 60°C respectively to achieve the safe level of 10^{-4} pppy. For *Ascaris*, post-treatment requires approximately 50 h, 24 h, and 12 h for 40°C, 50°C, and 60°C respectively.
- For S4-S6, norovirus requires 147 h, 161.5 h, and 170 h for the S4, S5 and S6 of the steel box respec-

tively to reach a safe level and *Ascaris* requires 51.5 h, 54 h, and 54.5 h for the bottom, middle and top of the metal box respectively.

Farmers should be educated on high risks associated with compost and how to be safe during post-treatment. Effective post-treatment could reduce risk of pathogens in compost from the composting toilet and may provide a cheaper alternative to fertilizer for rural household farmers. To reduce health risk from pathogens in rural communities it is important to create affordable methods for post-treatment.

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Function of Wood Chips for Composting of Sewage Sludge by Thermophilic and Aerobic Digestion

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ABSTRACT: Thermophilic and aerobic digestion (TAD) of sewage sludge was performed using *Chamaecyparis obtusa*, *Cryptomeria japonica*, and *Pinus densiflora* wood chips to confirm physical and chemical properties of woody bulking agents affecting digestion efficiency. About 60% of digestion efficiency was attained by the *C. obtusa* and *C. japonica* wood chips, while that by *P. densiflora* was only 40%. Neither the difference of porous structure nor inclusion of anti-bacterial substances accounted for the different digestion efficiencies. *P. densiflora* wood chips have the highest acid leachability, and we finally confirmed that lowering pH to 6 was responsible for the lowest digestion efficiency.

INTRODUCTION

The quantity of sewage sludge generated in Japan has increased year-by-year to amounts over 75 million per year in 2010 because of the widespread use of sewer systems. Incineration and landfilling is considered to be one of the major sewage sludge treatment systems e.g. [1], whereby sewage sludge is treated by dewatering, drying, and incineration, followed by landfill disposal of the resultant ash. Although this series of processes is widely used, it has disadvantages on operational cost and recovery of useful components such as phosphorus. Therefore, biological sludge digestion processes have attracted attention because of low operational cost and possible recycling of phosphorus included in the residues, though required treatment time is longer when compared to physicochemical treatment processes such as incineration.

As one of the promising biological treatment processes, this study focused on thermophilic and aerobic digestion (TAD), where activities of aerobic bacteria are promoted to enhance digestion of organic matters with aeration at high temperature (45–65°C) [2]. Since high temperatures support elimination of pathogenic and allergenic microorganisms [3,4], residue of TAD possesses potential of recycling for an agricultural purpose. For use as compost, phosphorus concentration in the residue is required to be more than 2%. Therefore, TAD processes should be designed to achieve sufficient removal of organic matters, though heavy metal content of the residue is the next question that should be addressed regarding its practical use [5].

Various factors such as moisture content, amount of air flow, and temperature may affect an efficiency of TAD [6,7]. In addition, bulking agents are normally used to maintain aerobic condition and moisture content of mixture in the TAD processes e.g. [8]. As bulking agents, wood chips and other plant materials such as wheat straw, cotton waste, bark chips, grape stalk, peanut shell, sawdust, paper, bagasse, olive leaves, and rice husk have been widely used [9–11], while other abiotic materials such as a recyclable plastic [12] and pumice [13] were recently examined as bulking agents. It is well known that size distribution of bulking agent and its mixing ratio to sewage sludge are the operational factors for successive digestion of organic matters [14]; however only limited information is available for the characteristics of wood materials that can influence organic matter digestion in TAD processes.

Wood plants of certain species contain organic compounds including antibacterial substances to protect themselves in nature [15]. Because of the antibacterial action, possible release of antibacterial substances may

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change the bacterial community responsible for the organic matter digestion. In addition, some of the organic compounds contained in wood plants are acidic. If released, pH value of the mixture of sewage sludge and bulking agent may be lowered. It is natural to expect that change of pH value may affect bacterial activities as well as bacterial community in the TAD processes because of possible generation of ammonium [16]. In addition to these chemical properties, there are physical properties of the bulking agents affecting TAD. One physical property is the porous structure of the bulking agent because of its capability to contain air and its provision of habitats to microorganisms. In this study, whether these chemical and physical properties affect a digestion efficiency of TAD was examined using wood chips of different tree species, and the property responsible for the different digestion performance by the different wood plant species was finally identified.

MATERIAL AND METHODS

Wood Chips, Sewage Sludge, and Inoculum

Commercially obtained square logs of *Chamaecyparis obtusa*, *Cryptomeria japonica* and *Pinus densiflora* were milled by using a cutter mill, and then the wood chips were sieved to obtain 1 cm × 1 cm size. In order to investigate an effect of fine pore structures on TAD efficiency, a hammer mill was used to produce *C. japonica* wood chips of which fine pore structures were crushed. To investigate an effect of antibacterial substances inclusion, we prepared the antibacterial substances-removed *C. japonica* wood chips, because *C. japonica* is known to have anti-bacterial activity [17]. Briefly, *C. japonica* wood chips produced by the cutter mill were immersed in ethanol at the ratio of 1:2 v/v, and after 24 hours the ethanol extract was replaced with fresh ethanol. The extraction was repeated five times to attain removal of the antibacterial activity as described later, and then the residue was dried at room temperature. An excess sludge cake with about 85% of moisture content was collected from Higashi-Hiroshima sewage treatment plant (Higashi-Hiroshima, Japan), while the inoculum for TAD was sampled from the composter in Yauchi drainage facilities of agricultural communities in Shoubara, Japan.

TAD

All TAD experiments were performed for 14 d using a 30 L composting device (Z-025, Kowa Emtech, Japan) equipped with a ribbon heater, thermo regulator, and churning paddle (Figure 1). 8 L of the wood chips, 300 g of an excess sludge cake and 45 g of the inoculum were put together into the device operated at 60 ± 5°C. Churning was carried out for 30 minutes every three hours. During 14 d of digestion period, an excess sludge cake was supplied to the device once a day at 300 ± 10 g/day. The moisture content of mixture in the device was maintained at 40–50% by spraying water. In the experiment to investigate an effect of pH lowering on TAD, phosphoric buffer was added to the mixture to maintain its pH value at 6 or 9.

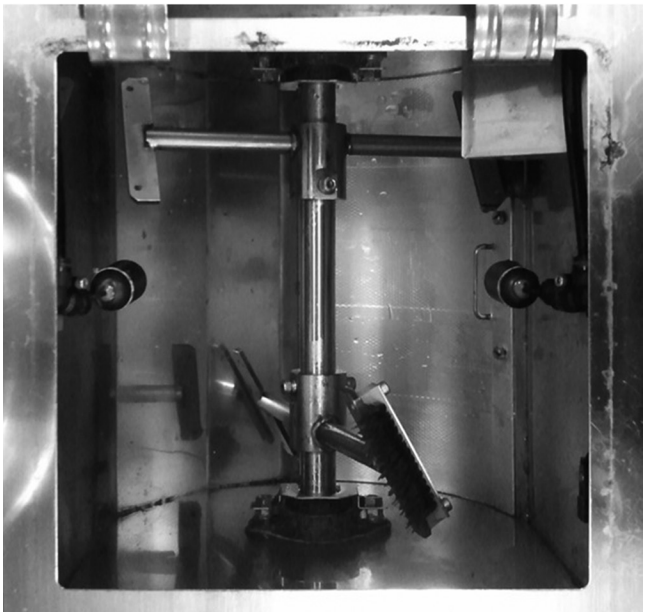

Figure 1. *Inside of the TAD device.*

About 15 g of samples were taken a day for analyses, while the total weight of mixture in the device was weighed. Based on the water content, the amount of solid matters in the mixture was determined, and digestion efficiency was calculated by Equation (1).

$$\text{Digestion efficiency [\%]} = \frac{\text{Total solid added [g]-Remaining solid [g]}}{\text{Total solid added [g]}} \times 100 \quad (1)$$

Assay of Antibacterial Activity

The antibacterial activity of the ethanol extract of *C. japonica* wood chips was checked by a paper disk assay using *Bacillus subtilis* (JCM 2499, Riken, Japan) in 20 replications. Briefly, *B. subtilis* was pre-cultured at 30°C in CASO-bouillon medium (CASO-Bouillon, Merck, Germany), and 100 μL of the culture solution

was uniformly applied to the surface of a standard count agar plate (Nihon Pharmaceutical, Japan). After putting a paper disk (∅ 6 mm, Advantec, Japan) on the agar plate, 10 µl of the ethanol extract was impregnated onto the paper disk. The agar plate was incubated at 30°C for 24 h, and then the diameter of clear zone on the agar plate was measured. In the control experiment, fresh ethanol was impregnated onto the paper disk. In order to confirm whether the residual wood chips of the ethanol extraction had antibacterial activity, the residual wood chips were further extracted using n-hexane. The antibacterial activity of the hexane extract was also tested by the same manner for the ethanol extract with an exception that n-hexane was used in the control experiment.

Analyses

For analyses of water content of the mixture of wood chips and excess sludge in the device, the sludge and wood chips were separated by scratching, and then the sludge was weighed and dried at 110°C. The pH value of the mixture was measured using the slurry prepared by adding 30 ml of MilliQ water to 3 g of the mixture. The acid leachability of the wood chips was compared on the basis of measured pH value in the aqueous solution prepared by adding the wood chips to water at 1:10 v/v% followed by 24 hours agitation at 60°C. The cutting surface and pore sizes of the wood chips were observed by a digital microscope (KH-1300, Hirox, Japan) after washing by sonication in MilliQ water at 45 kHz for 2 min to remove any debris.

The bacterial community structures were compared by the polymerase chain reaction- denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Bacterial DNA was extracted from the samples taken after 10 day of the digestion using ISOIL beads beating (Nippon Gene, Tokyo, Japan). DNA fragments of approximately 200 bp were PCR-amplified from a total genomic DNA sample of mixed populations using universal eubacterial oligonucleotide primers 341f-GC and 534r primer set [18] targeting the variable region 3 (V3) region of the 16S rRNA gene according to the condition described in a previous report [19]. All PCR-products were subjected to the D Code DGGE complete system (Bio-Rad Laboratories, Inc., California, U.S.), where the PCR-amplified DNA fragments were separated on 8% polyacrylamide gels containing urea and formamide as the denaturant from 25% to 65% at 100V for 12 h. As the size marker the DGGE Marker I (Nippon Gene, Tokyo, Japan) was used.

RESULTS AND DISCUSSION

TAD

The digestion efficiencies by the *C. obtusa* and *C. japonica* wood chips were about 60%; however that by the *P. densiflora* wood chips was only 40% [Figure 2(a)]. The digestion efficiency of 60% was good as compared to the reported value 30% [20], and the *C. japonica* and *C. obtusa* wood chips were suitable as bulking agents for TAD of the excess sludge. In contrast, the digestion efficiency by the *P. densiflora* wood chips was lower. On the other hand, the pH value of the mixture of the *P. densiflora* wood chips and sludge was maintained at about 6, whereas that of the other 2 species, *C. japonica* and *C. obtusa*, reached to about 9 [Figure 2(b)]. Since degradation of organic compounds in sludge may produce ammonium [21], one of the plausible explanations for the lower digestion efficiency might be the lowering pH by the *P. densiflora* wood chips by possible release of acidic compounds. In addition, *C. obtusa* and *C. japonica* are known to

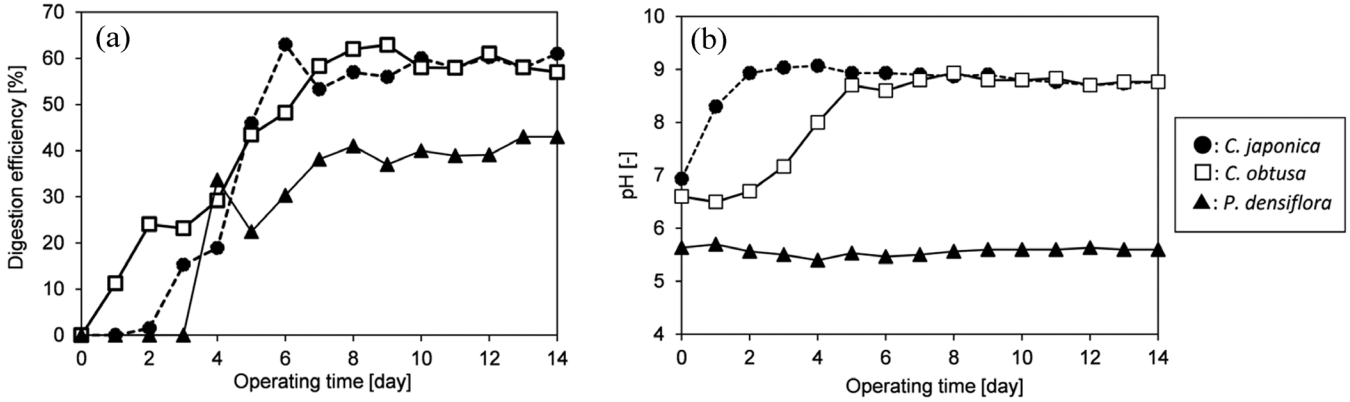

Figure 2. *Time course of digestion efficiency (a) and pH value of the mixture (b) in the TAD device.*

have antibacterial substances [17,22], and therefore it was also expected that the antibacterial activity might result in the higher digestion efficiency. Furthermore, an effect of different porous structures of the wood chips should also be tested as a possible cause for the different digestion efficiency.

Bacterial Community Structures in the TAD Devices

The PCR-DGGE analysis was carried out to compare the bacterial community in the TAD devices. Although the same bacterial community was used in all experiments, the DGGE profiles of each sample of *C. japonica*, *C. obtusa*, and *P. densiflora* were different after 10 day of the digestion (Figure 3). The common bacterial species emerging in the samples of *C. japonica*, *C. obtusa*, and *P. densiflora* appeared as the bands *b*, *g*, and *l*. On the other hand, the bands *a* and *f* appeared at the common positions in the samples of *C. japonica* and *C. obtusa*, whereas these bands didn't appear in that of *P. densiflora*. In addition, each sample had specific bands, such as *c* in the *C. japonica*, *d*, *e*, and *h* in *C. obtusa*, and *i*, *j*, and *k* in *P. densiflora*. Although the role of each bacterium was not investigated, the result confirmed that use of the wood chips of different tree species caused the different bacterial community structures, which might result in the different digestion efficiencies.

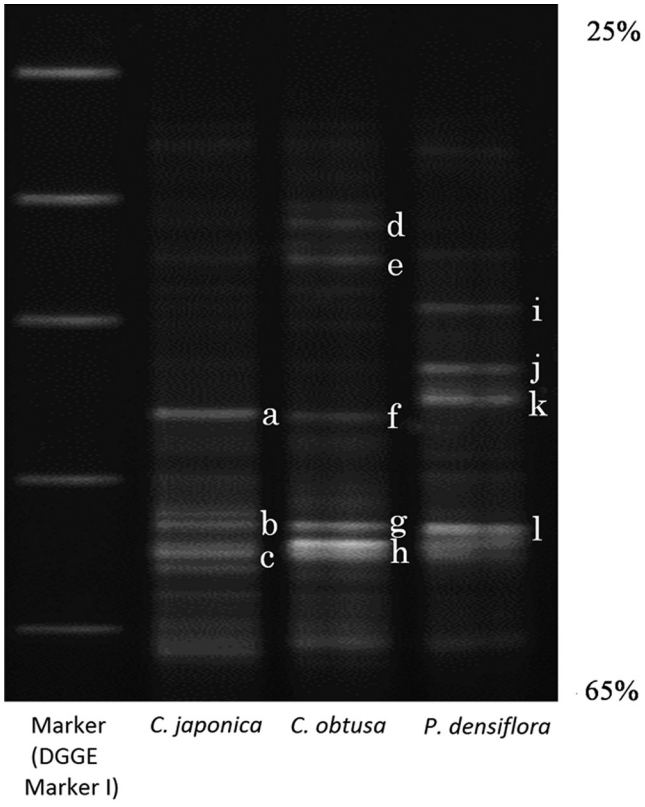

Figure 3. *DGGE patterns of the bacterial community structures in the mixtures containing C. japonica, C. obtusa, and P. densiflora wood chips.*

Effect of Porous Structure

Chang *et al.* [23] reported that the smaller porous size (< 0.4 mm) of poly(vinyl alcohol) (PVA)-derived porous media leads to higher moisture retention and higher bacterial adhesion. In addition, the PVA-derived porous media promoted better organic matter digestion than the wood chips. Since small pore size naturally results in large surface area of a bulking agent, an effect of surface area of bulking agent on the digestion efficiency of TAD was investigated by measuring its pore cross-section area.

The pore cross-section area of *C. obtusa*, *C. japonica*, and *P. densiflora* wood chips were about 500, 1,600, and 1,700 μm^2, respectively (Figure 4). Although no significant difference in pore cross-section area was observed between *C. japonica* and *P. densiflora* wood chips, the digestion efficiencies were different. In addition, the *C. obtusa* and *C. japonica* wood chips have different pore cross-section area; however the digestion efficiencies were same. Furthermore, a microscope observation confirmed the absence of sludge in the pore structure of all wood chips (not data shown), thereby indicating that the pore sizes of wood chips do not affect the digestion performance at the range from 500 to 1,700 μm^2 of pore cross-section area. Since the use of *C. japonica* wood chips produced by the hammer mill resulted in much lower digestion efficiencies,

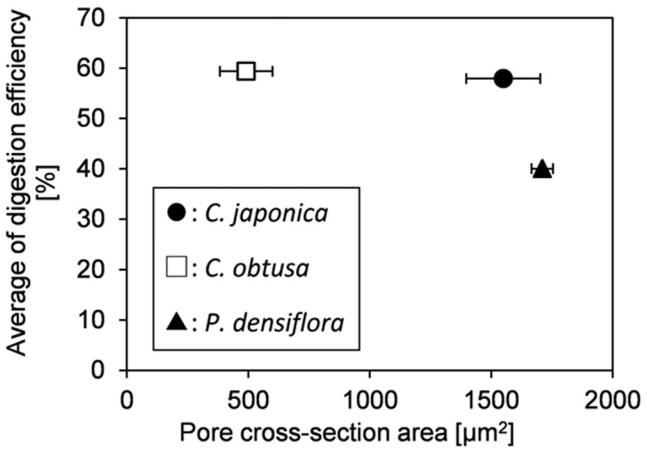

Figure 4. *Relationship between the pore cross-section area and digestion efficiency calculated as the average value from day 7 to day 14.*

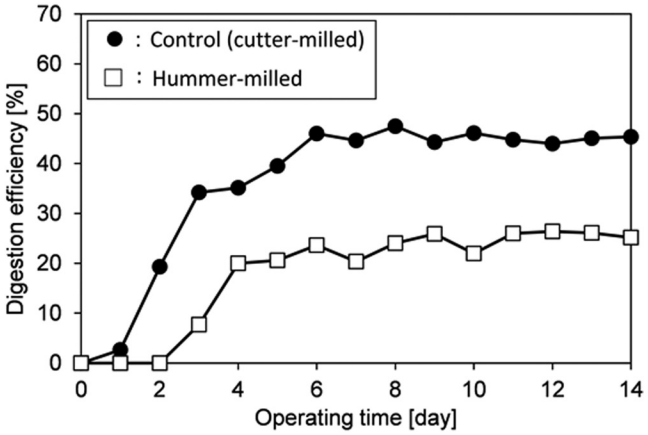

Figure 5. *Time course of the digestion efficiency in the TAD device using the C. japonica wood chips.*

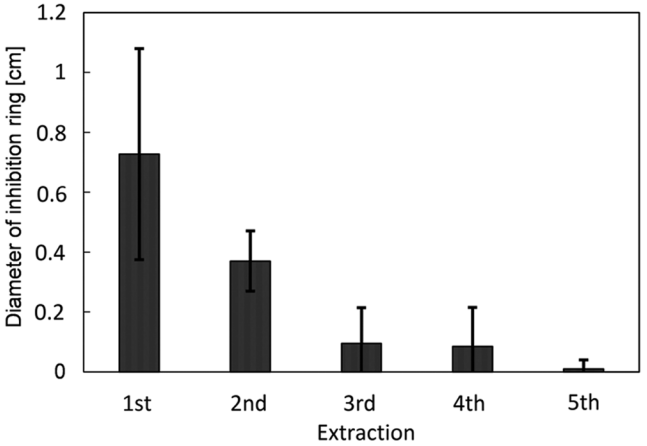

Figure 6. *Diameter of the inhibition ring by the ethanol extract of C. japonica wood chips.*

about 25% (Figure 5), the existence of pore structures in wood chips itself is necessary for TAD via some other mechanisms, such as control of moisture content and air supply.

Influence of the Antibacterial Activity

After the five times-ethanol extraction, the antibacterial activity of *C. japonica* wood chips became negligible (Figure 6). Furthermore, the hexane extract did not show any antibacterial activity (data not shown). When the antibacterial substances-removed *C. japonica* wood chips were used for the TAD experiment, surprisingly the digestion efficiency did not change (Figure 7). This result clearly indicates that the inclusion of antibacterial substances does not affect the TAD performance.

Influence of Lowering pH

The pH values of the mixture of the *P. densiflora* wood chips and sludge was about 6 and lower than that including the other wood chips [(Figure 2(b)]. In addition, the water extract of *C. obtusa*, *C. japonica*, and *P. densiflora* wood chips showed the pH values of 6.0, 5.3, and 4.6, respectively. This confirmed that the acid leachability of *P. densiflora* wood chips was the highest among the three wood species. We expected that the lowering pH to 6 as a result of acid leaching from *P. densiflora* wood chips might cause the lower digestion efficiency [Figure 2(a)].

When the mixture of *C. japonica* wood chips and excess sludge was adjusted to pH 9 with the phosphate buffer, the digestion efficiency was not changed and remained to be 60%. On the other hand, when the pH is adjusted to 6, the digestion efficiency was reduced to less than 40%, which was the same as that obtained by *P. densiflora* wood chips [Figures 2(a) and 8]. These results confirmed that the low digestion efficiency of *P. densiflora* wood chips was accounted by the lowering pH.

CONCLUSIONS

In this study, TAD of sewage sludge was performed using *Chamaecyparis obtusa*, *Cryptomeria japonica*, and *Pinus densiflora* wood chips as bulking agents. Digestion efficiencies varied with tree species of wood chips, though common bacterial species existed in the mixtures prepared using *C. obtusa*, *C. japonica*, and *P. densiflora* wood chips. About 60% of digestion efficiency was attained by *C. obtusa* and *C. japonica* wood chips while that by *P. densiflora* was only 40%.

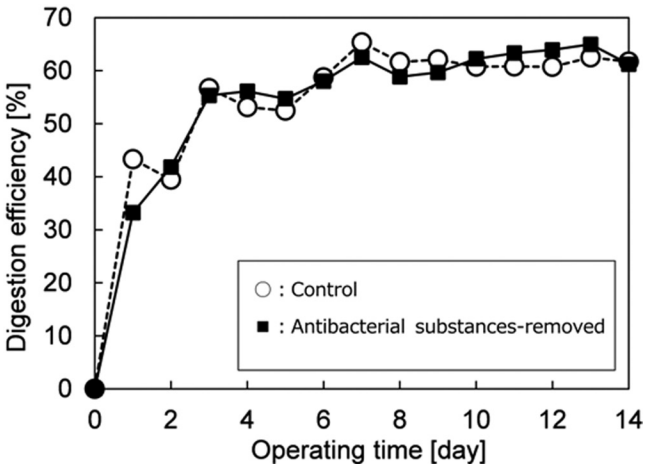

Figure 7. *Time course of the digestion efficiency in the TAD device using the C. japonica wood chips.*

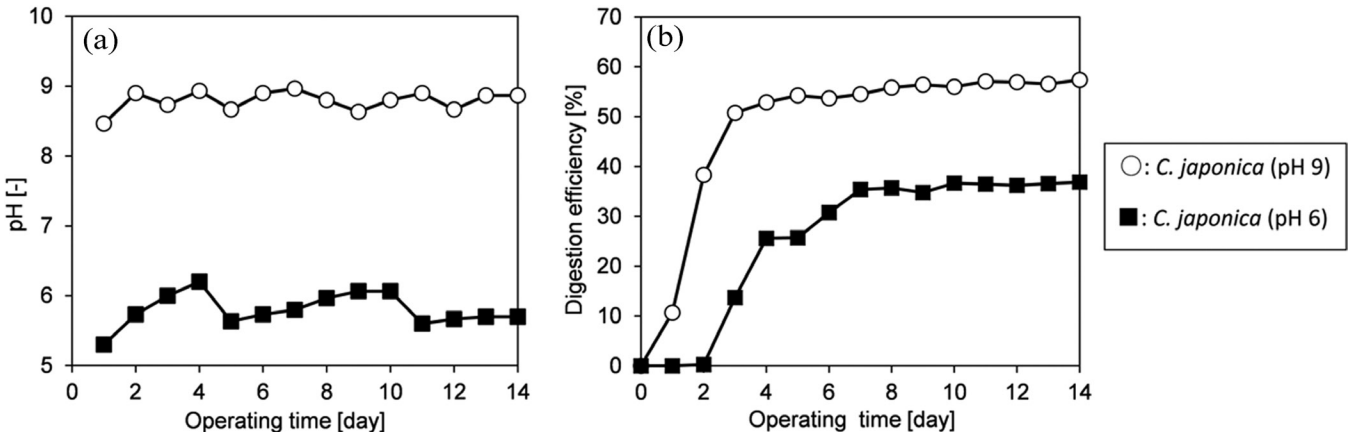

Figure 8. *Time course of the pH value (a) and digestion efficiency in (b) the TAD devices to which phosphoric buffer was added.*

Neither the porous structures of wood chips between 500–1,700 μm^2 of pore cross-section area nor the inclusion of antibacterial substances affected digestion efficiency. *P. densiflora* wood chips have the highest acid leachability and lowering pH to 6 was responsible for the lowest digestion efficiency by the *P. densiflora* wood chips.

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Effect of Sludge Particle Size on Uptake of Cs-137 by Two Leaf Vegetables

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ABSTRACT: Sludge generated in drinking water treatment plants in the Tohoku and Kanto regions has become contaminated with radiocesium (Cs-134 and Cs-137) released from the 2011 accident at the Fukushima Daiichi Nuclear Power Plant. If the sludge is reused as a material for potting mixes for home gardens, vegetables grown in the mixes will take up radiocesium. Since sludge contains various size particles, the effect of sludge particle size on the uptake of Cs-137 by leaf vegetables was determined. Active root uptake of Cs-137 was found in the experimental potting mix including powder particles of the contaminated sludge.

INTRODUCTION

THE Fukushima Daiichi Nuclear Power Plant (FDNPP) accident has caused serious radioactive contamination in the environment. Among the released radionuclides, the behaviour of Cs-137 has been of concern because a significant amount of Cs-137 (1.5×10^{16} Bq) was released into the atmosphere [1] and the radionuclide has a 30-year half-life. Sludge generated in drinking water plants in and around the Tohoku and Kanto regions has become contaminated with Cs-137. Before the accident, the sludge was effectively reused as materials for construction, playground soils, and potting mixes [2]. Various vegetables are planted in potting mixes in home gardens, and thus the contaminated sludge can be expected to be a source of Cs-137 for vegetables.

Since April 1, 2012, the Japanese government has applied the standard limit for radiocesium in general foods (100 Bq kg^{-1}) to agricultural crops to ensure their safety [3]. Local governments have been monitoring the presence of Cs-137 in locally grown agricultural crops, but measurement of Cs-137 in vegetables harvested in home gardens has generally not been carried out before consumption. Intake of Cs-137-contaminated vegetables causes internal radiation exposure, and thus the transfer of Cs-137 from potting mixes to vegetables must be kept to the minimum.

The authors recently demonstrated the root uptake of Cs-137 from a potting mix containing the contaminated sludge by *Brassica rapa* var. *perviridis* (Japanese name: komatsuna) [4] and *Brassica oleracea* spp. (Japanese name: mini cabbage) [5]. In those experiments, the particle size of the sludge that was used were not controlled although particle size is likely to affect soil-to-plant transfer factor [6,7]. In the present study, the effect of sludge particle size on the uptake of Cs-137 by two leaf vegetables, komatsuna and *Brassica rapa* var. *chinensis* (Japanese name: chingensai) is shown.

MATERIALS AND METHODS

Preparation of Cultivation Potting Mixes

The elemental composition of the sludge used in the cultivation potting mixes is listed in Table 1. The sludge was obtained from a drinking water treatment plant in Saitama Prefecture. Two kinds of sludge that differed in particle size, called untreated sludge and powdered sludge, were used. The untreated sludge had various particle sizes ranging to less than about 20 mm. For the preparation of a powdered sludge, 400 g of wet sludge was weighed out and then dried it. The dried sludge was passed through a 2-mm-mesh-sieve and then milling with a grinder (Labo Milser LM-PLUS, Osaka Chemical Co., Ltd.) A base soil mix was made by thoroughly mixing akadama-tsuchi, kuro-tsuchi, leaf-mould and cow manure at the volume ratio of 6:3:0.5:0.5, respectively. Eight grams of chemical fertilizer (8% nitrogen, 8% phosphorus, and 8% potassium by weight) was added to separate (400 g-wet weight) amounts of each

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Table 1. Elemental Compositions (mean ± standard deviation, n = 3) of Sludge.

Element	Concentration, mg/g
Al	95.24 ± 5.43
Ca	2.67 ± 0.36
Cu	0.09 ± 0.01
Fe	38.91 ± 0.54
K	6.85 ± 0.32
Mg	1.84 ± 0.42
Mn	3.05 ± 0.13
Na	6.12 ± 0.27
Sr	0.00 ± 0.00
Ti	2.41 ± 0.07
Zn	0.30 ± 0.01

of the two kinds of sludge and that was mixed into a 2-L amount of the base soil mix (about 1.4 kg) to get two cultivation potting mixes. These mixes contained either the untreated or the powdered sludge and were identified as the untreated potting mix and the powdered potting mix, respectively.

Cultivation Conditions and Analysis Items

Komatsuna and chingensai were grown in a greenhouse for 28 days and for 32 days from May 9, 2013, under natural light, respectively. The temperature was controlled at 25 ± 5°C during the cultivation periods. After the harvest, concentrations of radiocesium (Cs-137 and Cs-134) in vegetables and potting mixes were determined. In addition to the radioactivity analysis, fresh weight, chlorophyll *a*, water content, sugar content and ascorbic acid content were analyzed. Fresh weight and the concentration of chlorophyll *a* were determined to clarify the effect of the powdered particles of the sludge on the growth of vegetables. Water content was measured for the conversion of a dry basis radiocesium concentration to a wet basis one. Sugar content and the concentration of ascorbic acid served as indexes of the nutritional status of the vegetables.

Experiments were independently repeated two times.

Analytical Methods

For the analysis of radiocesium, the samples of vegetables (edible part) and cultivation potting mixes were weighed, and then they were dried and powdered using a grinder. To confirm homogeneity, three soil subsamples from each cultivation potting mix were prepared. Powdered samples were transferred into a U8 polystyrene container for gamma-ray spectrometry. The gamma-ray spectrometry was done using a Ge detector (SeikoEG&G). An efficiency calibration of the detector was made with volume radioactivity standard gamma sources (MX033U8PP, Japan Radioisotope Association). For the measurement of Cs-134, the counting losses caused by coincidence summing effects were calibrated using the relative efficiency of the Ge detector. The measurement values were corrected to the harvest date.

Juice from edible part of the vegetables was prepared immediately after the harvest. Sugar content of the juice was measured by using a refractometer (PAL-S, ATAGO Co., Ltd.) and expressed as Brix %. Brix is a relative density scale based on sucrose in distilled water.

Ascorbic acid was measured with a RQflex 10 reflectometer (Merck KGaA). Vegetables were homogenized in 5% metaphosphate, and the homogenate was centrifuged at 12000 g for 15 min to obtain a supernatant. An ascorbic acid test strip (Merck KGaA) was immersed in the supernatant, and the concentration of ascorbic acid was determined with the reflectometer.

Chlorophyll *a* was extracted from five vegetable leaf discs (∅18 mm) into the solvent *N,N*-dimethylformamide. The optical densities of the extracts at 750 nm, 663.8 nm, and 646.8 nm were measured using a Shimadzu UV-160A spectrophotometer. The concentrations of chlorophyll *a* were calculated according to the equation by Porra *et al.* [8].

Elution

Elution of radiocesium from sludge was determined. Four grams of each sludge type (untreated and powdered) was separately soaked in 40 mL of 1M ammonium acetate placed in a polypropylene tube. This tube was incubated with shaking (100 rpm) for 24 hours at 25°C in the dark, and then the supernatant was filtered through a 0.2 μm cellulose acetate membrane filter. The activity of radiocesium in the filtrate was measured with the Ge detector (Seiko EG&G).

Transfer Factor

The soil-to-plant transfer factors (TFs) were calculated as the ratio of the radiocesium concentration in the plant (Bq kg^{-1} dry weight) to its concentration in a cultivation potting mix (Bq kg^{-1} dry weight).

Table 2. Fresh Weights and Chlorophyll a Concentrations of Vegetables (mean ± absolute value of difference from mean, n = 2).

Vegetable	Cultivation potting mix type	Fresh weight, g/12 plants/pot	Chlorophyll *a*, μg/cm^2
Komatsuna	Untreated	187 ± 23	43.2 ± 0.9
	Powdered	206 ± 9	56.7 ± 0.4
Chingensai	Untreated	195 ± 10	20.3 ± 1.9
	Powdered	221 ± 1	22.7 ± 2.2

RESULTS AND DISCUSSION

Effect of the Powdered Sludge on the Growth of Vegetables

Soil texture and structure affect plant growth through their effects on water holding and gas exchange [9]. The powder sludge granules may cause a decrease in efficiency of gas exchange due to clogging of void structure in potting mixes, and this may influence the growth of the plant. To confirm the effect of the powder sludge on the plant growth, fresh weight at the harvest and chlorophyll *a* content were compared between plants grown in the two different cultivation potting mixes (Table 2). The fresh weights and chlorophyll *a* contents were higher for the vegetables grown in the cultivation mix of the powdered sludge and that suggested the powdered sludge did not inhibit the growth of the vegetables.

Radiocesium Contamination

Cesium-134 derived from the 1986 Chernobyl accident and past atmospheric nuclear testing mainly in the 1960s should have decreased to below the detection limit in the sludge because this radionuclide has a half-life of about 2 years. Therefore, the Cs-134 in the cultivation potting mixes (Table 3) should be contamination that resulted from the FDNPP accident.

Generally, powdered granules can easily move through the void structure in soils. The potting mix layer in the present experiments was about 10 cm deep, and thus there was the possibility for loss of the powdered granules during cultivation. Because the main source of radiocesium in the potting mixes is the sludge, a significant loss of the contaminated granules would mean a decrease in the concentration of radiocesium in the mixes. However, only slight differences in radiocesium concentrations between the untreated and the powdered potting mixes were observed (Table 3). These results suggest that there was no significant loss of the sludge granules from the pots during the cultivation periods.

Contamination of radiocesium in vegetables occurs mainly by root [10] and foliar uptake [11]. For foliar uptake, adsorption of radiocesium on vegetables is required. Thus, events such as fallout deposition promote foliar uptake. Because there was no fallout deposition of radiocesium in the greenhouse during the experimental period, the radiocesium detected in komatsuna and chingensai was taken up through their roots. Root uptake of radiocesium was also observed to occur by mini cabbages [5].

The concentration of radiocesium in chingensai was 130.1 Bq kg^{-1}-dry when this vegetable was grown in the powdered potting mix (Table 3). For the komatsuna samples, the concentrations were less than 100 Bq kg^{-1}-dry. Since the Japanese standard limit for radionuclides in general foods (100 Bq kg^{-1}) is based on the concentration on wet weight basis [3], the concentrations on dry weight basis were converted to those on the wet weight basis using measured water contents. The maximum concentration of radiocesium was 4.9 Bq kg^{-1}-wet for chingensai grown in the powdered potting mix. From these estimations, the radiocesium concentrations in vegetable samples in the experiments were well below the limit.

The Ministry of Health, Labour and Welfare permits

Table 3. Concentrations of Radiocesium in Cultivation Potting Mixes and Vegetables (mean ± absolute value of difference from mean, n = 2).

Vegetable	Cultivation Potting Mix Type	Concentration, Bq/kg-dry				Water content, %	Concentration, Bq/kg-wet	
		Mix		Vegetable			Vegetable	
		Cs-137	Cs-134	Cs-137	Cs-134		Cs-137	Cs-134
Komatsuna	Untreated	608 ± 50	294 ± 21	39.9 ± 3.7	21.8 ± 3.1	93.6 ± 0.8	2.6 ± 0.1	1.4 ± 0.0
	Powdered	556 ± 30	277 ± 13	52.9 ± 9.7	28.4 ± 1.1	94.6 ± 0.2	2.8 ± 0.4	1.5 ± 0.1
Chingensai	Untreated	568 ± 5	281 ± 5	53.9 ± 14	28.6 ± 8	96.2 ± 0.2	2.1 ± 0.4	1.1 ± 0.3
	Powdered	567 ± 69	282 ± 22	84.5 ± 19	45.6 ± 16	96.3 ± 0.1	3.2 ± 0.8	1.7 ± 0.7

the shipment of sludge with radiocesium of less than 400 Bq kg^{-1}-wet from drinking water treatment plants [12]. Sludge is generally mixed with various types of soils at the mixing ration of 10 to 20 weight percent as commercial potting mixes [12], and thus the concentration of radiocesium in such a potting mix was calculated to be about 80 Bq kg^{-1}-wet for 20 wt% sludge. In compliance with the basic concept of the efficient use of radiocesium-contaminated sludge, the concentration of radiocesium in vegetables, at least komatsuna and chingensai, will not exceed 100 Bq kg^{-1}-wet. Even if the radiocesium concentration is lower than the regulation value, it is necessary to minimize the contamination by radiocesium for the health safety of consumers.

It should be noted that vegetables which were grown in the powdered potting mix tend to have higher concentrations of radiocesium compared with those grown in the untreated potting mix (Table 3). These results suggest that particle size might affect the root uptake of radiocesium. The effect of particle size has also been observed for vermiculite as a Cs adsorbent [13]. The use of sludge having large particle sizes would minimize the uptake of radiocesium into vegetables. Even if the uptake of radiocesium is minimized, the intended purpose for eating the vegetables may not be achieved if their nutritional status is significantly changed for the worse. Sugar and ascorbic acid contents were measured as indexes of nutritional status. Slight differences for both indexes were observed under the experimental conditions (Table 4). The results support the effectiveness of the use of the sludge with large particle sizes to minimize the contamination from uptake of radiocesium.

Transfer Factors

Soil-to-plant transfer factor (TF) is regarded as one of the most significant parameters in environmental safety assessment for nuclear facilities [14]. In the present study, the TF values for Cs-137 were calculated (Figure 1) because this radionuclide has a half-life of about 30 years and remains in the environment for decades. TF values were from 0.066 to 0.15 and were within the range of previously published values (0.041–0.17) for leaf vegetables [15], which were found before the FDNPP accident.

Table 4. Contents of Sugar and Ascorbic Acid in Vegetables (mean ± absolute value of difference from mean, n = 2).

Vegetable	Cultivation Potting Mix Type	Sugar Content, Brix%	Ascorbic Acid, mg/100 g
Komatsuna	Untreated	2.9 ± 0.6	26.8 ± 5.1
	Powdered	2.4 ± 0.4	27.4 ± 7.4
Chingensai	Untreated	2.2 ± 0.1	18.4 ± 0.3
	Powdered	2.0 ± 0.1	18.8 ± 1.6

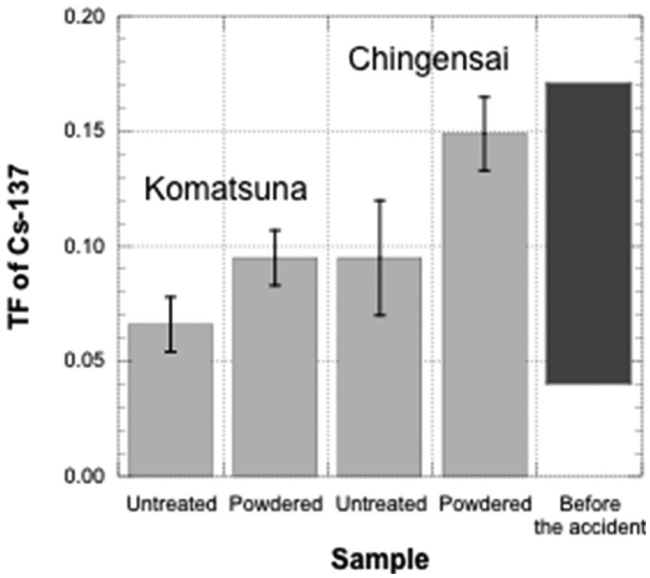

Figure 1. *Soil-to-plant transfer factors (TFs) of komatsuna and chingensai obtained in this study, and the range of TF of leaf vegetables before the FDNPP accident [15]. Error bars represent ± absolute value of difference from mean (n = 2).*

Effect of the Powdered Sludge

Pulverization of soils is often performed as a pretreatment for extracting organic substances and inorganic elements. The relatively higher concentrations of Cs-137 in the vegetables grown using the powdered potting mix may be caused by a high elution rate of Cs-137 from the powdered sludge, which was crushed physically. Therefore, elution of Cs-137 from the sludge in 1 M ammonium acetate was tested. No difference in the eluted Cs-137 activity was found between the untreated sludge and the powdered sludge: the concentrations of Cs-137 were 0.039 ± 0.0043 Bq mL^{-1}-supernatant for the untreated sludge and 0.037 ± 0.0035 Bq mL^{-1}-supernatant for the powdered sludge. No effect by pulverization of sludge was recognized. The surface area per unit volume of the sludge increased with decreasing particle size, and thus the contact areas of plant roots would increase; it was likely that this surface area difference caused the Cs-137 concentration difference.

CONCLUSIONS

Effect of sludge particle size on root uptake of Cs-

137 by komatsuna and chingensai grown in potting mixes were studied and values of sludge-to-plant transfer factors were within the range of values obtained before the FDNPP accident. The transfer of Cs-137 from sludge to vegetables is dependent on particle size of sludge. It is expected that use of sludge with large particle sizes minimizes radiocesium contamination in vegetables. These results provide information related to health and safety when considering reuse of drinking water treatment sludge in potting mixes.

ACKNOWLEDGEMENTS

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Experimental Study for Elemental Mercury Removal Using 5% Co-Ti-Pillared Clays Catalysts

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ABSTRACT: Experimental study on elemental mercury removal by 5% Co-Ti-Pillared clays catalysts was conducted in simulated flue gas with the addition of HCl. Effects of experimental conditions and gas components on the removal efficiency of zero-valent mercury (Hg^0) catalytic were investigated. Results show that the addition of HCl in flue gas would significantly promote Hg^0 removal efficiency. NH_3/NO in flue gas would inhibit the activity of catalyst. The adsorption of water vapor and Hg^0 would change into a competitive mechanism form which would influence Hg^0 catalytic removal efficiency.

INTRODUCTION

EMISSIONS of mercury in flue gas from coal-fired power plant are the main source of atmospheric mercury pollution [1], enriching mercury in the natural environment and posing threats to biological and human health [2,3]. At the beginning of this decades, the United States began to control mercury pollution by coal-fired power plants and promulgated coal mercury emission standards in the first place [4]. Main combustion coal adopted in China in the power plants is lignite [5], which contains a high percentage of mercury and causes serious air pollution. Therefore, mercury pollution in flue gas from coal-fired power plants needs to be addressed effectively in a timely fashion.

Mercury in flue gas is mainly in the form of three states, including particulate state (Hg_p), oxidation state (Hg^{2+}) and elemental state (Hg^0) [6]. Hgp is collected along with dust in the course of dust extraction. Hg^{2+} can be dislodged during the dust extraction or wet process of flue gas desulfurization (FGD) system. Hg^0 has stable character and does not dissolve in water and acid, which brings a challenge [7] to the removal of mercury in the flue gas. It is widely accepted that oxidize Hg^0 to Hg^{2+} can be measured effectively and the oxide form (Hg^{2+}) can be removed by the wet process of FGD. Studies show that [8] some of Hg^0 could be oxidized with certain reaction with the catalyst of Selective Catalytic Reduction (SCR) and a certain concentration of HCl in flue gas. In view of the fact that the electronic shell of transition metal is not full, oxidization of which could play a catalytic role. An experiment using fly ash [9] containing CuO was carried out to oxidize Hg^0. Results from their study showed that mercury catalytic removal efficiency was increased significantly due to CuO.

Some studies show that [10,11] pillared interlayered clays (PILCs) can be used as a supporter of SCR catalysts to achieve better reaction activity and selectivity. In addition, they are as a new molecular sieve catalyst material developed in recent years, pillared clays have several features, e.g., a large surface area, good resistance to sulfur, and strong surface acidity. It performs better than commercial metal oxide catalysts in the current load of substance denitration. Studies have shown that CoOx as a catalyst has higher catalytic oxidation activity [12,13]. While there are many studies in the literature focusing on the development of Co/TiO_2 in catalyst, use of Co-Ti-Pillared for mercury removal has not been studied.

This study uses an experimental approach to study the effectiveness of Co-Ti-Pillared clays as catalyst for mercury removal in flue gas. To further study the basis of Hg^0 removal mechanism, Hg^0 removal under different reaction conditions is examined.

EXPERIMENTAL METHOD AND EQUIPMENT

Preparation of Catalyst

Sodium chloride (NaCl) solution is first used to soak montmorillonite to prepare sodium montmorillonite, and then dip-molding is adopted to add a cer-

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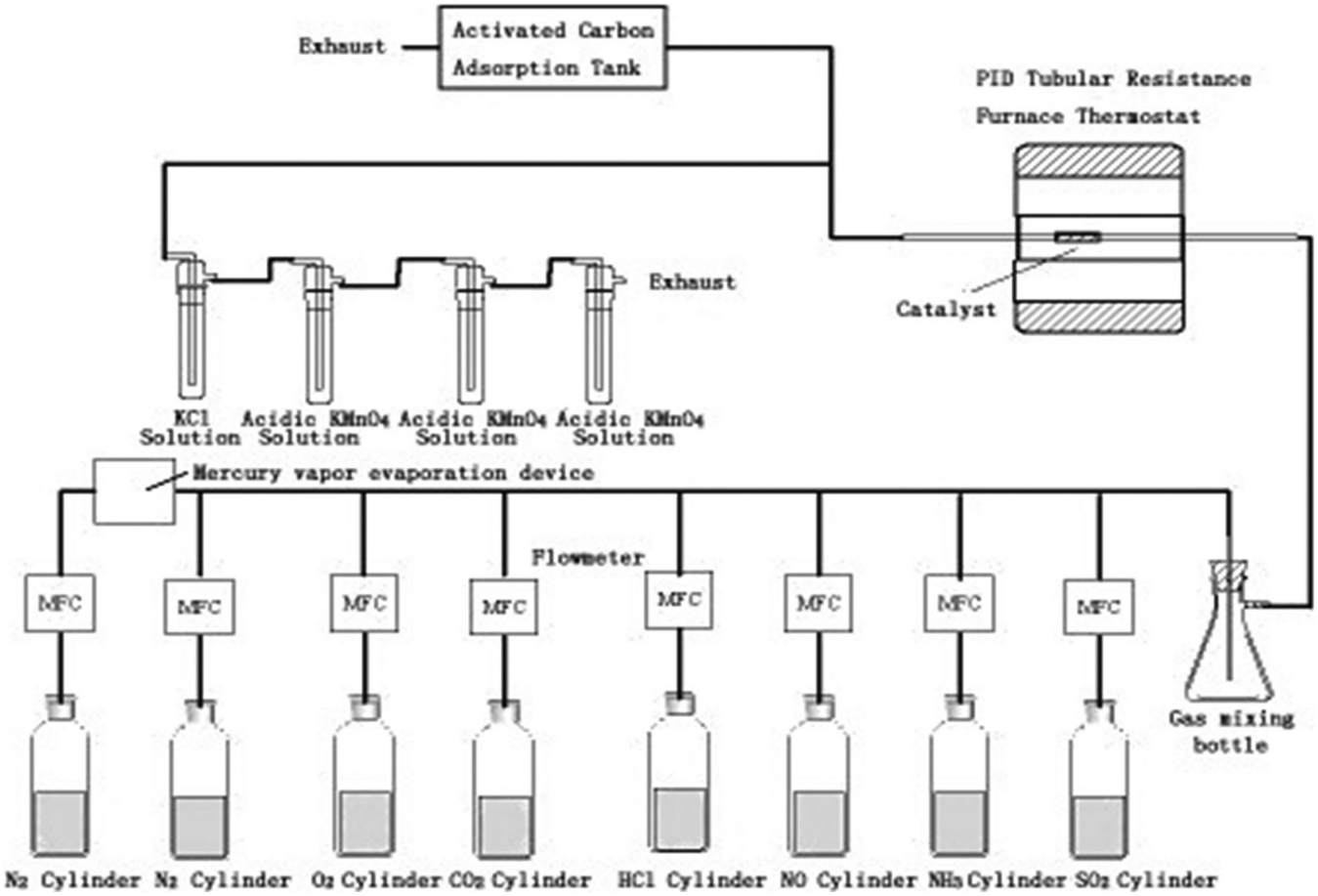

Figure 1. *Dynamic simulation system of mercury removal in flue gas.*

tain concentration of titanium crossing-linking agent to aqueous solution of sodium montmorillonite. It is fully mixed before the processes of sucking filtration, desiccation and calcination in order to prepare Ti-PILCs. Finally, cobalt nitrate is used as presoma and Ti-PILCs is added to cobalt nitrate solution in 60°C to conduct IX (ion exchange). Catalyst is dried in 110°C for 12 h; it is then transposed in close roaster in 400°C to prepare Co-Ti-PILCs catalyst.

Testing System and Equipment

The dynamic simulation system is made up of a gas distribution system, a mercury permeation evaporation system, a catalytic reactor, a temperature controlling system and a dynamic simulation system shown in Figure 1. The mercury permeation tube immerges in thermostatic oil bath, and it is able to offer a certain concentration of element mercury vapour under the carrier gas flow of N_2. The catalytic reactor is put in tubular electric furnace. It has a temperature programming and is filled with 3 g catalyst in quartz glass tube with inner diameter of 10 mm and length of 1000 mm. The mercury absorption device is made up of four large-scale bubble absorption tubes, absorption time of which is 15 min. A 5 mL KCl solution in 1 mol/L is used to intake from the reactor. The KCl solution with absorbed Hg^{2+} is then diluted with water in 25 mL. There are three large-scale bubble absorption tubes with 5 mL 5 wt % $KMnO_4$ solution, which are responsible for taking in Hg^0. The mixture is then diluted in 50 mL. The test method for mercury is atomic fluorescence analysis. Analytical instrument is AFS930 atomic fluorescence manufactured by Beijing JiTian instrument limited company.

Test Method

The catalyst weighed 3 g is placed in the reactor. The simulated flue gas is sent into the reactor until the concentration of the reactor's inlet and outlet is stable. This aims to eliminate the interference of adsorption on catalytic removal efficiency. The concentration of Hg^0 is in the reactor is 0.3 mg/m^3. HCl is passed into

the simulated flue gas. At the same time, the temperature of the tube furnace is regulated (200–400°C) to test Hg^0 concentration at the inlet and outlet near the reactor, which are used for the calculation of Hg^0 removal efficiency.

RESULTS AND DISCUSSION

Effect of Reaction Temperature on Catalytic Hg^0 Removal Efficiency

In the simulated reactor, the effect of different temperature on catalytic removal of under a defined condition is investigated. Under the defined condition, airspeed is 1.0×10^4 h^{-1}, HCl concentration is 10×10^{-6} (V/V) and catalyst is Co-Ti-PILCs of 5 wt.%. Removal efficiency of Hg^0 under different temperature is shown in Figure 2.

As shown in Figure 2, the catalytic activity of Co-Ti-PILCs shows a downward trend after the first rising as the reaction temperature increases. The most suitable reaction temperature was 300°C, of which removal efficiency reached to 86.7%. When reaction temperature ranged from 200°C to 300°C, Hg^0 removal efficiency increased from 65% to 86.5%. When reaction temperature ranged from 300°C to 400°C, Hg^0 removal efficiency decreased from 86.7% to 70%. At 300°C, the catalyst activity is best, promoting the conversion from HCl to Cl. Hence, more Cl [14] is involved in the oxidation reaction. However, when the temperature further rises, catalyst activity is limited. The produced Cl was decreased, resulting to reduction of efficiency of the catalytic oxidation.

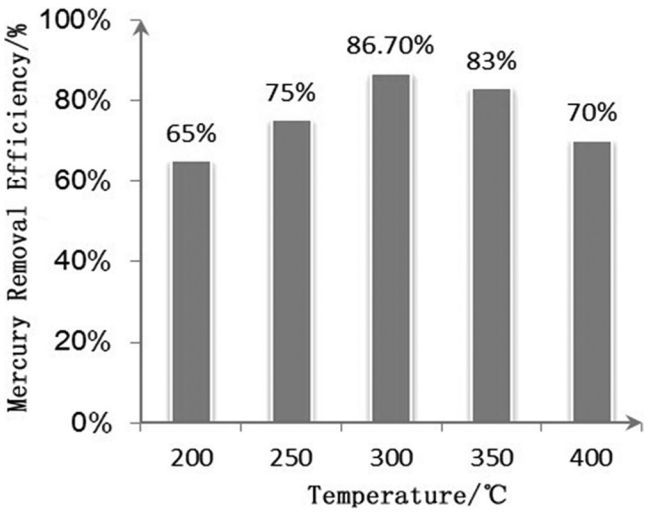

Figure 2. *Effect of temperature on Hg^0 removal efficiency.*

Effect of HCl in Flue Gas on Hg0 Removal Efficiency

Studies have shown that [15] HCl can be used as an oxidizing agent in the catalytic oxidation reaction of Hg^0. During catalytic oxidation, strong oxidizing substances (Cl_2, Cl atom and O atom) are produced by the reaction between O_2 and HCl. These strong oxidants can oxidize Hg^0 to Hg^{2+} [16]. To further examine this, the effect of HCl in flue gas on catalytic oxidation of Hg^0 is investigated in this study. The 5 wt% Co-Ti-PILCs Ti/clay is 15 mmol/g, calcinations temperature is 400°C) is used. Airspeed is maintained at 10,000 h^{-1}. HCl gas of different concentration is added to the simulated flue gas. The effect of HCl on catalytic removal of Hg^0 at 200~400°C is examined. Results are displayed in Figure 3.

The HCl in the flue gas can significantly improve the catalytic oxidation efficiency of the zero-valent mercury. During the catalytic process, HCl is converted to Cl and Cl_2 which have strong oxidization and promote the oxidation of Hg^0. When the concentration of HCl increased from 5×10^{-6} (V/V) to 10×10^{-6} (V/V), the removal efficiency of mercury was significantly enhanced. The largest mercury removal rate reached to 86.7%. The increasing concentration of HCl has led to a higher level of Cl and Cl_2 at the active sites of the catalyst, which improved the removal efficiency of the mercury. However, when the concentration of HCl further increased, the removal efficiency of mercury in flue gas barely increased. This is due to the fact that the concentration of HCl was already very high and the number of active sites of the catalyst was relatively small, limiting the catalytic oxidation of Hg^0.

Effect of NH_3/NO on Hg^0 Removal Efficiency

Flue gas condition may influence mercury catalytic

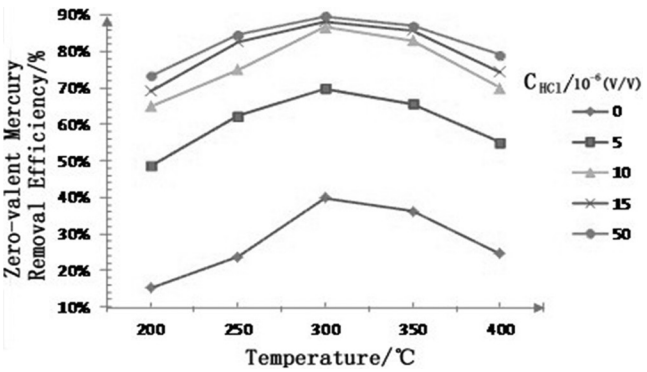

Figure 3. *Effect of HCl in flue gas on Hg^0 removal efficiency.*

removal efficiency, especially when abundant NH_3/NO exists in the SCR reactor. The experiment setup was as follows: 5 wt % Co-Ti-PILCs (Ti/clay as the tendency of 15 g, calcination temperature is 400°C) in simulated reactor with airspeed 10000 h^{-1}, HCl gas in the concentration of 10 ppm in the simulated flue gas, NH_3 and NO are in the concentration of 200×10^{-6}~600×10^{-6} (V/V). The results are shown in Figure 4.

In the presence of NH_3 and NO, the mercury removal efficiency reduced to some extent. With the increasing concentration of NH_3/NO, mercury removal efficiency dropped dramatically. Results show that NH_3/NO in flue gas have inhibited the catalytic activities. In the catalytic reaction system, NH_3 was used as reducing gas, and Hg^0 presents oxidation reaction in the catalyst has activated sites. The catalyst adsorbed NH_3, thus part of the active sites were occupied. This has limited the participation of Hg^0 and HCl in oxidation reaction in active sites. At the same time, some parts of NH_3/NO participate in catalytic reduction reaction in these active sites directly, resulting in competition with catalytic to oxidize Hg^0.

Effect of Water Vapor in Flue Gas on Hg^0 Removal Efficiency

The experimental setup is as follows: Co-Ti-PILCs at concentration of 5 wt.% and Ti/clay concentration is 15 mmol/g under calcination temperature of 400°C in simulated reactor with airspeed 10,000 h^{-1}; A certain concentration of HCl gas is added in the simulated flue gas (10 ppm). Under a certain concentration of water vapor, such experiment setup is used to study the effect of water vapor on the removal of Hg^0. The results are shown in Figure 5.

As shown in Figure 5, mercury removal efficiency in flue gas containing no water vapor is significantly higher than that of gas containing vapor. This demonstrates that water vapor can inhibit removal to some extent. Hg^0, HCl and H_2O molecules in flue gas are competitive in adsorption of Hg^0 in the active sites on the surface of the catalyst, resulting in a decline in the activities of the catalyst. It also shows that water resistant thermal performance of the catalyst is good, which may be related to its supporter Co-Ti-PILCs. It has good hydrophobic properties due to crosslinking column surface, resulting in strong water vapor resistant [17,18].

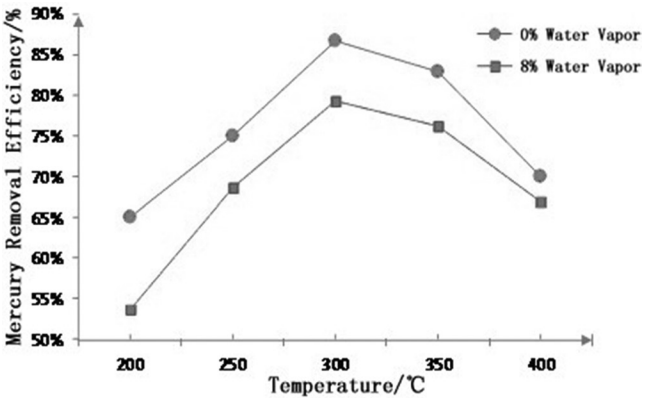

Figure 5. Effect of water vapor in flue gas on Hg^0 removal efficiency.

Effect of Airspeed on Hg^0 Removal Efficiency

Airspeed reflects the time flue gas spent on the surface of the catalyst, determining the degree of the reaction. This experiment adopts Co-Ti-PILCs 5 wt.% at 300°C with 10^{-6}(V/V) to study mercury removal efficiency in a simulated reactor in 6,000 h^{-1}~14,000 h^{-1}. The results are shown in Figure 6.

As shown in Figure 6, Hg^0 removal activity has gradually weakened with the increase of space velocity. The catalytic Hg^0 removal efficiency declined from 92.1% to 79.5% when airspeed increased from

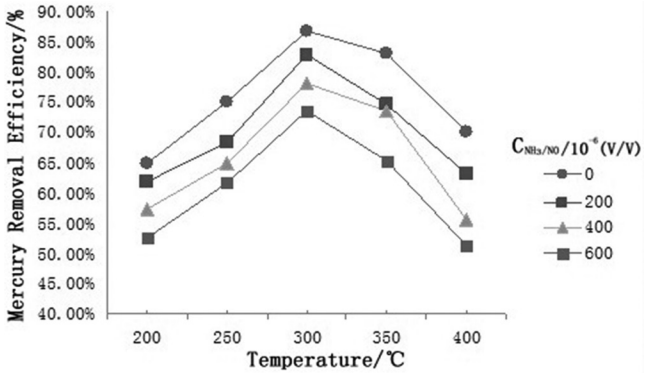

Figure 4. Effect of NH_3/NO on Hg^0 removal efficiency.

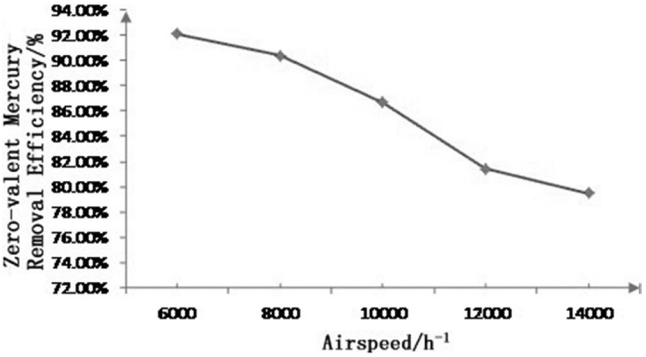

Figure 6. Effect of airspeed on Hg^0 removal efficiency.

6,000~14,000(10^{-6}V/V)h^{-1}. Because of the increasing airspeed, the contact time of catalyst and flue gas declined. Hg^0 was not fully spread to active sites on the catalyst.

CONCLUSION

The 5 wt.% Co-Ti-PILCs has a higher activity at 300°C. The working temperature of SCR is 300–350°C. The catalyst can be synchronized to remove the mercury in the SCR reactor which provides a reference for realizing mercury removal synchronously in SCR reactor.

HCl in flue gas can significantly promote Hg^0 removal efficiency. While NH_3/NO in the flue gas may limit the activity of the catalyst to some degree, water vapor in flue gas can form a significantly competitive mechanism with the adsorption of zero-valent mercury. They both inhibit mercury removal. In addition, with increasing airspeed the catalyst's performance of mercury removal gradually declines.

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Raw and Treated Coal Fly Ash Amendment Aiming for Water Holding Capacity Adjustment of Natural Soils

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ABSTRACT: The effects of raw and treated coal fly ash (FA) amending on water holding capacity (WHC) of natural soils were investigated. Apatite mineral was synthesized on FA particles or FA surface was modified by chitosan, sodium alginate, or guanidine hydrochloride. WHC was measured as cumulative water retention during 12-hour drying experiments at 40°C or under natural condition. Experimental results suggest that raw FA amendment at lower temperature is preferable to increase soil WHC. On the other hand, inorganic and organic treatments gave FA positive dependency on temperature. Such treatments are more effective to increase soil WHC at higher temperature. This research found no correlation between WHC and water repellency of FA.

INTRODUCTION

COAL is one of major electric power sources all over the world. About 40% of electricity is generated by coal-fired power plants in year-2010 global average [1]. 50% increase of global coal consumption is expected from 2010 to 2040 [1]. Fly ash (FA) and bottom ash (BA) are two major by-products from coal-fired power generation. Global FA generation reaches to 750 million Mg/yr [2]. China, United States, and India are largest coal consumers in the world [3]. For example, China consumes 1439.5 million Mg of bituminous coal per year [4] and produces about 100 million Mg of coal combustion by-products. FA management is a severe problem in particular for large coal-consuming countries like China. As the world average, FA recycle ratio is estimated to be less than 50% [2] or about 25% [5]. Thus, huge amount of FA has not been used but disposed in controlled landfill sites and/or open dumping sites. Its improper disposal has become an environmental concern according to potential emission of toxic elements contained in FA [2,6–7]. Therefore, it has motivated many researches on FA recycles such as soil amelioration, construction industry, ceramic industry, etc. [7–10]. This study focuses on FA application for soil amelioration, in particular water holding agents from the view point of anti-desertification. Desertification is a large social problem in some countries like China and the U.S., where huge amount of FA are produced by coal-fired power generation [11–13]. Although anti-desertification activities needs integrated approaches, water holding agent might be an effective method to reclaim degraded soil [14]. If FA can be recycled as water holding agent after necessary treatment for environmental safety, it will contribute into anti-desertification and FA management at the same time. Many works on FA amending to soil have been conducted to investigate the effect on plant growth [15–18], soil pH [19–21], soil toxic elements [19,22–25], soil salinity [20,25–26], soil fertility [27–30], and water holding capacity (WHC) [31–36]. FA amendment increases soil WHC as well as inorganic or organic amendments with FA [37–41]. In spite of many researches referred above, there still remains some uncertainty about the effect of FA amendment on soil WHC. Although soil WHC depends on organic carbon and soil texture [42–43], their effects are still unclear for FA-amended soil. In addition, temperature dependency of such effects is also uncertain. Therefore, this study aims for investigating the effect of FA amending on soil WHC focusing on their texture, organic carbon, and temperature. Surface-modified FA by inorganic or organic treatment was tested at different temperature to investigate its impact on WHC.

MATERIALS AND METHOD

Soils and Coal Fly Ash Properties

In this research, two kinds of soils were tested,

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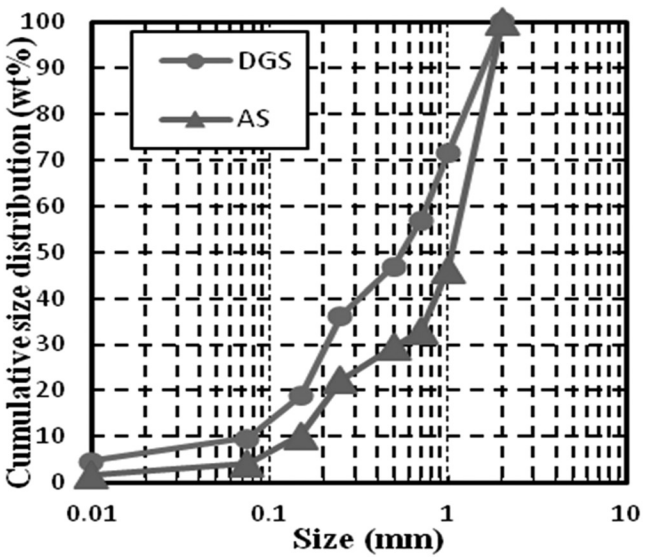

Figure 1. *Cumulative size distribution of two tested soils (Unit: wt%) (DGS: Decomposed granite soil, AS: Akadama soil).*

which were Decomposed Granite Soil (DGS) and Akadama Soil (AS). Coal fly ash (FA) used in this study was taken from a coal-fired power plant in Japan. 100 g of each sample was sieved to measure cumulative size distribution. The cumulative size distribution is plotted as weight percentage and shown in Figure 1. Both soils were comprised primarily of sand-sized particles, whereas most of the fly ashes were primarily fine silt-sized particles. DGS has smaller particle sizes than AS. Coefficients of Uniformity (ASTM D2487) of DGS and AS are 10 and 7.7, respectively. Coefficients of Gradation (ASTM D2487) of DGS and AS are 0.78 and 1.4, respectively.

Elemental content of tested FA, analyzed by Energy Dispersive X-Ray Fluorescence spectrometer, is shown in Table 1. Major component elements of FA are Si, Al, Fe, and Ca, which are the same with other researches [44].

Pretreatment of FA

Soil WHC depends on organic carbon and soil texture [42–43]. In this study, inorganic and organic treatments of FA for its textural modification were tested to investigate its impact on soil WHC when treated FA is amended in soils. In inorganic treatment (basic phosphate treatment), apatite was synthesized on FA particle surface. 400 g of raw FA was mixed with 80 ml of 0.2 mol/L Na_3PO_4 solution first, and then mixed with 80 ml of saturated $Ca(OH)_2$ solution. In organic treatments, three types of organic compounds, chitosan, sodium alginate, and guanidine hydroxide, were tested. 160 ml of 2 wt% of chitosan solution (chitosan treatment), 1 wt% of sodium alginate (alginate treatment), or 160 mL of 3.87 wt% of guanidine hydrochloride (guanidine treatment) was mixed simply with raw FA to increase organic content on FA surface. Raw and treated FA were dried at 105°C for over 24 hours, crushed softly, and then utilized for WHC experiments.

Surface Observation

Microscopic surface conditions of soil, raw FA, and treated FA particles were observed by scanning electron microscope (SEM; JSM-6610LA, JEOL Co.).

Leaching Experiment

Leaching experiments were conducted according to Japan Leaching Test 46 (JLT46). In JLT46 experiments, pure water was used as extractant medium. The weight ratio of liquid to solid (FA sample) is 10. After 6-hour leaching at shaking rate of 200 rpm without any pH adjustment, leachate was filtrated with 0.45 μm mesh membrane and then digested with HNO_3 at above 120°C for longer than 12 hours. Leached elements were measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES; SPS5510, SII-NT Co.).

Water Holding Capacity Measurement

Water holding capacities (WHC) of soil, raw FA, and treated FA were measured by drying experiments. 7.5 g of pure water was mixed with 17.5 g of each dried sample to adjust initial water content as 15 wt%. Moistened samples were dried under natural condition (around 20°C) for 12 hours or dried isothermally in an oven at 40°C for 12 hours. The weights of moistened samples were measured at 1 hour intervals to monitor water retention in the sample. They are repeated twice or three times to check data reproducibility and

Table 1. Elemental Content of FA.

Element	Si	Al	Fe	Ca	Ti	K	Ba	Mg	Sr	S	P	Zr	Mn	Co	Zn	Ni	Cr	Cu	Sn
Content (wt%)	46.9	19.6	15.2	5.86	3.66	3.17	1.44	1.21	0.615	0.504	0.41	0.398	0.186	0.119	0.118	0.10	0.093	0.081	0.060

Table 2. Leaching Concentrations of Toxic Elements from Raw and Treated FA (JLT46 leaching test).

Element	Unit	As	B	Cd	Cr	Cu	Se
Soil standard (Japan)	mg/L	< 0.010	< 1.0	< 0.010	< 0.050	< 125	< 0.010
Raw FA	mg/L	< 0.40	18.0	< 0.050	0.26	< 0.050	< 2.0
Basic treatment	mg/L	< 0.40	12.6	< 0.050	0.26	< 0.050	< 2.0
Chitosan treatment	mg/L	< 0.40	25.9	< 0.050	< 0.050	< 0.050	< 2.0
Alginate treatment	mg/L	< 0.40	13.5	< 0.050	0.16	< 0.050	< 2.0
Guanidine treatment	mg/L	< 0.40	12.9	< 0.050	0.15	< 0.050	< 2.0

experimental errors. According to drying experimental data, water retention curves were drawn, which are weight-based relative percentages of remained water in the sample at different drying times. It sets 100% at the beginning of drying experiment. 50% means that half amount of water is remained in the sample and 0% means complete drying. The concept of WHC calculation is illustrated in Figure 2. In this study, WHC was calculated as the area of water retention curve within 12 hours. In order to investigate the impact of raw- and treated-FA amending on soil WHC, FA-amended soil was also tested by the same drying experiments. 7.5 g of distilled water was added to 17.5 g of FA-amended samples with weight mixing ratio of 10 wt%, 20 wt% or 30 wt% to adjust initial water content as 15 wt% and then dried under natural condition or in the oven at 40°C.

Water Drop Penetration Time Measurement

Water drop penetration time (WDPT) was also measured to investigate its correlation with WHC. Distilled water was dropped on the surface of samples and time that dropped water absorbed into the surface completely was measured. WDPT is a common and simple method to distinguish obvious hydrophobic soils and others [45–46]. If FA amending changes soil hydrophobicity and FA amending changes plays an important role of WHC, it might give strong correlation between WHC and WDPT.

RESULTS AND DISCUSSION

The Impact of FA Pretreatment on Toxic Element Immobilization

The results of JLT46 leaching experiments are summarized in Table 2. The Soil environmental standard of Japan, which is leaching concentration of toxic elements, is also listed in Table 2. It should be noted that As, Cd and Se measurement in this study could not meet necessary analytical accuracy for As, Cd and Se owing to limitedly available samples. Therefore, As, Cd and Se might exceed the standard even when measurement data is less than each quantification limit. Although raw FA meets Cu standard, leaching concentrations of

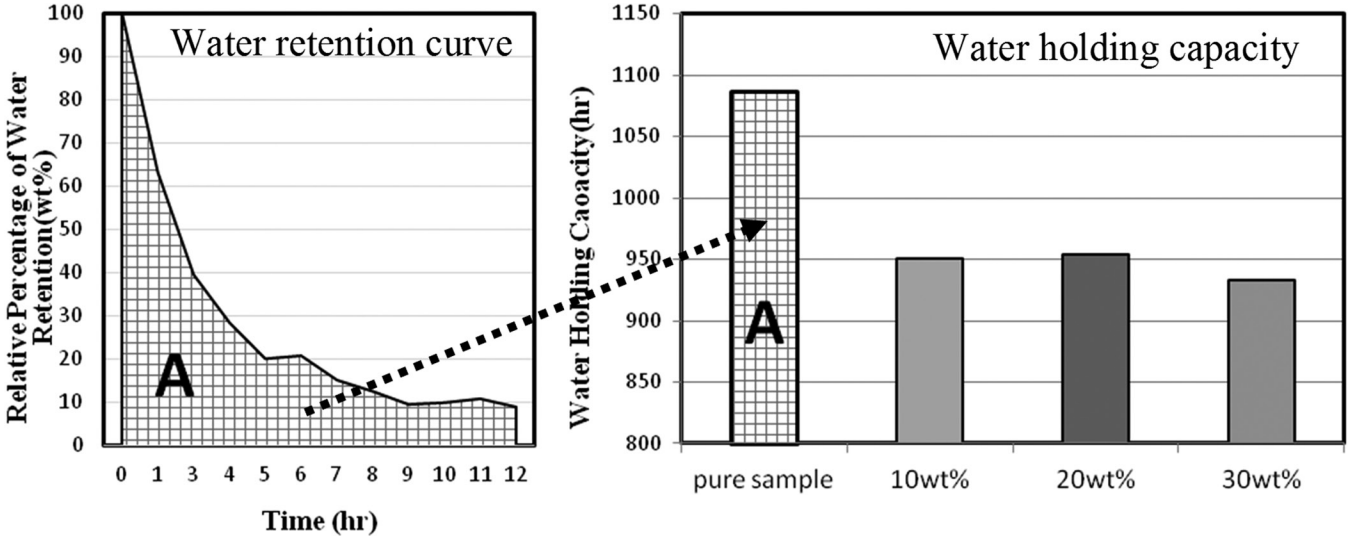

Figure 2. *Calculation concept of WHC based on water retention curve.*

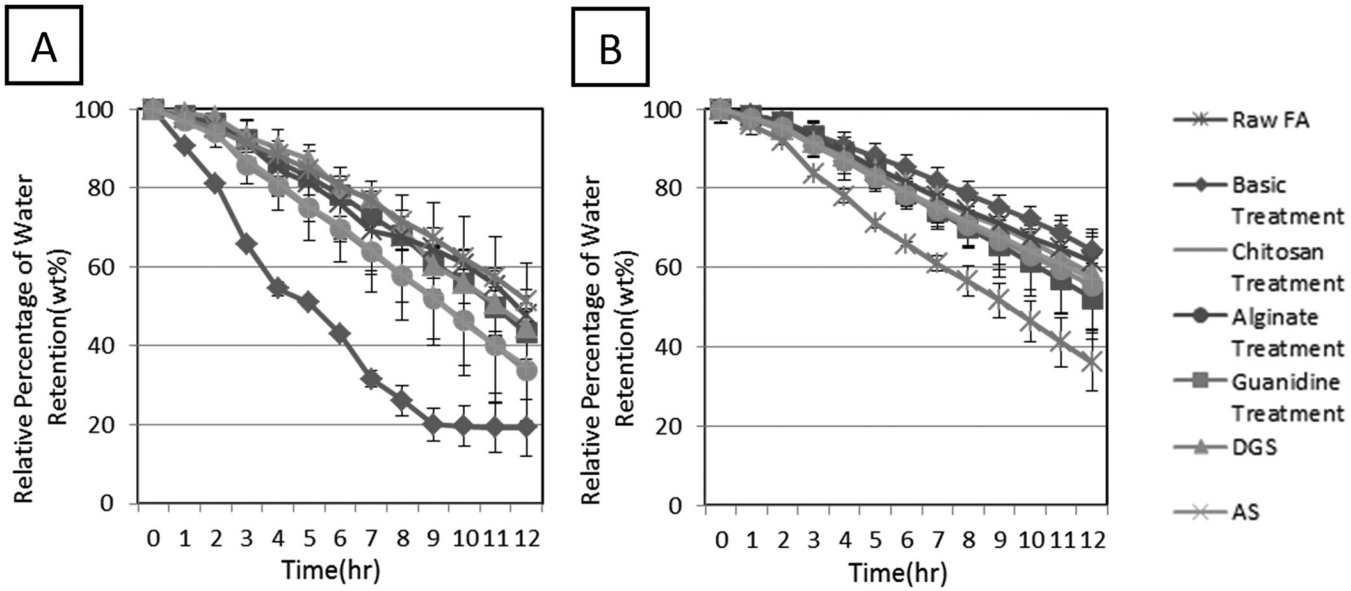

Figure 3. *Water retention curves of soils, raw and treated FA ([A] 40°C, [B] Natural condition).*

B and Cr for raw FA exceed the standard obviously. Because basic treatment synthesizes apatite mineral on FA particle surfaces, it was expected to enable mineralogical and/or physical immobilization of toxic elements like B and Cr and thus decrease leaching concentrations. However, basic treatment gave only 30% reduction and no obvious reduction for B and Cr leaching concentration, respectively. Such insufficient effect of basic treatment on B and Cr immobilization might be caused by incomplete surface coverage of apatite mineral and/or its low durability against 200 rpm shaking during leaching experiments. On the other hand, both alginate- and guanidine-treatments gave about 25% and 39% reductions for B and Cr leaching concentration, respectively. Although chitosan-treatment gave no impact on B immobilization, it is very effective on Cr immobilization and decreased Cr leaching concentration less than the standard. This can be explained by Cr sorption to chitosan [47–49]. A simple pretreatment, which was just only mixing FA with organic/inorganic

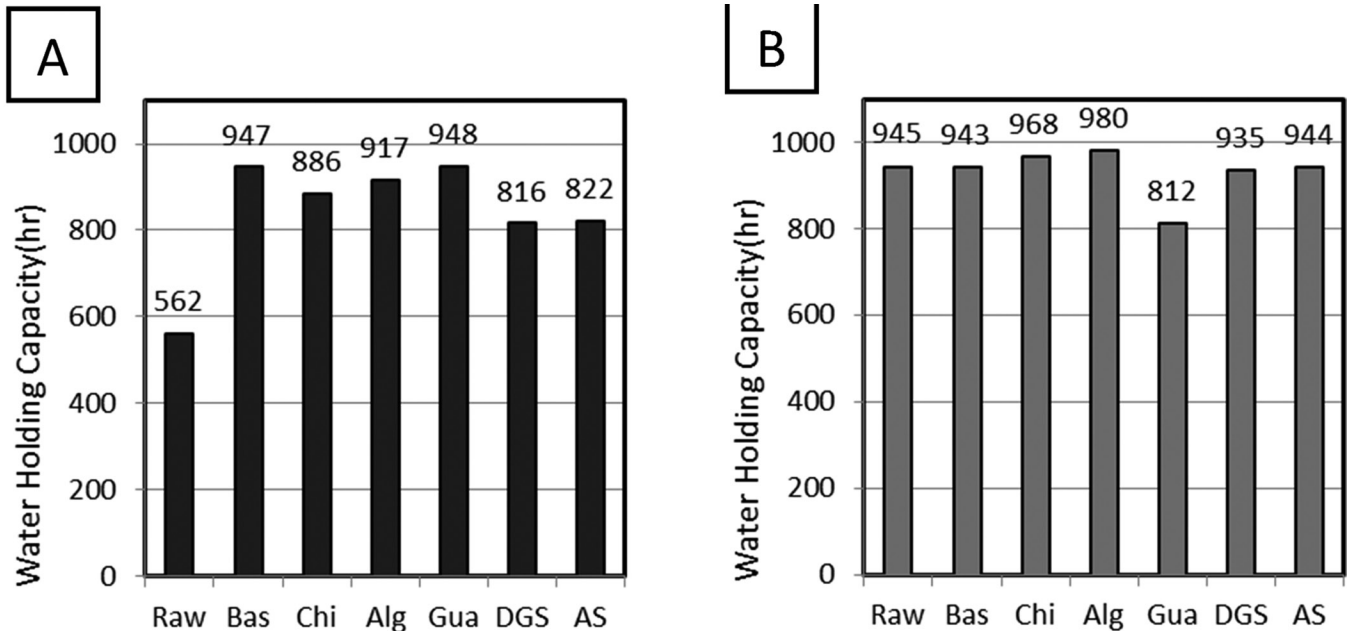

Figure 4. *Water holding capacity of soils, raw and treated FA (Raw: raw FA, Basic: Basic treatment FA, Chi: Chitosan-treated FA, Alg: Alginate-treated FA, Gua: Guanidine-treated FA, DGS: Decomposed granite soil, AS: Akatama soil) ([A] 40°C, [B] Natural condition).*

reagents, was tested in this study from the viewpoint of technical feasibility in real applications. However, Table 2 shows clearly that more appropriate pretreatment is necessary for FA to meet soil environmental standards.

WHC of Soils, Raw FA and Treated FA

Water retention curves of soil (DGS and AS) and FA are shown in Figure 3. WHC of these samples are shown in Figure 4. Figure 4(a) shows clearly that raw FA has almost the same WHC with DGS and AS under natural condition (around 20°C). Although basic treatment, chitosan- and alginate-treatment gave no significant impact on WHC, guanidine-treatment gave about 15% reduction for WHC. On the other hand, both organic and inorganic treatments are obviously effective on WHC increase at higher temperature (40°C). Although WHC of raw FA at 40°C is lower than those of DGS and AS, basic treatment, chitosan-, alginate-, and guanidine-treatment increased WHC of FA 8–16% higher than those of DGS and AS. Increase of WHC of FA by organic amendment with FA is also reported [50]. Such WHC increase by organic amendment seems to be explained firstly by size fraction change. Coarser size fractions of FA in general have higher water holding capacities than the finer ones [51]. Basic treatment and organic treatment in this study likely changes FA size distribution larger by binding FA particles. On the other hand, this study shows newly the temperature dependency of organic amendment effect on WHC of FA. Although this seems to be related to textural change including surface hydrophobicity by inorganic and organic treatment, it needs further study for mechanism-based explanation.

Effect of Raw/treated FA Amending on Soil WHC

Experimental Results

Water retention curves of raw or treated FA-amended soils with 10 wt%, 20 wt%, or 30 wt% mixing ratio at room temperature (natural condition) or 40°C are shown in Figure 5. Water holding capacities of these samples and pure soils are shown in Figure 6. Comparison of the effects of FA amending on soil WHC is difficult because the differences of temperature, soil type, FA mixing ratio, and pretreatment type should be taken into consideration at the same time. Therefore, the effects of FA amending would be discussed step by step focusing on highest WHC comparison, temperature dependency, soil type dependency, and mixing ratio dependency.

Comparison of the Highest WHC Cases

In order to compare the effects of FA amendment on soil WHC, the highest WHC at optimum mixing ratios are selected and its relative changes from pure soil WHC are summarized in Table 3. In this comparison, more than 5% increase and decrease are regarded as positively effective and negatively effective, respectively. Raw FA amending gave positive effect on WHC of both soils under natural condition. Increase of soil WHC by raw FA amendment is also reported [31–36]. In contrast to natural condition, however, raw FA amendment decreased WHC of both soils by about 13% at 40°C. The effect of raw FA amendment on soil WHC shows large temperature dependency. On the other hand, basic treatment gives FA opposite temperature dependency. Although basic treatment FA amendment gives negligible impact on soil WHC, it increases soil WHC at 40°C by 19.4% for DGS and 15.5% for AS and, respectively. Chitosan treatment gives FA comparable effect on WHC increases and the same temperature dependency with basic treatment. Alginate treatment gives FA both temperature and soil type dependency. Although it is effective for DGS regardless of temperature, it is effective for AS only under natural condition. Guanidine treatment showed negative effects on soil WHC under natural conditions. It decreased soil WHC by more than 4.0%. However, it increases WHC significantly for both soils at 40°C by more than 10%. These results suggest that the effect of treated FA amendment on soil WHC depends on many

Table 3. Relative Changes of WHC of FA-amended Soils at Optimum Mixing Ratios Based on Non-amended Soil WHC.

		Relative Change of WHC (%)	
Soil	**Treatment Type**	**Natural Condition**	**40ºC**
DGS	Raw FA	+1.71	–13.0
	Basic Treatment	+2.46	+19.4
	Chitosan Treatment	+5.35	+8.33
	Alginate Treatment	+6.20	+14.3
	Guanidine Treatment	–12.0	+11.4
AS	Raw FA	+1.27	–11.8
	Basic Treatment	+3.18	+15.5
	Chitosan Treatment	+4.98	+5.23
	Alginate Treatment	+6.78	+2.43
	Guanidine Treatment	–4.03	+10.2

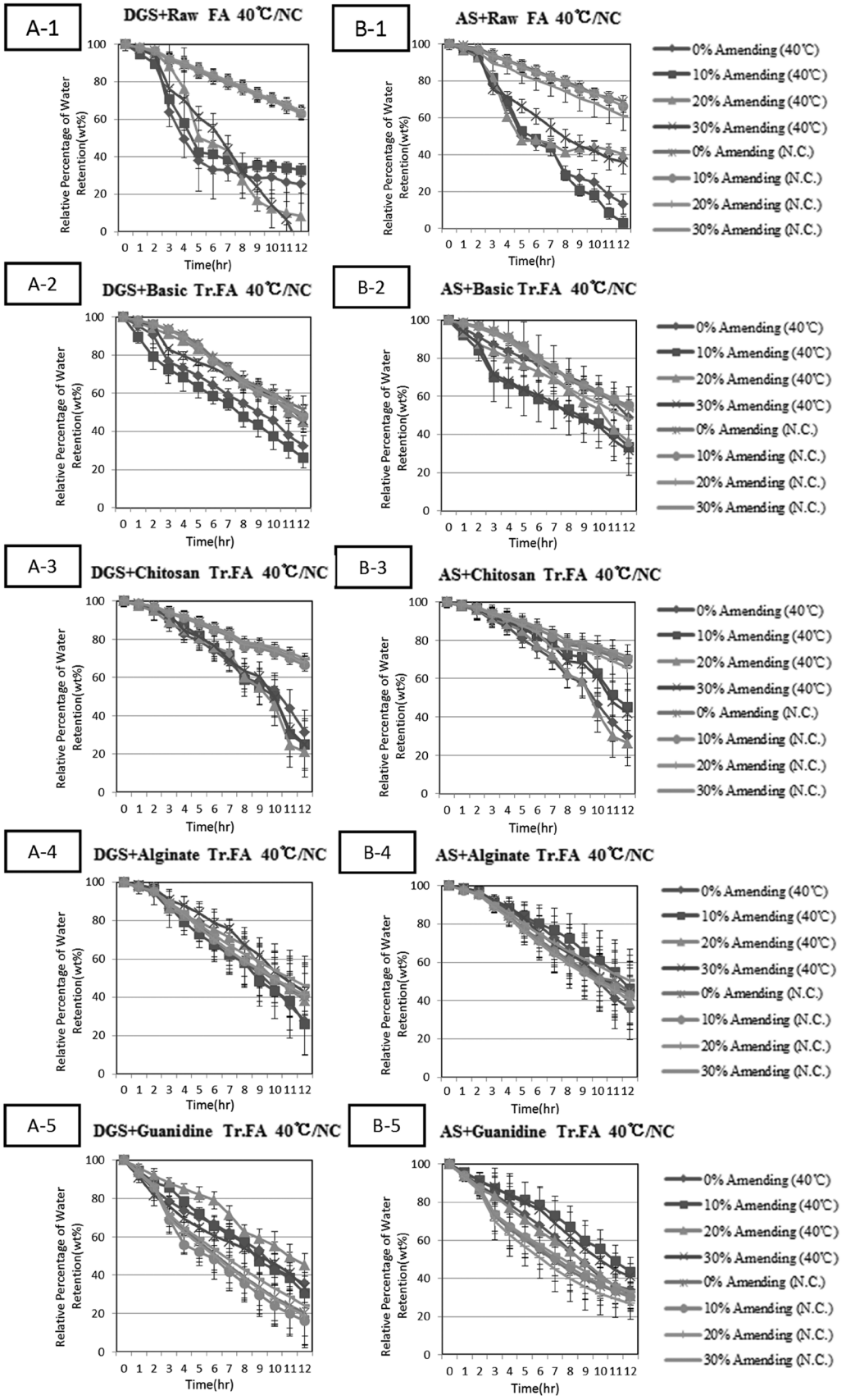

Figure 5. *The difference of the highest WHC in each pretreatment between natural condition and 40°C ([A] Decomposed granite soil, [B] Akatama soil).*

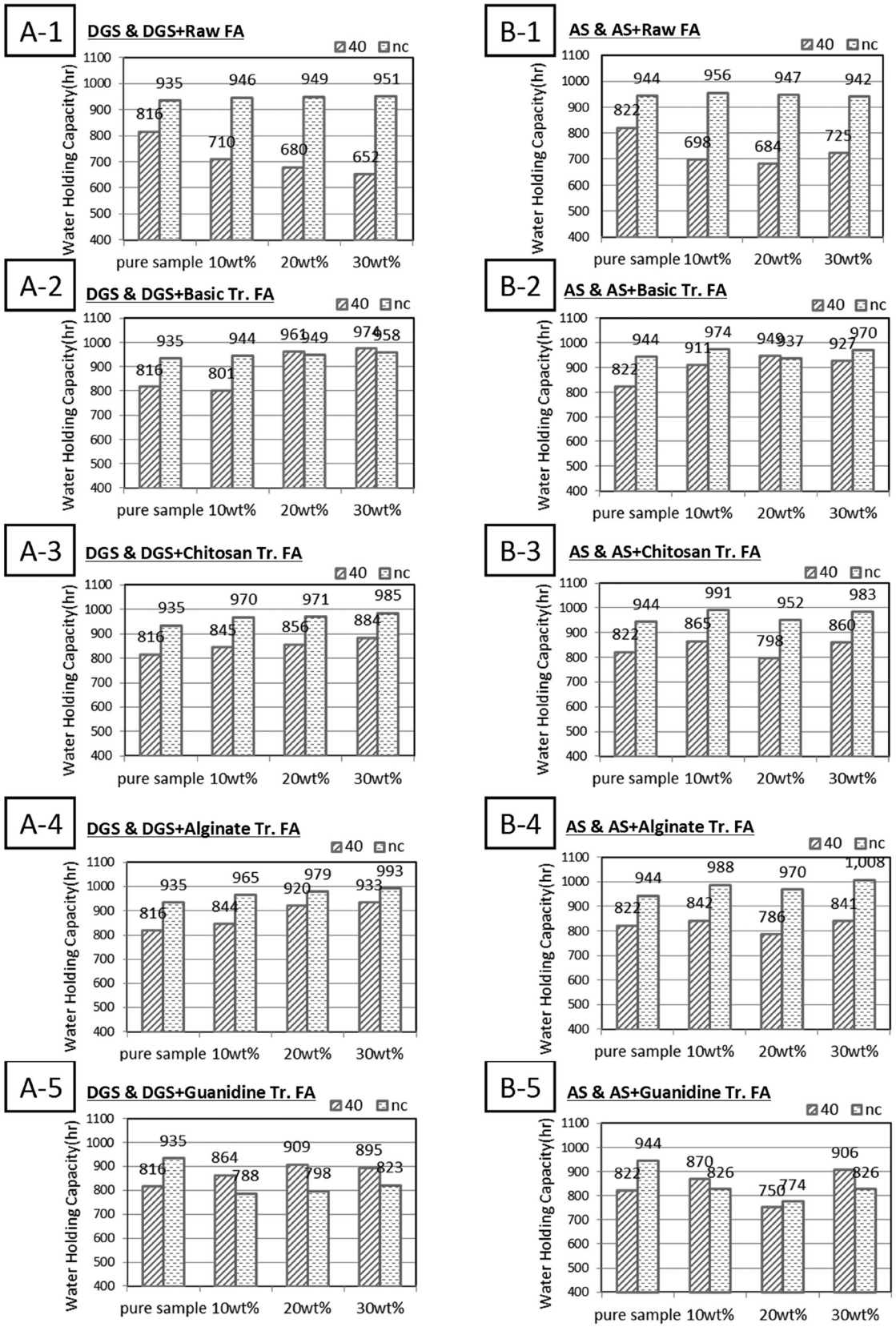

Figure 6. *Water holding capacities of FA-amended Decomposed granite soil (DGS) and Akatama soil (AS) ([A-1] DGS amended with raw FA, [A-2] DGS amended with basic treatment FA, [A-3] DGS amended with chitosan-treated FA, [A-4] DGS amended with alginate-treated FA, [A-5] DGS amended with guanidine-treated FA, [B-1] AS amended with raw FA, [B-2] AS amended with basic treatment FA, [B-3] AS amended with chitosan-treated FA, [B-4] AS amended with alginate-treated FA, [B-5] AS amended with guanidine-treated FA).*

factors interactively and it makes the dependency highly complicated.

Temperature Dependency

In order to make temperature dependency of FA amendment effect on soil WHC more understandable, the difference of the highest WHC in each treatment type and soil type between natural condition and 40°C are illustrated in Figure 7. Positive value in Figure 7 means that the effect of FA amendment on increase of soil WHC becomes larger at higher temperature. Negative value means that FA amendment decreases soil WHC more and more at higher temperature. Figure 7 shows that raw FA amending has an obviously negative dependency on temperature. Raw FA amendment at lower temperature is preferable to increase soil WHC. On the other hand, some inorganic and organic treatments give FA positive dependency on temperature. Such treatments are more effective to increase soil WHC at higher temperature.

Soil Type Dependency

Raw FA shows almost no dependency on soil type. Its amendment gives non-negligible increase of soil WHC under natural condition and large decrease at 40°C regardless of soil type. Basic and chitosan treatment also show negligible dependency on soil. Basic treated FA gives negligible decrease/increase of soil WHC under natural condition and more than 15% increase at 40°C for both soils. Chitosan treated FA showed similar tendency but less effective on WHC increase than basic treated FA. In addition, guanidine treatment shows obvious soil dependency although effects given to FA by guanidine treatment under natural condition are different from those of other treatment. Guanidine treatment is negatively effective to increase soil WHC under natural conditions. As described previously, alginate treatment shows both temperature and soil type dependency. It is effective to increase soil WHC for DGS regardless of temperature and for AS only under natural condition. Except for alginate treatment, these results are summarized that the effects of raw and treated FA amendment on soil WHC depend on temperature more strongly than soil type. The results of alginate treatment suggests that soil type dependency can be non-negligible possibly compared with temperature dependency when certain treatments are used.

Mixing Ratio Dependency

According to Figure 6, linear dependency of soil WHC on mixing ratio appears when chitosan- and alginate-treated FA were amended with DGS. Chitosan- and alginate-treated FA amendment at higher mixing ratio gives higher soil WHC. In other cases, optimum

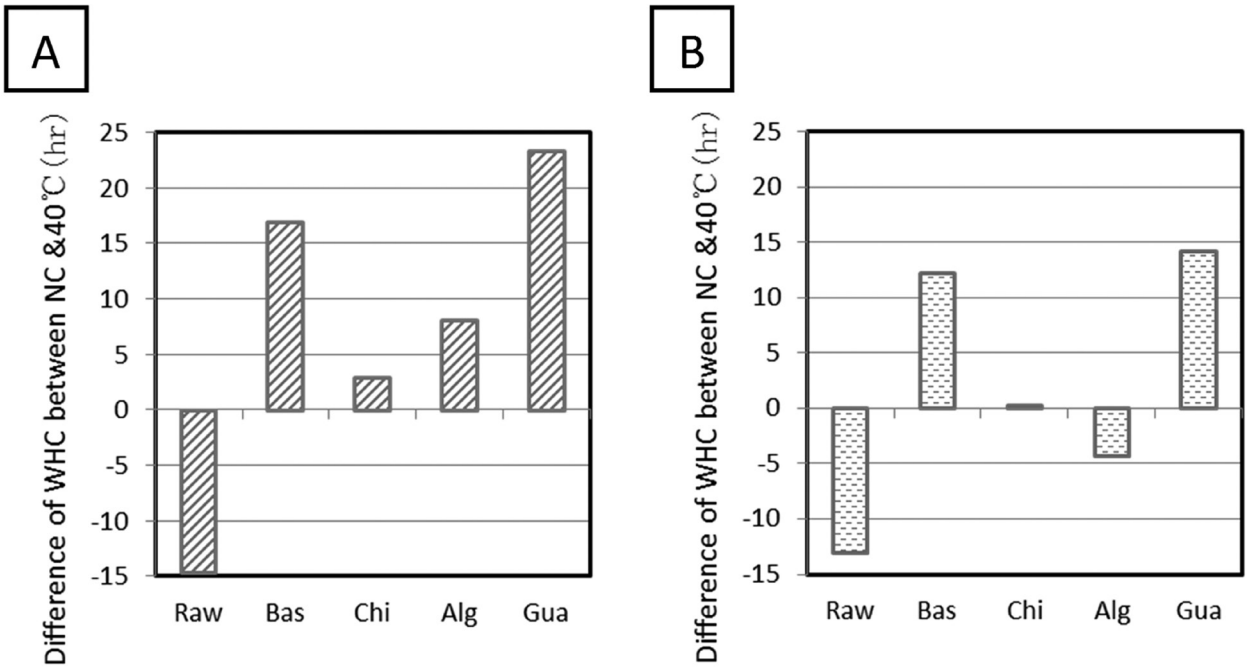

Figure 7. *The difference of the highest WHC in each pretreatment between natural condition and 40°C ([A] Decomposed granite soil, [B] Akatama soil).*

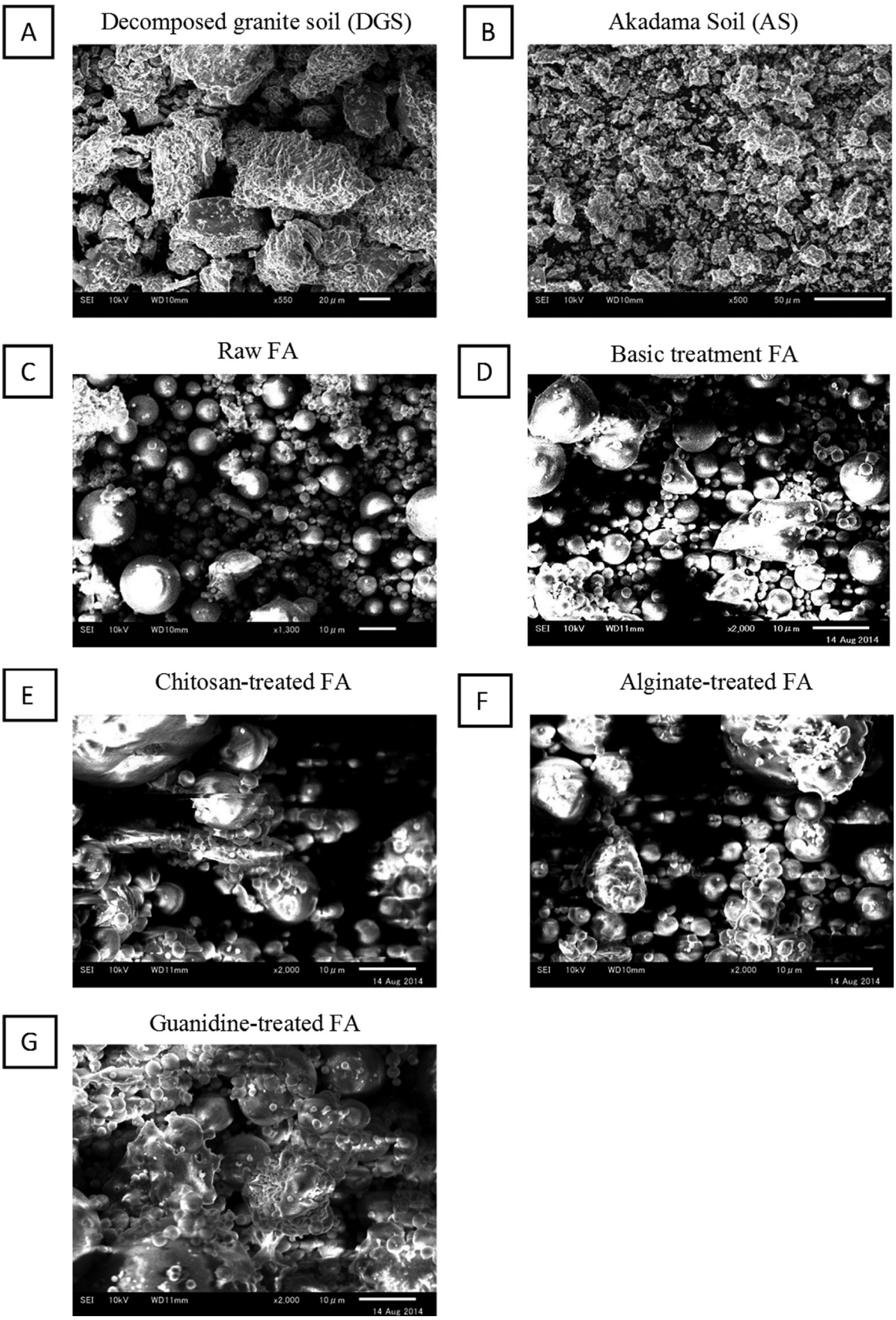

Figure 8. *SEM photographs of soils, raw and treated FA ([a] Decomposed granite soil (DGS) [B] Akatama soil (AS), [C] Raw FA, [D] Basic treatment FA, [E] Chitosan-treated FA, [F] Alginate-treated FA, [G] Guanidine-treated FA).*

Table 4. Water Drop Penetration Time of Raw/treated FA and Raw FA-amended Soils at 0–70 wt% Mixing Ratios.

Sample	WDPT (s)	Sample	WDPT (s)	Sample	WDPT (s)
Raw FA	12.3 ± 5.04	DGS	4.19 ± 2.22	AS	12.8 ± 3.90
Basic treated FA	2.86 ± 0.43	+10 wt% Raw FA	5.55 ± 4.17	+10 wt% Raw FA	40.2 ± 18.7
Chitosan treated FA	2.21 ± 0.49	+20 wt% Raw FA	4.16 ± 1.62	+20 wt% Raw FA	13.1 ± 3.06
Alginate treated FA	2.56 ± 0.32	+30 wt% Raw FA	7.13 ± 5.47	+30 wt% Raw FA	18.5 ± 7.52
Guanidine treated FA	2.86 ± 0.65	+50 wt% Raw FA	21.0 ± 8.10	+50 wt% Raw FA	10.8 ± 2.26
		+70 wt% Raw FA	27.1 ± 12.7	+70 wt% Raw FA	13.0 ± 3.33

mixing ratios were found depending on temperature, soil type, and treatment type. Almost no significant dependency of raw FA amendment at 40°C for both soils should be noted. When raw FA is amended at 10 wt% or more, it decreases soil WHC at 40°C by more than 12% regardless of soil type and mixing ratio.

Water drop penetration time (WDPT)

WDPT of raw and treated FA, and raw FA-amended DGS and AS are listed in Table 4. Standard deviations of measurement data are also listed. Inorganic and organic treatments decrease WDPT by more than 76%, which means that water repellency of FA was decreased by pretreatments. When WDPT listed in Table 4 and WHC of raw/treated FA and soils shown in Figure 4, no clear correlation between WDPT and WHC can be found. It might imply that water repellency of FA is not a controlling factor of WHC. Although WDPT of raw FA-amended DGS increase with increase of mixing ratio, WDPT at 50 wt% mixing ratio or more exceeds original WDPT of raw FA. In the case of AS, no clear dependency of WDPT on mixing ratio can be found and WDPT are higher than the original WDPT of raw FA and AS at some mixing ratios. Although WDPT experiments were repeated more than 10 times for each sample, this might suggest soil sample heterogeneity can give large experimental uncertainty.

SEM Observation of Soils, Raw and Treated FA

To confirm the texture and surface conditions of soils, raw and treated FA, SEM observation was applied for all samples. They are shown in Figure 8. Both soils have more rough and rugged surface than raw FA. Although basic treatment does not change the texture of FA particles so much, coagulation of some FA particles are found. Organic treatments changes FA particle surface more rough and promotes coagulation of FA particles. In particular, guanidine-treatment promotes the coagulation. In previous section, the authors suggest that size distribution change to larger side by binding some FA particles might explain the increase of WHC of organic treated FA. These observation results support size distribution change to larger side.

CONCLUSION

The effects of raw and treated FA amending on soil WHC were investigated in this study. Apatite mineral was synthesized on FA particles (basic treatment) or FA surface was modified by chitosan, sodium alginate, and guanidine hydrochloride. WHC was measured as cumulative water retention during 12-hour drying experiments at 40°C or under natural condition. Although raw FA has comparable WHC with soils under natural condition, it has lower WHC than soil at 40°C. Inorganic and organic treatments increase WHC to the same level with soils. This can be explained by FA coagulation after inorganic/organic treatments. In contrast to previous researches, raw FA amendment decrease soil WHC by more than 13% at 40°C regardless of soil type and mixing ratio. On the other hand, the effects of treated FA amending on soil WHC have interactive dependency on temperature, soil type, treatment type, and mixing ratio. The amendment of basic treatment FA or chitosan-treated FA gives negligible impact on soil WHC under natural conditions. However, it increases soil WHC at 40°C in non-negligible level if mixing ratio is optimum. Alginate-treated FA has temperature and soil type dependency. It is effective for Aakatama soil only under natural conditions and for Decomposed granite soil at both room temperature and 40°C. Guanidine treatment showed negative effects on soil WHC under natural conditions. It decreased soil WHC by more than 4.0%. However, it increased WHC of both soils at 40°C by more than 10%. These results can be summarized that raw FA amendment at lower temperature is preferable to increase soil WHC. On the other hand, some inorganic and organic treatments give FA positive dependency on temperature. Such treatments are more effective to increase soil WHC at

higher temperature. In addition, this research found no significant correlation between WHC and water repellency of FA.

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Screening of Heavy Metal Tolerant Microbes in Sludge and Removal Capability of Lead

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ABSTRACT: The sludge in solid waste dismantling area is often seriously polluted by heavy metals, while some microbes are resisting to heavy metal pollutants due to their life activities such as gene mutations, secretion change and physiology adjustment. This paper identifies two kinds of microorganisms, namely Penicillium citrinum and Aspergillus niger, which can resist heavy metals in the sludge in solid waste dismantling area. By discussing the removal capacity of these two microorganisms on Pb^{2+} under different conditions of pH value, microbial quantity and initial concentration. Results show that these factors have great influence on adsorption of Pb^{2+}, when the initial concentration is 30 mg/L and the bacterium fluid volume is 2 ml. The removal rate is the highest under such condition. The comparative experiments show that removal capacity of Penicillium citrinum is better than Aspergillus niger. Heavy metal ions can be effectively removed by both types of microorganisms.

INTRODUCTION

THE presence of heavy metals in solid waste dismantling area is becoming a severe environmental problem and poses great risks to organisms in the sludge [1–3]. The sludge in solid waste dismantling area has been long-term seriously polluted by heavy metals and other pollutants [4,5]. Heavy metals are non-biodegradable, toxic and carcinogenic even at very low concentrations, which can easily enter into the food chain and accumulated in vital organs to the high levels. Accordingly, it can pose damage or even threat to poisoning in human health with severe damage to organs and leads to deadly diseases, thereby posing the greatest danger to the living [6–8].

In recent years, due to the toxic action of heavy metal pollution is more and more harmful to environment and human health, research on the aspects of heavy metal pollution has drawn increasing attention in the research community, especially the field of bioremediation has become a hotspot [9–12]. As a new processing technology among different treatment techniques, bioremediation has a very broad development prospect in the aspect of wide application in treating wastewater treatment with heavy metal wastewater treatment of low concentration of heavy metal [13–16]. But in the aspect of heavy metal pollution in sludge, the study primarily focuses on phytoremediation [17–18] rather than microbial adsorption and repair mechanism [19].

The solid waste dismantling area has been serious polluted by heavy metals for ages, the heavy metals enter into sludge and then into human body through migration and transformation, so as to constitute a serious hazard to human health. The microorganisms in sludge occupy an important position in the process of geochemical cycle, and play a very important role on the transition and transformation of organic pollutants, toxic heavy metals and their compounds in sludge. Since heavy metals are toxic to microorganisms, a portion of microorganisms gradually become dysfunctional. On the other hand, some microorganisms could adapt to changing environment, and remain normal function under a certain concentration level of heavy metals. It is therefore important to identify such microorganisms that are resistant to heavy metals in contaminated sludge.

SCREENING OF HEAVY METAL TOLERANT MICROBES IN SLUDGE

Materials

Sludge samples are collected in a typical solid waste dismantling area. Samples are naturally dried in a cool ventilated place, sifted with size of 20 meshes and then

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loaded in the reagent bottle and sealed for further analysis.

Experimental Instruments

The experimental instruments are listed below.

1. High pressure steam sterilization pot;
2. Constant temperature oscillation incubator;
3. Constant temperature incubator;
4. Sterile workbench;
5. Atomic absorption spectrophotometer;
6. Electric stove, Flat dish, Alcohol lamp, Inoculation loops, Electronic balance, Pipette, Beaker, Conical flask, etc.

Experimental Medium

The culture medium is a substrate for microbial growth, and each microorganism requires different culture medium to cultivate. This experiment selects solid culture medium as potato culture medium and another fluid medium as basal culture medium.

1. *Solid medium*: 20 grams of glucose, 15 to 20 grams of agar, and 300 to 500 g of potato are used as the raw materials. Potatoes are first washed and peeled, and further cut into pieces, which are put into the gauze. 500 ml water is added to the potato. The solution is then boiled in water for 15 minutes. The gauze is then removed. Potato solution is diluted with distilled water to 1 liter under natural pH value.
2. *Liquid medium*: It is comprised of 20 grams of glucose, 10 grams of peptone, 0.2 grams of sodium chloride, 0.05 grams of sodium bicarbonate, 0.5 g of potassium hydrogen phosphate, 0.1 grams of potassium chloride, 0.1 grams of calcium chloride, 0.25 grams of magnesium sulfate, 0.005 grams of iron vitriol. The solution is then diluted by distilled water to 1L under natural pH value.

Microbial Screening Method

The microorganisms are usually domesticated to become characteristic ones under certain conditions when microbes with certain special functions can be identified. Since the study area has been polluted for a long time, microorganisms in sludge have been naturally domesticated. Therefore, there is no need for additional domestication. Separation and purification should be carried out immediately when sludge samples are retrieved from the study area. The following five steps are implemented to prepare a solution for further analysis.

1. Sterilization

   After the potato culture medium is prepared, it is sterilized in a high pressure steam pot for 20 minutes and then cooled down and transferred to the ultra-clean workbench. Then it is disinfected by ultraviolet light for 20 min.
2. Preparation of sludge diluents

   After sifting the contaminated sludge sample with 2 mm sieve, 1 gram of sludge is mixed with 9 ml of sterile water. The mixed solution is put on a table concentrator to shake for 10 minutes to well mix sludge and water. Five test tubes, each containing 9 ml sterile water, are used. Different levels of concentration, e.g., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} are prepared.
3. Cultivation

   The solid medium of 40°C is poured into tablets. Inoculating loops lining are used on them with different cooling gradient of liquid sludge. Sealing film is used to seal and label them for cultivating in a constant temperature incubator at 30°C for 7 days.
4. Separation and purification

   Different forms of single colony are mixed with potato culture mediums and put on the tablets again. Cultivation is continuing until bacterial colonies grow with a single form and preliminary considered as one kind.
5. Preservation

   The bacteria is inoculated to slant medium at 30°C for 5 to 7 days. The medium is then bind up the slant medium and stored in a refrigerator at 4°C.

Microbial Identification

Different microbial colonies grow up after lining tablet with sludge suspension, and a single colony which grows well with same characteristics from tablets is identified. Lining for separation and purification is continued until the microbial colonies have the same characteristics. After continuous purification, two different forms of microbes in sludge are screened out, as

can be seen in Figure 1(a). Bacterium 1 is dark green with smooth and white edges, arranging as a string of beads. Bacterium 2 is brown-black, beaded globular and smooth.

After separating and purificating these two types of bacteria, ITS sequences and 16 srDNA testing experiment were conducted at Beijing Sunbiotech co., Ltd. Identification result shows that Bacterium 1 is fungus called Penicillium citrinum, and Bacterium 2 is fungus called Aspergillus niger.

REMOVAL CAPACITY OF MICROBES TO LEAD

Microbial Strains Processing

Microbial Strains Enrichment Culture

Experimental strains are Penicillium citrinum and Aspergillus niger isolated and screened from polluted sludge. These two microbes are put into a constant temperature incubator for cultivation for about three to five days at 30°C. After bacterial growing in good conditions, second phase of inoculation is carried out under the same condition as the first time. Strains in good conditions are identified and put into the potato medium on tablets. A third time of purification maintains for about three to five days. After three rounds of purification and cultivation, the two microbes Penicillium citrinum and Aspergillus niger are shown in Figure 1(b) and (c).

Bacterial Suspension Preparation

Two kinds of bacteria with stable growth are identified and put into the constant temperature incubator. Bacterial spores are washed with 50 ml sterile distilled water, and shaked well in sterile conical flask. Spore

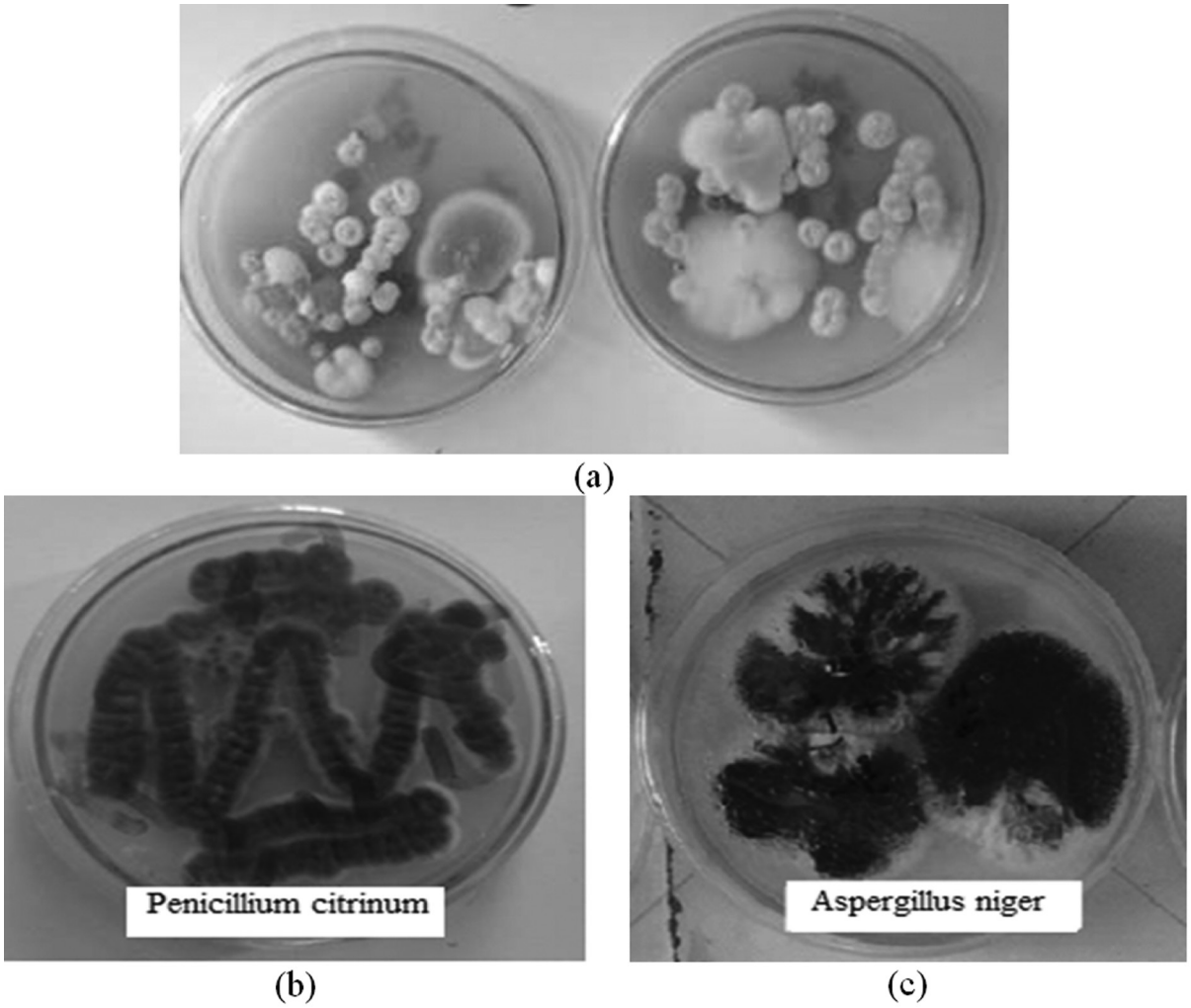

Figure 1. *(a) Microorganisms of cultivated in sludge (b) Penicillium citrinum after three generation of enrichment culture (c) Aspergillus niger after three generation of enrichment culture.*

suspension is then obtained. The numbers through the blood counting chamber is counted. The solution is then diluted properly to prepare the spore suspension with number of about 1.0×10^7 per millimeter.

Adsorption Experiment

Different pH Values

1. The medium is poured into two groups of sterilized conical flasks under aseptic conditions, and Pb^{2+} solution is then added to prepare 100 ml liquid culture medium with concentration of 50 mg/L.
2. 2 ml of suspension liquid of Penicillium citrinum and Aspergillus niger is added into conical flasks. The initial pH value is adjusted with HCl of 1 mol/L and NaOH of 1 mol/L solution, to reach different levels of pH values, e.g., 2.0, 3.0, 5.0, 7.0, 9.0, 11.0.
3. The sealing film is used to seal the conical flasks and put them in constant temperature oscillation incubator to cultivate for 4 days at 28°C and 130 r/min speed. After reaching the adsorption equilibrium, supernatant liquid is then centrifuged and for measuring the concentration of heavy metals.

Different Microbial Solution Volume

1. The medium is poured into two groups of sterilized conical flasks under aseptic conditions. Pb^{2+} solution is then added to prepare a 100 ml liquid culture medium with concentration of 50 mg/L.
2. Different volumes of suspension liquid of Penicillium citrinum and Aspergillus niger, e.g., 0.5 ml, 1 ml, 2 ml, 4 ml and 6 ml, are added into different conical flasks.
3. The sealing film is used to seal the conical flasks, in which are put in constant temperature oscillation incubator to cultivate for 4 days at 28°C under the speed of 130 r/min. After reaching the adsorption equilibrium, supernatant liquid is centrifuged for testing the concentration of heavy metals.

Different Initial Concentration

1. (1) Liquid mediums are sterilized and then put into two groups of conical flasks. Different concentrations of Pb^{2+} solution, e.g., 30 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L are added to prepare for 100 ml liquid culture medium.
2. Add 2 ml suspension liquid of Penicillium citrinum and Aspergillus niger into the corresponding conical flasks, under the natural pH value.
3. Use the sealing film seal the conical flasks, and put them in constant temperature oscillation incubator for 4 day for cultivation at 28°C and 130 r/min speed. After reaching the adsorption equilibrium, centrifuge the flasks and pick up supernatant liquid to test the concentration of heavy metals.

All media, glassware and heavy metal solutions used in this study should be sterilized, and all the experimental operations should be carried out in a sterile environment.

Calculation

The concentrations of Pb^{2+} are determined by the flame atomic absorption spectrophotometric method. This method is to intake sample or digested sample directly to flame, and then determine the heavy metal concentration of the solution by comparing the absorbance of solution under test with the absorbance of the standard solution. Different levels of concentration of standard solution for Pb^{2+} are 0, 0.20, 0.40, 0.80, 1.20 and 2.00 mg/L, respectively.

After microbial adsorption on heavy metals achieves equilibrium, Pb^{2+} concentration could be determined in solution. Adsorption rate can be calculated based on the equation described below:

$$\omega = (C_0 - C_1)/C_0 \times 100\% \quad (1)$$

where C_0 is initial concentration of heavy metal Pb^{2+} in the solution (mg/L) and C_1 is the concentration of heavy metal Pb^{2+} in solution after adsorption equilibrium (mg/L).

RESULTS AND DISCUSSION

Effect of Different pH value on Pb^{2+} Adsorption

After microbial adsorption on Pb^{2+} achieves equilibrium, Pb^{2+} concentration in solution is determined. Adsorption rate can also be calculated, as shown in Table 1.

As can be seen from Figure 2, pH of solution has strong influence on Pb^{2+} adsorption of microbes. Under different pH conditions, the Pb^{2+} adsorption capacities

Table 1. Effect of pH Value on Pb^{2+} Adsorption.

pH	$C_0(Pb^{2+})$ (mg/L)	Penicillium Citrinum C_1(mg/L)	Absorbtion Rate (%)	Aspergillus Niger C_1 (mg/L)	Absorbtion Rate (%)
2	50	42.29	15.42	44.63	10.74
3	50	31.62	36.76	38.92	22.16
5	50	29.89	40.22	37.53	24.94
7	50	29.82	40.36	38.13	23.74
9	50	32.77	34.46	38.29	23.42
11	50	40.1	19.80	40.94	18.12

of these two kinds of microbes are not identical. When pH is less than 5.0, Pb^{2+} adsorption rate of Penicillium citrinum increases with the rise of pH; In the range of 5.0 to 7.0, the Pb^{2+} adsorption rate reaches its maximum; and when pH is higher than 7.0, the adsorption rate of Penicillium citrinum decreases with the increasing of pH. When pH is less than 5.0, $Pb2^{+}$ adsorption rate of Aspergillus niger increases with the increasing pH; In the range of 5.0 to 9.0, adsorption effect reaches the optimum; while pH is higher than 9.0, Pb^{2+} adsorption rate of Aspergillus niger decreases with the increasing pH. From Figure 3, it can be concluded that the best pH range of Pb^{2+} adsorption by Penicillium citrinum and Aspergillus niger is 5.0 ~ 7.0 and 5.0 ~ 5.0, respectively.

Therefore, pH has a great impact on the microbial adsorption to heavy metals. This is primary due to the fact that pH value does not only affect physical and chemical properties of heavy metals, but also affect adsorption properties of the bacterium cell walls. When pH value is lower, there are more H^+ in solution. Increased number of ions would compete for adsorption occurred at the cell walls of microbes. Therefore, adsorption rate of strains is smaller at lower pH value. With the rise of pH, the number of both H^+ and OH^- irons in the solution gradually decreases. This leads to electrical changes on the surface of cell wall and strengthens the removal capacity for heavy metals. But when the pH value is higher than 7, heavy metal ions could produce precipitation. The adsorption rate, therefore, decreases correspondingly.

Effect of Microbial Solution Volume on Pb^{2+} Adsorption

After reaching the adsorption equilibrium, the adsorption effect of these two kinds of microbes on heavy metal Pb^{2+} is examined and listed in Table 2.

Figure 3 shows that with the change of microbial solution volume, the adsorption effect of the two kinds of microorganisms on heavy metal Pb^{2+} is different. For Penicillium citrinum, with the increase of the microbial quantity, the adsorption rate of Pb^{2+} increases. When the microbial solution volume is 2 ml, the adsorption rate is the highest (40.58%), which is four times more than that of solution volume of 0.5 ml. When solution volume is 4 ml, adsorption reaches equilibrium. For Aspergillus niger, it is the same that with the increase of microbial volume, the adsorption rate gradually increases. When solution volume is 2 ml, the adsorption rate is 26.16% and reaches the maximum. When bac-

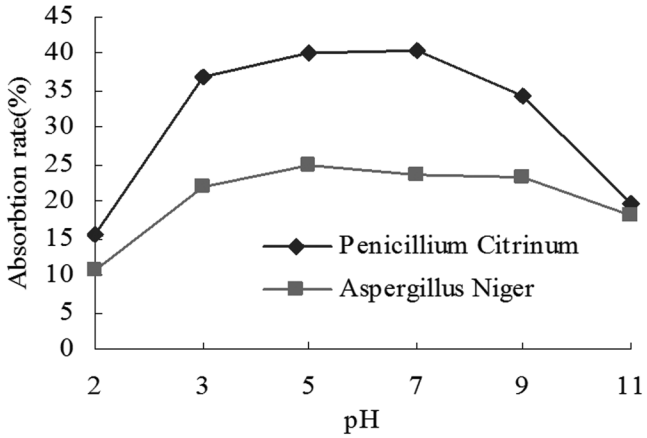

Figure 2. *Effect of pH value on Pb^{2+} adsorption.*

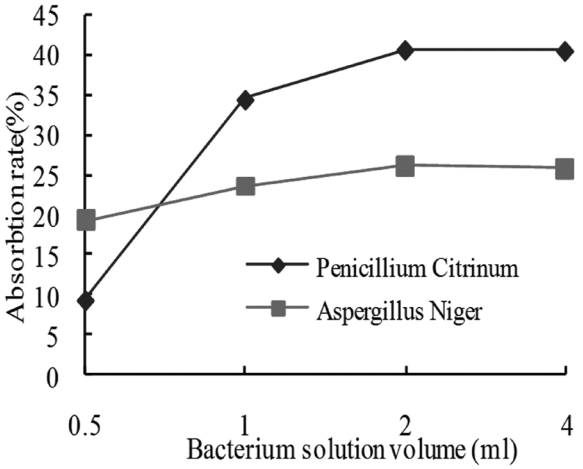

Figure 3. *Effect of microbial solution volume on Pb^{2+} adsorption.*

Table 2. Effect of Microbial Solution Volume on Pb^{2+} Adsorption.

Microbial Solution Volume (ml)	$C_0(Pb^{2+})$ (mg/L)	Penicillium Citrinum C_1(mg/L)	Absorbtion Rate (%)	Aspergillus Niger C_1 (mg/L)	Absorbtion Rate (%)
0.5	50	45.3	9.4	40.43	19.14
1	50	32.81	34.38	38.28	23.44
2	50	29.71	40.58	36.92	26.16
4	50	29.77	40.46	37.27	25.66

terium solution volume is 4 ml, the adsorption rate decreases to 25.66%.

This is primary due to the fact that when microbial solution volume is added at the beginning, microbial cells interact with heavy metals. There are plenty of functional groups and adsorptive sites on cell walls that can adsorb heavy metals. When the volume is increased to a certain amount, adsorption reaches equilibrium. However, if too much volume is added, part of microbes between the microbial cells reunite, and specific surface area of adsorption reduces. Hence, the adsorption rate decreased. It is found that the turbidity of the solution is higher if there are more microbial cells. This is due to the fact that the adsorption of heavy metal often occurs at cell walls. Reduced specific surface area leads to increasing of repulsion between cells, which makes portion of heavy metals resolute in the solution.

Compared to the adsorption effect of these two kinds of microorganisms on heavy metal Pb^{2+}, the adsorption ability of Penicillium citrinum is much higher than Aspergillus niger. Such difference reflects different functional groups of the two kinds of microbial cell walls.

Effect of Initial Concentration on Pb^{2+} Adsorption

After reaching the adsorption equilibrium, the adsorption effects of these two kinds of bacteria to heavy metal Pb^{2+} are investigated and listed in Table 3.

As can be seen from figure 4, with the increase of initial concentration of heavy metals, the adsorption rates gradually reduce for both microbial cells. For Penicillium citrinum, when the concentration of heavy metals is 30 mg/L, the adsorption rate is as high as 61.08%. When the concentration of heavy metals is 50 mg/L and 100 mg/L, adsorption rates are 43.4% and 40.67%, respectively. Surprisingly, when the initial concentration of Pb^{2+} is more than 100 mg/L, the adsorption efficiency drops dramatically, which is only 25.90% of the adsorption rates when the ion concentration is 200 mg/L. For Aspergillus niger, when the concentration of heavy metal is 30 mg/L, the adsorption effect is also the best, and the adsorption rate is at its highest (43.08%). But when concentration is higher than 50 mg/L, the adsorption rate reduces rapidly. When the initial concentration is higher than 100 mg/L, the adsorption reaches equilibrium and the adsorption rate tends to be stable eventually. It can be concluded that the lower the ion concentration, the better the microbial adsorption effect.

This is due to the fact that that the characteristics of the functional groups in microbial cell wall are combined with heavy metals. When the concentration of heavy metal ions in solution is lower, there are relatively more functional groups and binding sites existing for the same area of microbial cell wall. The adsorption rate at lower concentration is hence higher. With the increase of the concentration of heavy metals, increased number of heavy metal ions promotes its adsorption of microorganisms. The adsorption rate gradually decreases relatively to the increasing concentration. It is found that high concentration of heavy metal ions is poisonous and resistant to microorganisms.

Table 3. Effect of Initial Concentration on Pb^{2+} Adsorption.

Microbial Solution Volume (ml)	$C_0(Pb^{2+})$ (mg/L)	Penicillium Citrinum C_1(mg/L)	Absorbtion Rate (%)	Aspergillus Niger C_1 (mg/L)	Absorbtion Rate (%)
2	30	9.73	61.08	17.08	43.08
2	50	28.3	43.4	34.24	31.52
2	100	59.33	40.67	80.54	19.46
2	150	107.58	28.28	123.48	17.68
2	200	148.19	25.90	163.18	18.41

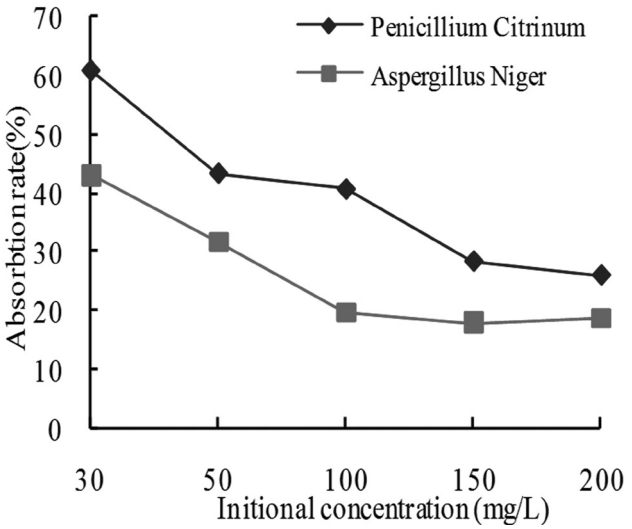

Figure 4. *Effect of initial concentration on Pb^{2+} adsorption.*

CONCLUSIONS

First, two kinds of heavy metal tolerant microorganisms are screened from the sludge in typical solid waste dismantling area, which were biologically identified as Penicillium citrinum and Aspergillus niger.

Second, experiment results show that pH value of solution, microbial solution volume and initial concentration of Pb^{2+} all have great impact on adsorption. When initial concentration is 30 mg/L and microbial solution volume is 2 ml, the adsorption rate is the best.

Finally, comparative experiments show that the adsorption effect of Penicillium citrinum is better than that of Aspergillus niger for lead. Both can be used to remove heavy metal ions effectively. This research provides technical reference for heavy metal pollution control.

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Pollutant Removal by Gravel Contact Oxidation Treatment System in Taipei

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ABSTRACT: The Guiyang gravel contact oxidation treatment system was constructed to purify combined sewage and rainwater in Taipei. In this study, influent and effluent were sampled monthly for the analyses of dissolved oxygen (DO), suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen and nitrate. Results showed that removal rates of BOD, SS and NH_3-N ranged 85–95% (averaged 90%), 70–95% (averaged 85%), and 70–90% (averaged 80%), respectively. Therefore, the gravel contact oxidation system is quite effective in pollution control for combined sewage and rainwater. However, microbial denitrification was not effective due to high concentrations of dissolved oxygen in non-aeration zones of the gravel contact oxidation treatment system. Although the concentration of NH_3-N decreased quickly, nitrogen was not removed by microbial degradation. It was only a migration process among different forms of nitrogen. Therefore, it is important to effectively control aeration in the front zone of the gravel contact oxidation treatment system to improve the removal efficiency of total nitrogen.

INTRODUCTION

THE gravel contact oxidation system, among many other kinds of ecological engineering technologies, is a conventional method to strengthen the natural water purification process. The gravel contact oxidation with the packed medium of gravel acts as a biofilm carrier [1,2]. Pollutants are removed by biofilm attached to the filter surface through adsorption, absorption, sedimentation and metabolic effects, etc [3]. The purification process of gravel contact oxidation system includes physiochemical process and biological process. When the sewage flows through the gravel bed for a long time, biofilm forms on the surface of gravel and pollutants are degraded by biofilm absorption and used for the metabolism of the biofilm. In short, biofilm plays a key role on sewage purification in the gravel contact oxidation system. As a subsurface flow system, a gravel contact oxidation system provides a large contact surface area between the sewage and the biofilm. Purification effect is, therefore, better than that of surface flow system [4]. To treat sewage more effectively, a gravel contact oxidation system is often filled with gravels of varying tightness and different sizes. It can be generally divided into two layers. The upper layer of gravel is often arranged loosely, where sewage flows fast to maintain aerobic condition. The lower layer of gravel is, however, packed tightly. Sewage passes through slowly and aeration pipes are laid on the bottom of the gravel bed to keep aerobic condition. In addition, sewage with high concentration of suspended solids flows directly into the gravel bed, easily blocking the pores and reducing the hydraulic loading and decreasing the removal efficiency. Thus, a grit chamber unit was added to remove suspended solids in sewage to reduce clog before the sewage passing through the gravel bed.

MATERIAL AND METHODS

Site Description

The Guiyang gravel contact oxidation treatment system is located in the high beaches of the upstream of Zhongxing Bridge, Taipei. It covers an area of 3,850 m^2, of which the area of the gravel contact oxidation unit is about 1,930 m^2 (excluding road area). The influent, which is combined sewage and rainwater, is collected from Guiyang pumping station.

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The catchment area of the pumping station is about 69,100 m^2, ranging from the north of Guangzhou Street to the south of Neijiang Street, Chongqing South Road to the west of Roosevelt Road, till the riverside of Tamsui River. Treatment capacity of the treatment system is about 10,000 m^3d^{-1}, including all sewage collected from Guiyang pumping station. In the collected catchment area, the discharge pipe line is in network distribution, connected to Guiyang Pump Station. Sewage is then sent to the gravel contact oxidation system (Figure 1).

Primary water quality parameters of the designed sewage quality are listed in the following: BOD 40 mgL^{-1}, NH_3-N 18 mgL^{-1} and SS 40 mgL^{-1}. The designed removal rate is 75% for BOD, SS and NH_3-N; or BOD < 10 mgL^{-1}, SS < 10 mgL^{-1} and NH_3-N < 5 mgL^{-1}.

The system includes the following four parts, the influent unit, the pre-treatment unit, the gravel contact aeration oxidation unit, and the effluent unit (Figure 2). The combined sewage flows into the pre-treatment unit under gravity. The rectangular grid at the front of the pre-treatment unit with grid space of 20 mm is used to remove coarse suspended solids. A set of automatic water quality analyzer is placed at inlet to monitor SS and other water quality parameters. When SS concentration is too high, the influent is stopped automatically to avoid high concentrations of SS entering into gravel processing unit, which could decrease porosity and reduce the service life of the gravel contract oxidation system. The sewage then flows into the grit chamber, the purpose of which is to remove sand with particle size larger than 0.2 mm. The design capacity of the grit chamber is 1800 $m^3m^{-2}d^{-1}$.

Gravel Contact Aeration Oxidation Treatment Unit

The Guiyang gravel contact oxidation treatment system was divided into two identical parallel treatment parts considering the space limit. The two units were filled with gravel, the particle size of which is 10–25 mm. The gravel contact aeration oxidation treatment units also consist of two separate parts to deal with high concentrations of ammonia nitrogen in the influent. The front part is an aeration zone, with an effective area of 2,346 m^2. The latter is non-aeration zone, with an effective area of 782 m^2. The hydraulic retention time of the aeration zone is 4.5 h, and it is 1h for the non-aeration zone. Therefore, the total hydraulic retention time of the gravel contact aeration oxidation units is 5.5 h. Treatment processes such as organic matter degradation and ammonia nitrogen nitrification occurred in the aeration zone. In the non-aeration zone, processes such as settling of total suspended solid and nitrogen removal by denitrification were also expected.

Water Sampling

From June 2012 to January 2014, influent and effluent were sampled monthly for analyses of DO, NH_3-N, NO_3-N, BOD, COD, and SS. All water quality parameters were analyzed using standard methods described in APHA-AWWAWPCF [5].

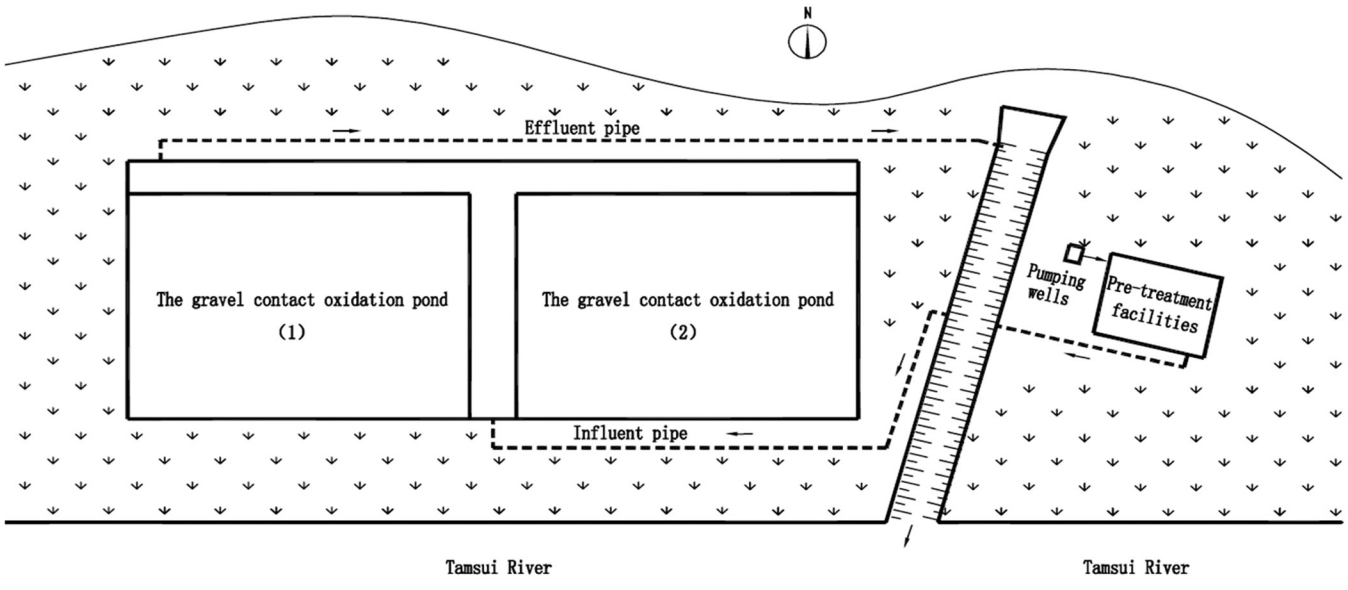

Figure 1. *Map of the gravel contact oxidation treatment system site.*

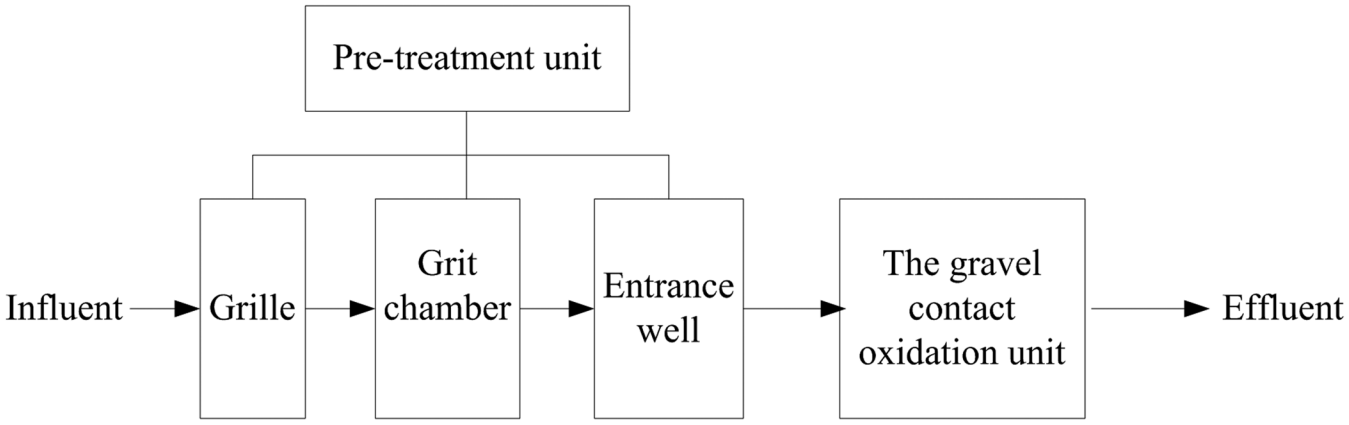

Figure 2. *Flow chart of the gravel contact oxidation treatment system.*

RESULTS AND DISCUSSION

SS Removal

SS was removed mainly by precipitation and filtration in the gravel contact oxidation treatment process. According to Figure 3, after pre-treatment of the grit chamber, SS concentration of the effluent entering into the gravel contact oxidation unit ranged 10–30 mgL^{-1}, which was not too high. The effluent SS concentration ranged 2–4 mgL^{-1} and the removal efficiency was 70–95%.

BOD, COD Removal

Some organic matter could be removed quickly through sedimentation and filtration in the gravel contact oxidation system. Soluble organic materials could also be removed by microorganism adsorption and degradation in aerobic conditions. Figure 4 demonstrate the variation of COD and BOD concentration in the influent and effluent. COD concentrations of ranged 30–95 mgL^{-1} of the influent and 10–20 mgL^{-1} of the effluent. BOD concentrations of the influent were 13–22 mgL^{-1} and 1.0–4.2 mgL^{-1} in the effluent. The removal rate was as high as 85–95%. The concentrations of COD in both influent and effluent were much higher than BOD. In Figure 5, DO concentrations of influent and especially effluent were high, indicating that biodegradable organic matter was removed sufficiently with adequate dissolved oxygen. BOD concentrations in the effluent were very low. The large difference in BOD and COD concentrations showed that the combined sewage contained much non-biodegradable organic compound. It was difficult to remove such organic compounds just by the gravel contact oxidation system, although COD removal efficiency was high.

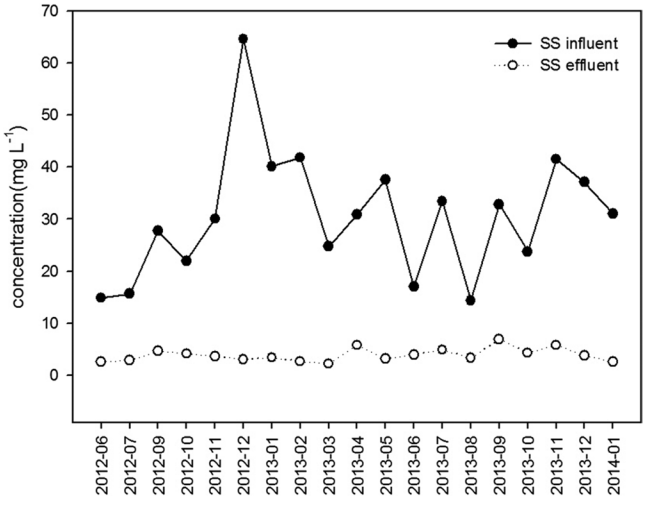

Figure 3. *Influent and effluent SS concentrations of the treatment system.*

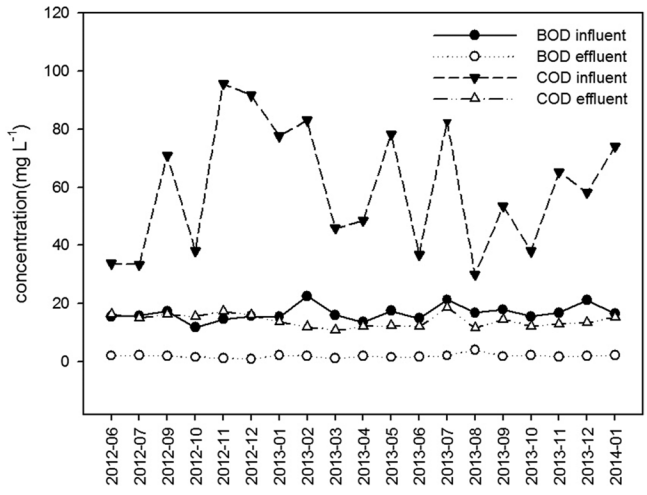

Figure 4. *Influent and effluent BOD and COD concentrations of the treatment system.*

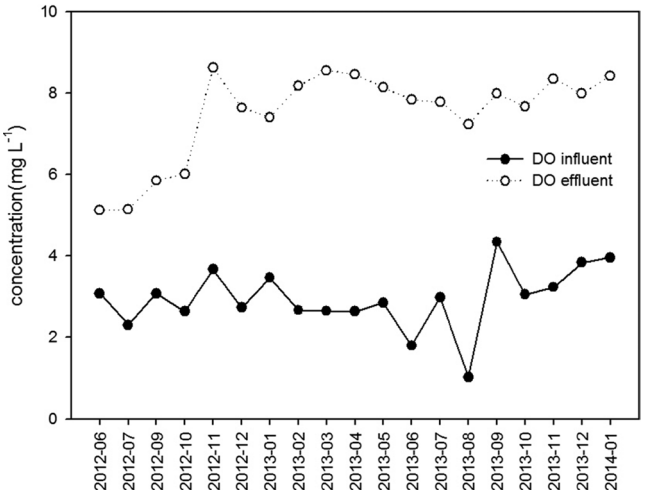

Figure 5. *Influent and effluent DO concentrations of the treatment system.*

Ammonia Nitrogen Removal

Removal mechanisms for ammonia nitrogen by the gravel contact oxidation system include adsorption, filtration and sedimentation, microbial nitrification and denitrification, and ammonia nitrogen volatilization. Among these, microbial nitrification and denitrification play an important role in nitrogen removal process [6–8]. Figure 6 demonstrates the ammonia nitrogen purification effect of the gravel contact oxidation system. Results show that, the ammonia nitrogen concentration decreased quickly. The concentrations of ammonia nitrogen in the influent were 3–7 mgL^{-1}, while the concentrations of ammonia nitrogen in the effluent decreased to 1 mgL^{-1}. The removal rate reached 70–90%. The average nitrate concentration of influent was 1.0 mgL^{-1}, while the average effluent concentration was 4.28 mgL^{-1}. In the first half of the gravel contact oxidation treatment system (aeration zone), microbial nitrification was accomplished completely. Then the combined sewage flowed into the non-aeration zone with a large amount of dissolved oxygen (Figure 5), although the system was not aerated. The denitrification process could not complete successfully. Ammonia nitrogen has converted into nitrate and nitrite under aerobic conditions. Therefore, ammonia nitrogen concentrations in the effluent were very low, while nitrate concentrations increased considerably. Nitrogen in the system was not removed through nitrification and denitrification processes.

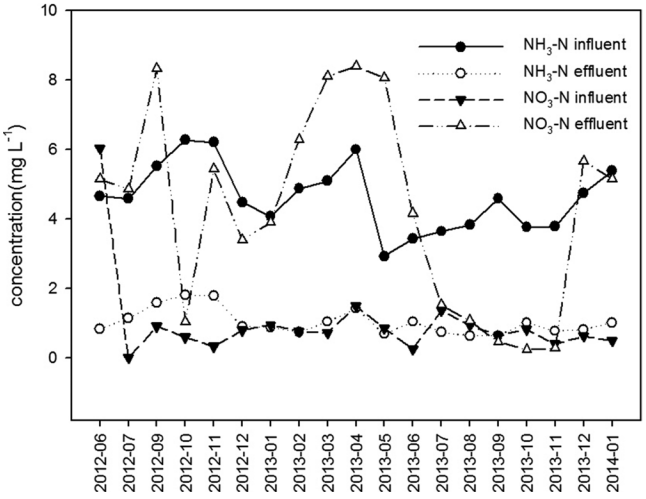

Figure 6. *Influent and effluent NH_3-N and NO_3-N concentrations of the treatment system.*

CONCLUSIONS

The gravel contact oxidation system is quite effective for combined sewage and rainwater system. The removal efficiency of BOD and SS ranged from 75–80% and from 70–95%, respectively. There was no seasonal variation for contaminants removal in the system due to the small temperature difference in Taipei throughout the year.

A gravel contact oxidation system was divided into two zones, aeration and non-aeration. For excessive aeration in the first half part, the sewage still contained large amounts of oxygen when it flowed through the non-aerated area. Microbial denitrification was relatively poor. Although ammonia nitrogen concentration decreased, nitrate concentration increased simultaneously. Nitrogen was not removed by the system. It was just a migration process among different forms of nitrogen. Therefore, aeration in the front zone of the gravel contact oxidation treatment system should be controlled in order to improve the removal efficiency of nitrogen.

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Components of a Bioflocculant for Treating Tannery Wastewater

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ABSTRACT: A prepared bioflocculant previously shown to be effective in treating tannery wastewater was examined to detect its components. Qualitative analysis revealed that the bioflocculant contained saccharides and proteins, which might have existed as glycoprotein, in addition to osamines and fats, but not nucleic acid. Quantitative analysis showed the mass composition of the bioflocculant to be as follows: saccharides (0.8963 g/g); proteins (0.0206 g/g); osamines (0.0081 g/g); and fats (0.055 g/g). It is expected that these results will contribute to future research into the mechanism by which the bioflocculant removes contaminants from tannery wastewater.

INTRODUCTION

SOME complex components are contained in tannery wastewater such as nitrogen-containing compounds within a large volume of darkly colored liquor. Many physical, chemical and microbial methods are available for treating tannery wastewater. [1] It is important that treatment methods are both economical and effective. Recently, the use of bioflocculants to treat tannery wastewater has become the focus of new research. It is well known that bioflocculants produced by microbes under unique cultural conditions consist of polymers that readily coalesce to form flocs. Due to their potential for easily treating wastewater and their unique effects on the biodegradation of pollutants, bioflocculants have received increasing attention from researchers [2].

To date, there have been several studies of treatment of tannery wastewater with bioflocculant. Zhang *et al.* treated tanning wastewater using bioflocculant produced from a kind of bacterium which was signed C-62 [3]. Li *et al.* isolated six species of bacteria producing bioflocculant and used one to treat leather effluent [4]. Chai *et al.* isolated two species of bacteria that produced bioflocculant and used them to treat tannery effluent [5]. Qin used microorganisms as flocculants to treat leather effluent and found they effectively removed the chemical oxygen demand (COD), suspended solids (SS) and Cr^{3+} from leather effluent [6]. Wang *et al.* selected four species of bacteria producing bioflocculants and found that the color, turbidity and COD of the tannery wastewater were significantly reduced after using the composite bioflocculant [7]. Liu *et al.* summarized the mechanism and research status of bioflocculant during treatment of tannery wastewater [8]. Rajan *et al.* found that *Trichococcus flocculiformis* and *Pseudomonas fluorescens* isolated from polluted tannery soil had flocculation ability [9].

However, there is a lack of research about the removal of total nitrogen (TN) in tannery wastewater using a bioflocculant. Previous experiments in our laboratory revealed that the bioflocculant produced from *Bacillus cereus* CZ1001, which was isolated from the activated sludge of a wastewater plant treating tannery effluent, had an ability to remove TN from tannery wastewater. The TN removal rate was about 35% when the bioflocculant concentration was 1.0 g/L and the initial concentration of total nitrogen was 350 mg/L [1]. Despite this relatively low removal rate, it is necessary to ascertain the components of this new bioflocculant so that the conditions under which it is produced can be optimized to generate a bioflocculant with improved ability to remove TN. In the present study, the components of the bioflocculant were detected through qualitative and quantitative analysis.

EXPERIMENTAL PROCEDURE

Materials

Biochemical-grade yeast extract, peptone and bovine serum albumin were purchased from Beijing AoBoXing

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Leiverseen Biotech Co. Ltd (Beijing, China). Wastewater was obtained from a tannery in Sichuan (China). All other reagents used were of research-grade quality.

Bacterial Strains

B. cereus CZ1001 was isolated from the activated sludge of a wastewater facility treating tannery effluent, and preserved in our laboratory. Before use, the organism was cultured in Luria broth.

Preparation of Bioflocculant

First, the culture medium was prepared with: KCl 0.5 g, K_2HPO_4 1 g , sucrose 30 g, $MgSO_4$ 0.5 g, $NaNO_3$ 2 g, $FeSO_4$ 0.01 g and water 1L. The pH of the culture medium was nature and the medium was sterilized at 121°C (0.105MPa) for 20 min before used. Then, the domesticated *B. cereus* CZ1001 was inoculated to the sterilized conical flasks containing 100 mL of the culture medium. Next, the flasks were incubated at 35°C while shaking at 180 r/min. After 24h, the cells were then centrifuged at 8000 rpm for 10 min at 4°C, after which the supernatants were amended with absolute ethyl alcohol that had been pre-cooled to 4°C at 3 times the volume of the supernatants. The mixture was then incubated at 4°C for 6 h, after which it was centrifuged at 10,000 rpm for 10 min. The precipitate was then collected and washed with distilled water, after which it was centrifuged at 8000 rpm for 10 min. Finally, the centrifuged precipitate was freeze-dried under a vacuum, which yielded the bioflocculant.

Qualitative Analysis of Bioflocculant [10–11]

Identification of Saccharide Content

The commonly used Molisch reaction test was employed for the identification of saccharides. The reaction is positive (indicated by a color change) when saccharide is present, whether in free or combined form. The detailed procedure was as follows. First, 5 g of α-naphthol were dissolved in 50 mL of 95% (w/w) ethanol to prepare an α-naphthol (Molisch) reagent. Then, 1 mL of fresh (i.e., unreacted) bioflocculant solution and two drops of α-naphthol reagent were added to a test tube, and the contents mixed uniformly. Then, 1 mL of concentrated sulfuric acid (H_2SO_4) was slowly and carefully added to the tube along the tube wall. Lastly, the interface between the two layers that formed (consisting of concentrated H_2SO_4 and sample, respectively) was observed to detect whether a purple ring appeared.

Identification of Protein

The Biuret reaction test was used to identify whether there was protein in the bioflocculant. First, 2 mL of sodium hydroxide (NaOH) solution was added into 1 g/L of the bioflocculant solution to create an alkaline environment. Then, Biuret reagent was prepared using 0.15 g of copper sulfate ($CuSO_4$) and 0.6 g of potassium sodium tartrate dissolved in 50 mL of distilled water; to this solution was added 30 mL of 10% (w/w) NaOH solution and 0.1 g of potassium iodide (KI) and the mixture was then diluted to a final volume of 100 mL. Next, 2 mL of sample (bioflocculant) solution and ten drops of Biuret reagent were mixed and observed for a change in color. If the resulting solution turned purple, the presence of protein was confirmed.

Identification of Glycoprotein

The Anthrone reaction test was used to identify whether there was glycoprotein in the bioflocculant. First, 3 mL of Anthrone solution were added to 1 mL of 1g/L bioflocculant solution. The resulting mixture was cooled rapidly in cold water, and then placed into a boiling water bath for 10 min. If the solution turned blue, hexose and penturonic acid were confirmed to be present. If the solution turned red, the presence of tryptophan was confirmed. If the solution turned violet, glycoprotein was confirmed to be present.

Identification of Osamine

The Elson–Morgan method was used for the identification of osamine. First, 2.5 mL of 2% (w/w) alkaline acetyl acetone solution were mixed with 0.5 moL/L of sodium carbonate solution (as a solvent) and added to 1 mL of sample (bioflocculant) solution. Then, the mixture was heated at 100°C for 20 min. After the mixture cooled, it was mixed with 1 mL of dimethylamine benzaldehyde and 1 mL of anhydrous alcohol. Next, this mixture was heated at 60°C for 90 min. As the mixture cooled, it was observed for a change in color. If a magenta color developed, osamine was confirmed to be present in the bioflocculant.

Identification of Fat

The presence of fat in the bioflocculant was mea-

sured using the well-known Soxhlet extraction method. A small mass (0.2 g) of dry bioflocculant was put into the round-bottom flask of a Soxhlet Extractor and weighed. After the extraction process was completed, the flask was re-weighed. If the weight of the flask increased, fat was confirmed to be present in the bioflocculant.

Identification of Nucleic Acid

The presence of nucleic acid in the bioflocculant was detected using two methods. The first method was as follows. A quantity of 0.2 g/L bioflocculant solution was scanned under the wavelength of 200 to 500 nm using a UV/VIS spectrophotometer. If there was an absorption peak at 260 nm, nucleic acid was confirmed to be present in the bioflocculant.

The second method used electrophoresis to generate an electrophoregram of the bioflocculant solution. If there was a strip for the bioflocculant solution compared with the marker in the electrophoregram, the presence of nucleic acid in the bioflocculant was confirmed.

Quantitative Analysis of Components in Bioflocculant [12]

The content of saccharides in bioflocculant was detected colorimetrically by the phenol-sulfuric acid method. The content of proteins in bioflocculant was detected by the quantitative Biuret method. The content of osamines in bioflocculant was measured by the Elson-Morgan method. The content of fats in the bioflocculant was measured with the Soxhlet extraction method.

RESULTS AND DISCUSSION

Analysis of Saccharide in Bioflocculant

The prepared bioflocculant is shown in dried form in Figure 1. In the Molisch test, a purple ring developed between the layer of concentrated H_2SO_4 and the layer of bioflocculant sample in the test tube (Table 1). Saccharides in the bioflocculant sample reacted and generated furfural or its derivatives through dehydration by the concentrated H_2SO_4; the derivatives then reacted with α-naphthol and generated purple substances [12]. This color change indicated that the bioflocculant sample reacted positively in the Molish reaction because it contained saccharides.

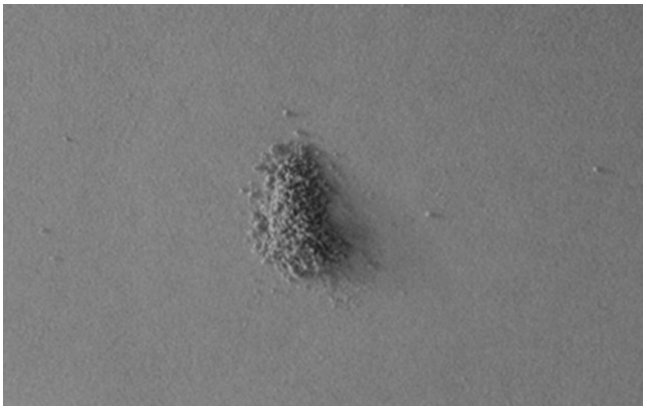

Figure 1. *A dried sample of prepared bioflocculant.*

Through the subsequent phenol-sulfuric acid quantitative analysis, it was found that there was 0.17926 g of saccharides in 0.2 g of bioflocculant which meant that the total content of saccharides in bioflocculant was determined to be 0.8963 g/g (Table 2).

Analysis of Protein in Bioflocculant

In the Biuret reaction test, the solution in the test tube became purple (Table 1), which confirmed there were proteins in the bioflocculant [12]. Through the quantitative part of the Biuret test, it was found that there was 0.00824 g of proteins in all in 0.4 g of bioflocculant which meant that the total content of proteins in the bioflocculant was determined to be 0.0206 g/g (Table 2), which was much less than the saccharides content.

Analysis of Glycoprotein in Bioflocculant

In the Anthrone reaction experiment, the solution turned violet (Table 1) confirming the presence of glycoprotein. The reaction indicated that saccharides and proteins in the bioflocculant were joined by cova-

Table 1. Component Identification of Bioflocculant.

Identification Experiment	Observed Phenomenon	Interpretation
Molisch reaction	Had purple ring	Contained saccharides
Biuret reaction	Solution became purple	Contained proteins
Anthrone reaction	Solution turned violet	Contained glycoprotein
Elson–Morgan method	Solution turned magenta	Contained osamine
Soxhlet extraction method	Weight of flask increased	Contained fat

Table 2. Content of Components in Bioflocculant.

	Saccharide	Protein	Osamine	Fat
Content (g/g)	0.8963	0.0206	0.0081	0.055

lent bonding into glycoprotein. Usually, glycoprotein has covalent bonding with short oligosaccharides and polypeptide chains. The Anthrone test showed that the saccharides in the bioflocculant contained short oligosaccharides and that the proteins contained polypeptide chains.

Analysis of Osamine in Bioflocculant

Osamine is an aminal formed when amine is linked to carbohydrates with a N-glycosidic bond. In the Elson–Morgan test, a magenta color appeared in the solution (Table 1), which confirmed that there was osamine in the bioflocculant. These results suggested that many amines existed in the bioflocculant and could react with the saccharides, thus forming osamine. Through the quantitative element of the Elson-Morgan test, it was found that there was 0.00405 g of proteins in all in 0.5 g of bioflocculant which meant that the total content of osamine in the bioflocculant was determined to be 0.0081 g/g (Table 2).

Analysis of Fat in Bioflocculant

In the Soxhlet extraction test, the weight of the round-bottom flask increased (Table 1), confirming the presence of fat in the bioflocculant. The quantitative analysis showed that there was 0.01102 g of fats in all in 0.2 g of bioflocculant which identified the total content of fats in bioflocculant to be 0.0551 g/g (Table 2).

Analysis of Nucleic Acid in Bioflocculant

During spectrophotometric scanning of the bioflocculant sample solution no absorption peak was observed at 260 nm (Figure 2) and thus this test indicated that there was no nucleic acid in the bioflocculant. In addition, as shown in Figure 3, electrophoresis failed to identify any strip for the bioflocculant solution compared with the marker in the electrophoregram. This result provided further confirmation that there was no nucleic acid in the bioflocculant.

Through the analyses described above, we found that the bioflocculant contained saccharides and proteins, which might have existed as glycoprotein, as well as osamines and fats, but no nucleic acid. We found the bioflocculant to be comprised of saccharides, proteins, osamines and fats in the following mass ra-

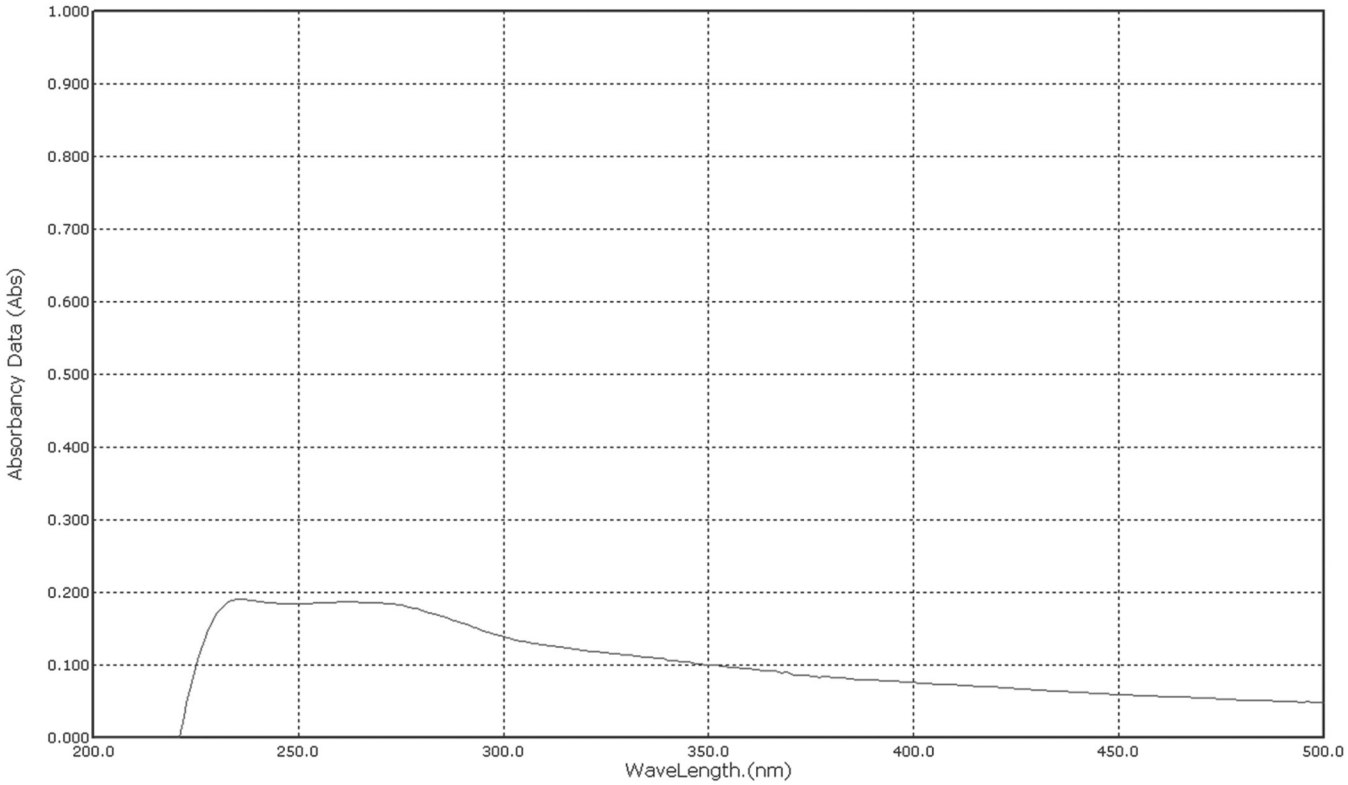

Figure 2. *Wavelength of the bioflocculant solution from 200 to 500 nm.*

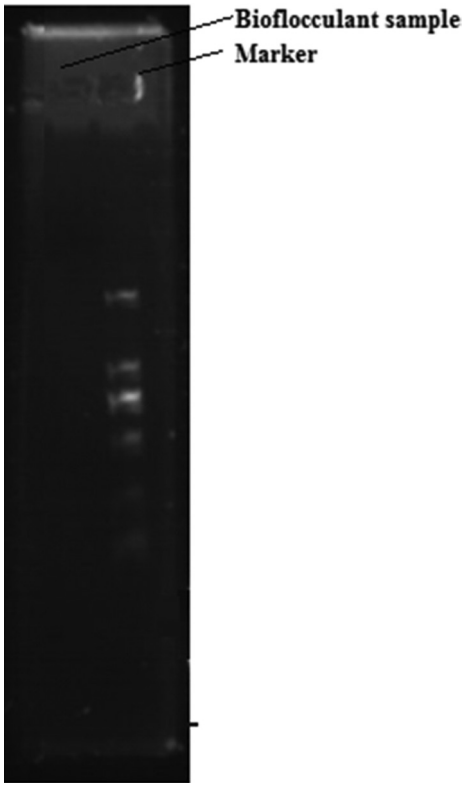

Figure 3. *Electrophoregram for solution of bioflocculant.*

tios: 0.8963 g/g, 0.0206 g/g, 0.0081 g/g and 0.055 g/g, respectively. The qualitative and quantitative analysis of components in the bioflocculant we examine was very important for the future mechanism study of the bioflocculant for removing tannery wastewater.

CONCLUSIONS

This study confirmed that the prepared bioflocculant studied contains saccharides and proteins, which might have existed as glycoprotein, as well as osamines and fats, in the following amounts: 0.8963, 0.0206, 0.0081, and 0.055 g/g, respectively. It is expected that the results will contribute to future research on the mechanism by which the bioflocculant can remove contaminants from tannery wastewater.

ACKNOWLEDGEMENTS

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Effects of Heavy Metal and Nutrients on Benthic Microbial Communities in Freshwater Sediment of Poyang Lake (China)

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ABSTRACT: The aim of this work was to relate sediment microbial communities with geochemical processes in a freshwater lake. Surface sediment samples were collected from seven sites (i.e., main basin and tributary estuaries of the lake) at Poyang Lake, China in May of 2011. Sediment environmental parameters such as concentration of nutrients and total contents of heavy metals (copper, zinc, lead, and cadmium) were measured. Profiles of bacterial communities were generated using DGGE (denaturing gradient gel electrophoresis). Pearson correlation analysis and CCA (canonical correspondence analysis) were employed to determine effects of sediment properties on benthic microbial communities. Experimental results showed that marginal and compound pollutions, tolerable for most benthic organisms, were found in sediments of Poyang Lake according to contents of nutrients and heavy metals. However, pollutions was severe in Rao and Xin River estuaries and especially copper pollution. Also, bacterial communities in the sediments had apparently horizontal heterogeneity and genotypic richness and diversity of microbial communities were predicted by sediment nutrients. Finally, total phosphorus (TP), heavy metal copper (Cu), and cadmium (Cd) in sediment were considered to be key factors driving spatial variation of bacterial community structures in Poyang Lake. This study suggested heavy metals together with phosphates may jointly affect benthic microbial communities and metal-reduction was an important microbial activity in freshwater sediment.

INTRODUCTION

FRESHWATER sediment, one of the most diverse microbial habitats [1], functions both as sink and source for heavy metals, nutrients, and other organic pollutants. Microbes regulating and influencing many ecosystem processes such as nutrient transformation and organic matter decomposition [2] could function as potential bio-indicators of pollution and environmental variation. All of this makes a freshwater sediment system a suitable environment to study the mechanism of complex geochemical recycling processes.

Several studies have been conducted in sediment of freshwater environments to determine links between benthic microbial community structures and environmental factors. Huang *et al.* [3] found that distribution of bacteria was affected by the combined effects of various dissolved inorganic matter in sediments of Pearl River, China. Total phosphorus and pH were characterized as significant environmental factors correlated with bacterial communities in the sediment of eutrophic lake [4]. Furthermore, it was reported that heavy metals have accumulative toxic effects on cells and they tend to be bound on sediments and removed from the water [5].

Poyang Lake, located in the middle-to-lower reaches of the Yangtze River in Jiangxi Province, is the largest freshwater lake in China. It is generally considered as a mesotrophic and graben-type lake. The lake receives flows from the Xiu, Gan, Fu, Xin, and Rao Rivers and exchanges water with the Yangtze River. Recently, anthropogenic activities threaten the water resources and biodiversity of Poyang Lake by deteriorating trophic status and releasing heavy metals [6], which makes the lake a possible locality for study on polluted freshwater sediment properties and its microorganisms. In the present study, following issues were expected to be answered: (1) the nutrient and heavy mental pollution status of the sediment (2) the spa-

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tial variations of bacterial community structure and diversity; (3) the major factors affecting the microbial communities.

MATERIALS AND METHODS

Site Description and Sediment Sampling

Surface sediment (0–5 cm) samples were collected in May 2011 from seven sites which were chosen and characterized based on their geographic locations (Figure 1). Site 1, in Songmen Mountain Region, and Site 4, in Nanjishan Region, are two sites in main southern basin of the lake and sites 2, 3, 5 and 6 are in the estuaries of Xiu, Rao, Fu and Xin River to Poyang Lake, respectively. Site 7 (Sanjiangkou Region) is the interface of Fu River, Xin River and the southern branch of Gan River.

Triplicate sediment samples were taken in each site with a grab sampler, then brought to the laboratory in an ice-cooled box. In the lab, samples were subdivided for molecular analysis (stored at –80°C) and physicochemical and heavy metals analyses (at 4°C).

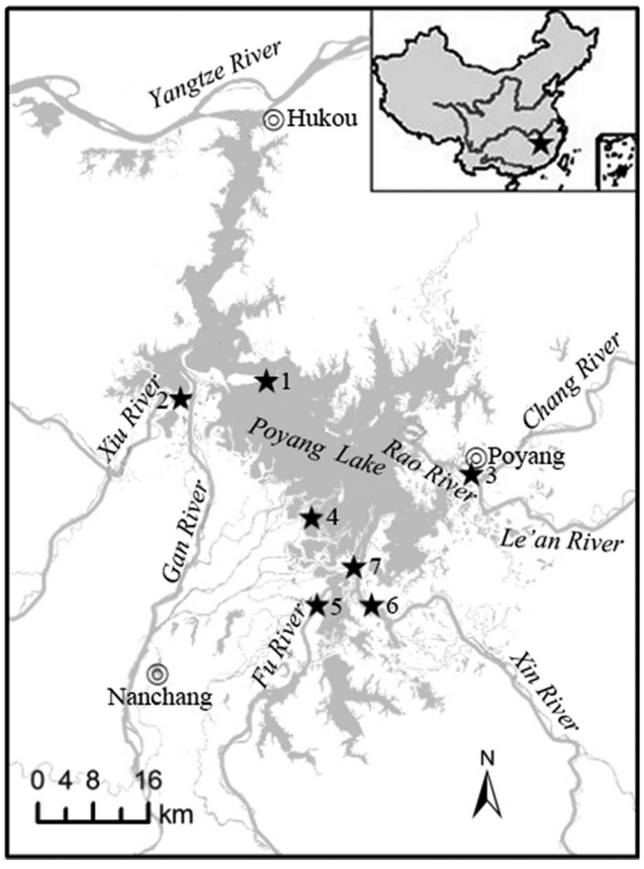

Figure 1. *Distribution of sampling sites in Poyang Lake, China.*

Sediment Properties Analyses

A detailed description of the sediment physicochemical measuring methods including experiment setup can be found elsewhere [7]. Briefly, Ash-free-dry-mass (AFDM) was determined by weighing and burning at 500°C for 5 h in muffle furnace (BF51800, Thermal). Total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) contents of sediments were analyzed by the Walkley-Black wet oxidation, the Kjeldahl and the molybdenum-stibium colorimetric methods, respectively. Furthermore, Total concentrations of copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) were quantified on an atomic absorption spectrophotometer (AA800, PerkinElmer) after wet-digestion with nitric acid, hydrofluoric acid, and perchloric acid, respectively.

DNA Extraction and PCR Amplification

Microbial genomic DNA was extracted from 0.5 g sediment using the PowerSoil DNA extraction kit (Mo-Bio, USA) by following the manufacturer's instructions. The V3-V5 region of the bacterial 16S rDNA was amplified with the primers F341 (adding a 40-bp of GC-clamp) and R907 on a Peltier Thermal Cycler (ABI 9700, USA). The PCR reaction volume and cycling condition were found in the work of [8]. Duplicate PCR amplicons from same site were gotten and purified with a DNA purification kit (Tiangen, China).

Denaturing Gradient Gel Electrophoresis (DGGE)

Bacterial community profiles were obtained using denaturing gradient gel electrophoresis (DGGE) to analyze PCR amplicons via a Dcode Universal Mutation Detection System (Bio-Rad) [9]. A volume of 10 μL amplicons mixed with equal volume of loading buffer was run on a 6% polyacrylamide gel (acrylamide-bisacrylamide ratio, 37.5:1) with 40–60% urea/formamide gradient in 1 × TAE buffer at 60°C 85 V for 16 h. After electrophoresis, the gel was silver stained.

Statistical Analyses

SPSS 17.0 was employed to test spatial heterogeneity of sediment properties. Background values of heavy metals in Poyang Lake were introduced to assess the contamination status [10]. Sediment quality was evaluated by Ontario sediment criteria [11].

DGGE banding patterns, in which each band generally stands for a group of bacterial species, were used

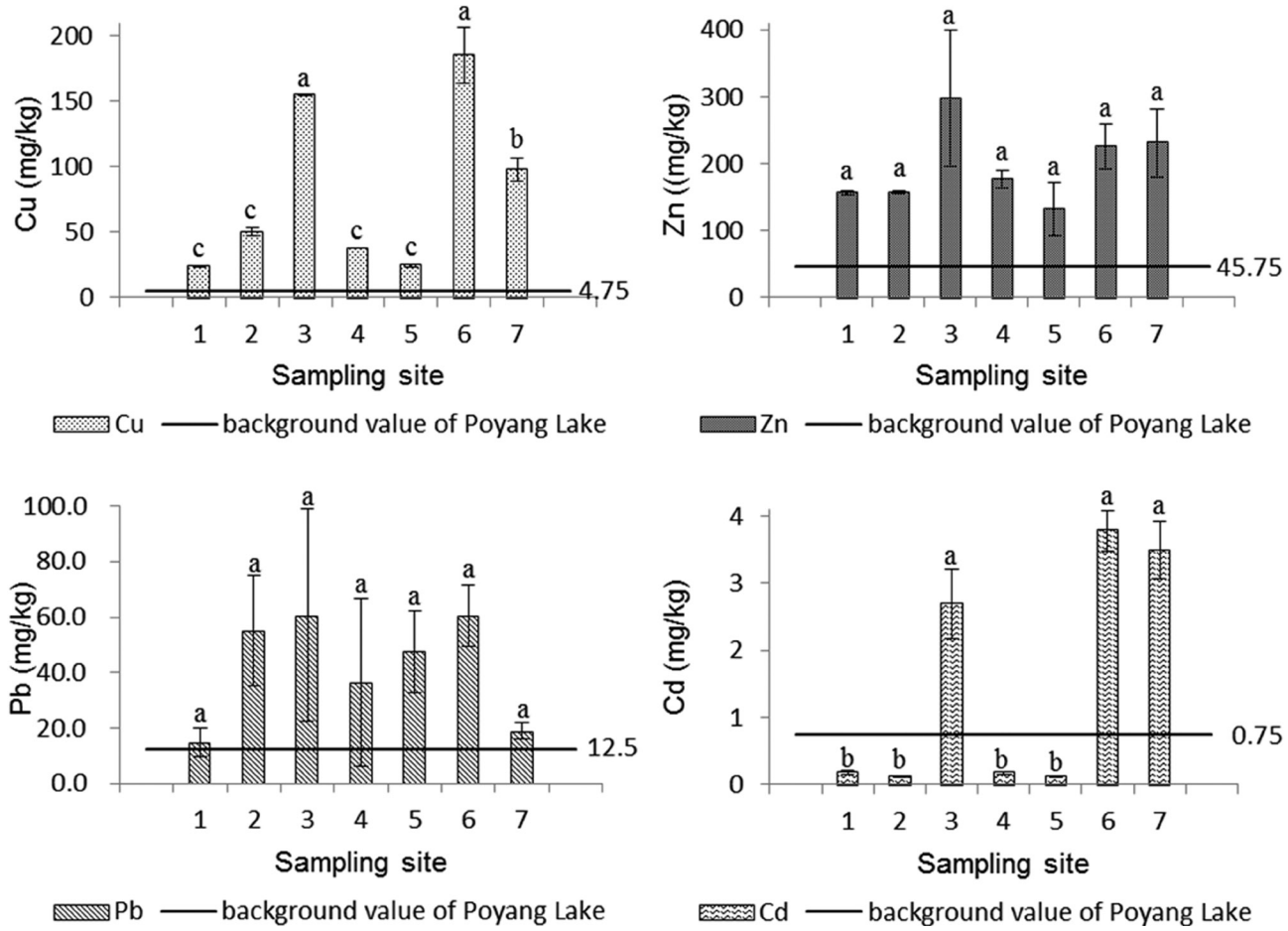

Figure 2. *Concentrations of heavy metals from surface sediment in Poyang Lake, China. Different letters in the same histogram mean significant difference at 0.05 level.*

to determine bacterial community structure by Quantity One software. The DGGE profiles obtained were analyzed by clustering via the unweighted pair group method with mathematical averages (UPGMA) [12]. The bands' position and intensity data were used to perform calculation of Shannon-Weaver diversity index (*H'*).

Pearson coefficient correlations of genotypic richness and Shannon-Weaver diversity index with environmental parameters were respectively performed by SPSS 17.0. Canonical correspondence analysis (CCA) was carried out to reveal relationships between sediment bacterial communities and measured sediment properties by Canoco for Windows 4.5 (Biometris, Netherlands).

RESULTS

Physicochemical Parameters of Sediments

Physicochemical properties were summarized in Table 1 and Figure 2. There was horizontal heterogeneity in several parameters of the Poyang Lake sediments. Among sampling sites, the contents of TP ($p < 0.001$), AFDM ($p < 0.01$), Cu ($p < 0.001$) and Cd ($p < 0.001$) were significantly different. The TOC and TP values were high at sites 3 and 6, while these two values were low at site 5. The TN content also reached a peak at site 3 and a bottom at site 5. Sediment pH ranged from 5.99 to 6.54 (Table 1).

The contents of Cu, Zn, and Cd were relatively high at sites 3, 6, and 7; while low at site 1 and 5 (Figure 2). In comparison with the background values, average contents of measured heavy metals in all sites established a contamination order as follows: Cu > Zn > Pb > Cd. Generally, sites 3, 6 and 7 were heavily polluted according to contents of all measured heavy metals, whereas sites 2 and 5 suffered a moderate or mixed state of pollution and sites 1 and 4 were relatively unpolluted.

Cluster Analysis of DGGE Profiles

The DGGE profiles of bacterial community struc-

Table 1. Physicochemical Parameters and Bacterial Diversity and Richness.

							Bacteria	
	Sampling Site	**pH**	**AFDM%**	**TOC (g/kg)**	**TN (g/kg)**	**TP (g/kg)**	***S***	***H'***
1	Songmen Mountain Region	6.18 ± 0.26a	3.39 ± 0.43b	9.30 ± 008a	0.86 ± 0.01a	0.62 ± 0.01b	18.0	2.37
2	Xiu River Estuary	6.44 ± 0.18a	4.80 ± 0.77ab	7.94 ± 0.05a	0.86 ± 0.01a	0.53 ± 0.00b	17.0	2.42
3	Rao River Estuary	6.49 ± 0.09a	5.98 ± 0.16a	11.90 ± 0.05a	1.09 ± 0.01a	0.99 ± 0.00a	23.5	2.50
4	Nanjishan Region	6.54 ± 0.33a	6.74 ± 0.57a	11.22 ± 0.35a	0.99 ± 0.05a	0.49 ± 0.01b	20.0	2.41
5	Fu River Estuary	5.99 ± 0.10a	4.82 ± 0.54ab	6.62 ± 0.12a	0.39 ± 0.01a	0.40 ± 0.01b	17.0	2.41
6	Xin River Estuary	6.33 ± 0.10a	6.54 ± 0.55a	12.28 ± 0.07a	0.88 ± 0.01a	1.02 ± 0.00a	21.0	2.79
7	Sanjiangkou Region	6.37 ± 0.09a	5.08 ± 0.08ab	9.92 ± 0.06a	0.77 ± 0.01a	0.65 ± 0.00b	19.5	2.67

Notes: Values are mean ± standard deviation, AFDM=ash free dry mass. Data in the same column followed by different letters means significant difference at 0.05 level. *S* and *H'* were bacterial richness and Shannon indexes.

ture and dendrogram of UPGMA in the 7 sediment sampling sites (sites 1 and 4 from main basin; other sites from estuaries) of Poyang Lake were presented in Figure 3. As seen in Figure 3(a), the bands were dispersed across the entire gel gradient and all lanes shared several strong dominating bands and a large number of fainter, well-resolved and unresolved bands in the background, indicating that the microbial community structure of sediment profiles in Poyang Lake were rather complex. UPGMA clustering graph was to describe the similarities of the bacterial communities in the different samples [Figure 3(b)]. The dendrogram revealed that samples were grouped into 2 defined clusters, the patterns of sites 6 and 7 formed a separate cluster sharing 49% similarity with DGGE patterns of the other five sites. The remaining five sites' patterns formed a joint cluster with four sub-clusters. Among them site 1 and site 4 were clustered together at a 74% similarity and sites 2 and 5 were assigned to a group at a similarity of 79%.

Genotypic richness (*S*) and Shannon-Weaver diversity index (*H′*) of sediment bacterial communities based on DGGE fingerprint were summarized in Table 1. The genotypic richness (*S*) varied from 17.0 to 23.5. The highest genotypic richness was found at site 3 while the lowest at sites 2 and 5. Bacterial communities *H′* varied from 2.37 to 2.79. The highest bacterial *H′* was observed at site 6 and the lowest at site 1. Richness (S) and diversity indexes (*H′*) of bacterial communities were significantly correlated with TP (r = 0.84 or 0.57), total Cu (r = 0.76 or 0.73) and Cd (r = 0.73 or 0.86), respectively.

Canonical Correspondence Analyses

The CCA of the bacterial 16S rDNA DGGE data explained 54.3% of the variation in the first two axes and confirmed the distinct separation of sampling sites according to environmental factors (Figure 4). The samples from estuarial sites (2, 3, 5 and 6) and the main basin of Poyang Lake (sites 1, 4 and 7) were separated by the first axis. Given the heavy metal pollution, the relatively unpolluted sites (1 and 4) were separated from the other polluted sites in the biplot. In the first axis, main positive correlation factors were TP (r = 0.40), Cu (r = 0.52) and Cd (r = 0.76). The second axis was negatively correlated to TP (r = –0.50), Cu (r = –0.61), Zn (r = –0.46) and Cd (r = –0.50). Monte Carlo test showed that Cu ($P < 0.05$) and Cd ($P < 0.01$) were strongly related to bacterial community composition in the sediments of Poyang Lake.

DISCUSSION

Trophic Status and Heavy Metal Pollution in Sediment of Poyang Lake

In this study, the average contents of TOC, TN and TP in the sediment of Poyang Lake were 9.88, 0.84 and 0.67 (g/kg), respectively, which suggested an accumulation trend compared to previous study (9.37, 0.66, 0.40 (g/kg), respectively) [13]. High contents of nutrients and heavy metals were found at Rao (site 3) and Xin River estuaries (site 6), attributing to heavily domestic and industrial wastewater discharge. Rao and Xin River estuaries are the main industrial locations such as aquatic farms and phosphate mines. Furthermore, copper mines are distributed along the southern branch of Rao River and in the middle reaches of Xin River.

According to the Ontario criteria, average contents of TN, TP, Cu, Zn, Pb and Cd in sediments of Poyang Lake exceeded the lowest effect level, indicating that the sediment of Poyang Lake was marginally polluted

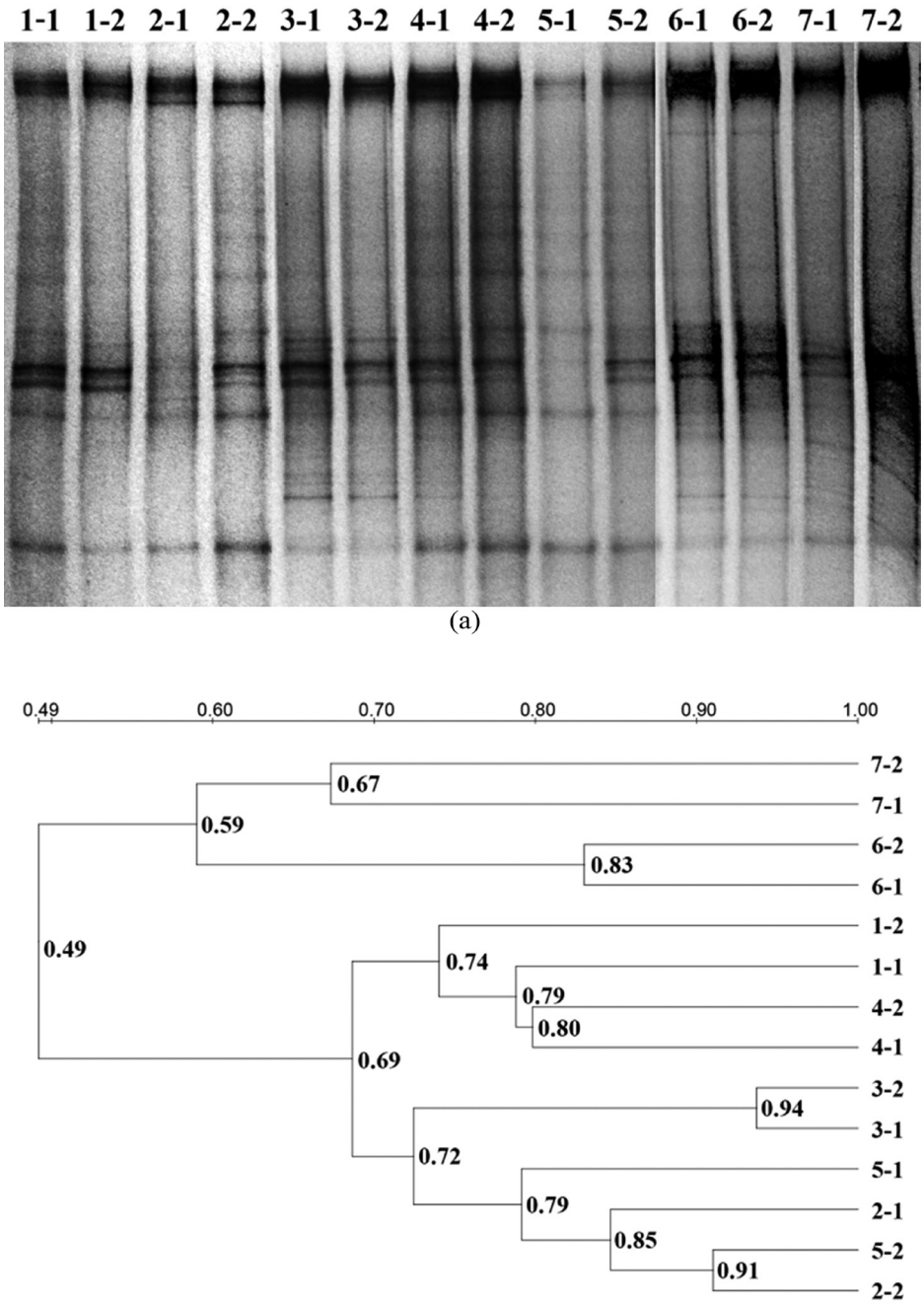

Figure 3. *DGGE fingerprint (a) and cluster analysis (b) of different sediment samples in Poyang Lake, China. Lanes: 1–7 represent different sampling sites. –1 and –2 mean in duplicate of same site.*

and can be tolerated by the majority of the sediment-dwelling organisms [14]. It was remarkable that concentration of all nutrients and heavy metals at Rao River Estuary (site 3) and Xin River Estuary (site 6) exceeded the lowest effect level and Cu contents indeed exceeded the severe effect level. Taking the water quality status into consideration, it is urgent to take measures to prevent further ecosystem degradation in Poyang Lake, especially due to the copper pollution.

Microbial Community Structure and Diversity in Sediment of Poyang Lake

In this study, parallel distribution patterns of bacterial community and nutrients were revealed: relatively, the genotypic richness and diversity of bacterial communities were high at Rao River estuary (site 3) and Xin River estuary (site 6) where high contents of TOC, TN and TP were found. Meanwhile, low levels of ge-

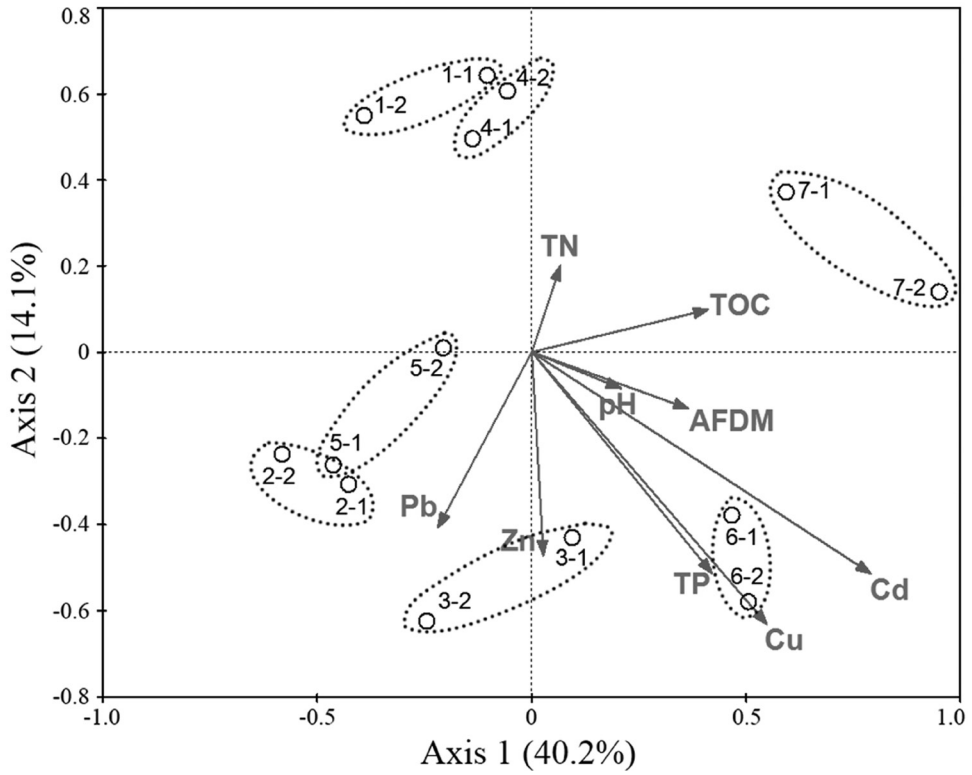

Figure 4. *Canonical correspondence analysis biplots of bacterial communities associated with environmental variables. Bacterial communities were presented by circles. Environmental parameters were indicated by arrows. Each sampling site was conducted in duplicate.*

notypic richness and diversity of bacterial community were detected at Fu River estuary (site 5) where the nutrient contents were low. This result was in agreement with our previous work, in which the richness and diversity of bacterial community was related to the nutrient status in the surface water column of Poyang Lake [15]. It may be due to the theory that sediment fertility and substrate availability influences microbial process rates [16]. Namely, more fertile sediment, with higher nutrients concentrations, may support higher rates of microbial processes as well as higher diversity. There may also be the possibility that the aerobic anoxygenic phototrophic bacteria (AAPB) were the dominated microbial contributor for the parallel distribution patterns with nutrients. Because AAPBs were found to be abundant (10~80% of bacterial biomass) in oligotrophic to mesotrophic lakes like Poyang Lake[17] and show higher abundance in high-nutrient media with a higher level of organic matter content [18].

Controlling Environmental Factors on Microbial Community

Our results suggested that bacterial communities were significantly affected by environmental factors. Notably, TP, Cu and Cd were important factors affecting bacterial community in surface sediment of Poyang Lake. TP played an important role in driving the changes in water column and sediment bacterial communities of freshwater Lake [4,15]. And it has been reported that phosphorus concentration is associated with the phosphate-dissolving and phosphate-decomposing bacteria in the sediments [19]. Also, previous work has demonstrated that changes in microbial diversity and function can be attributable to heavy metal contamination: Bouskill *et al.* [20] revealed that Cu and Cd strongly influenced the microbial community in river sediment. These may related to the selective pressure of heavy metals on microorganisms, some of which readily adsorb or accumulate metal ions [21] and finally adapt a variety of heavy metal tolerance mechanisms. These metal-tolerance mechanisms could be spread throughout a microbial community by lateral gene transfer [22]. Thus, heavy metals may function as important selective agents driving the evolution of microbial communities.

CONCLUSION

This work demonstrated an apparent spatial shift in both pollution status and microbial communities in sediments of Poyang Lake. Although there was a com-

pound of interest, it was generally microbial-tolerable pollution. The spatial distribution pattern of benthic microbial community varied identically along with that of nutrient level. Multivariate analyses indicated that TP, Cu and Cd had remarkable effects on bacterial community structures.

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Table 5. Comparison of state-of-the-art matrix resins with VPSP/BMI copolymers.

Resin System	Core Temp. (DSC peak)	T_E	Char Yield, %
Epoxy (MY720)	235	250	30
Bismaleimide (H795)	282	>400	48
VPSP/Bismaleimide copolymer			
C379: H795 = 1.9	245	>400	50
C379: H795 = 1.4	285	>400	53

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