Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Feasibility of sulfate-calcined eggshells for removing pathogenic bacteria and antibiotic resistance genes from landfill leachates

Mao Ye [a,b,*], Mingming Sun [c,d], Xu Chen [c], Yanfang Feng [e], Jinzhong Wan [f], Kuan Liu [c], Da Tian [c], Manqiang Liu [c], Jun Wu [c], Arthur P. Schwab [d], Xin Jiang [a,*]

[a] Key Laboratory of Soil Environmental and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, PR China
[b] Department of Civil Engineering, University of Nebraska-Lincoln, 844 North 16th Street, Lincoln, NE 68588-6105, United States
[c] Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, PR China
[d] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 88743, USA
[e] Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China
[f] Nanjing Institute of Environmental Science, Ministry of Environmental Protection of China, Nanjing 210042, PR China

ARTICLE INFO

Article history:
Received 31 October 2016
Revised 1 March 2017
Accepted 3 March 2017
Available online xxxx

Keywords:
Eggshells
Adsorption
Pathogenic bacteria
ARGs
Landfill leachate

ABSTRACT

High abundance of human pathogen and antibiotic resistance genes (ARGs) in landfill leachate has become an emerging threat against human health. Therefore, sulfate- and calcination-modified eggshells as green agricultural bioresource were applied to test the feasibility of removing pathogenic bacteria and ARGs from leachate. The highest removal of *Escherichia coli* (*E. coil*) and gentamycin resistant gene (*gmrA*) from artificial contaminated landfill leachate was achieved by the application of eggshell with combined treatment of sulfate and calcination. The 16S and *gmrA* gene copies of *E. coil* declined significantly from 1.78E8 ± 8.7E6 and 4.12E8 ± 5.9E6 copies mL^{-1} to 1.32E7 ± 2.6E6 and 2.69E7 ± 7.2E6 copies mL^{-1}, respectively, within 24 h dynamic adsorption equilibrium process ($p < 0.05$). Moreover, according to the Langmuir kinetic model, the greatest adsorption amount (1.56×10^9 CFU *E. coil* per gram of modified eggshells) could be obtained at neutral pH of 7.5. The optimal adsorption eggshells were then screened to the further application in three typical landfill leachates in Nanjing, eastern China. Significant decrease in species and abundance of pathogenic bacteria and ARGs (*tet*, *sul*, *erm*, *qnr*, and *amp*C) indicated its great efficiency to purify landfill leachates. This study demonstrated that sulfate-calcined eggshells can be an environmentally-friendly and highly efficient bioadsorbent to the management of reducing dissemination risk of pathogen and ARGs in landfill leachate.

1. Introduction

In most developed countries, the technology for the treatment of landfill leachate has been maintained well and monitored strictly. However, for the developing countries, waste classification and sealed management systems have not yet been perfectly established due to the lack of waste recycling legislation, technique equipment, and public awareness. A large amount of domestic waste, animal feces, and industrial waste with high contents of antibiotics has been dumped into landfills (Lu et al., 2016; Sun et al., 2016; Wu et al., 2015; Zhang et al., 2016). The existence and continuous input of such antibiotics could exert selective pressure on the indigenous bacteria and make the antibiotic resistant bacteria (ARB) the dominant communities in landfills (Brooks et al., 2016; Cui et al., 2016; Hwang et al., 2016; Lin et al., 2016; Salcedo and Kim, 2017). Moreover, the frequency of horizontal gene transfer between pathogenic bacteria and ARB would be stimulated with the assistance of mobile genetic elements, making the landfill the hotspot of pathogenic bacteria and ARGs (Miller et al., 2016; Warnes et al., 2012; Subirats et al., 2016). This is especially true for the landfills without proper seepage control facility (Kirmizakis et al., 2014; Zhang et al., 2013; Zolfaghari et al., 2016). In these sites, the mixed pollutants (ARB and ARGs) migrate along with landfill leachate, and contaminate the underground water or even surface water, resulting in the serious threat against human health and environmental safety (Calero-Cáceres and Muniesa, 2016; He et al., 2016; Marti et al., 2014). Consequently, it is urgent to develop controlling technology to reduce the abundance of pathogenic bacteria and ARG dissemination.

* Corresponding authors at: Key Laboratory of Soil Environmental and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, PR China (M. Ye).
E-mail addresses: yemao@issas.ac.cn (M. Ye), Jiangxin@issas.ac.cn (X. Jiang).

http://dx.doi.org/10.1016/j.wasman.2017.03.005
0956-053X/

For the controlling technology development, the preferential option is usually to screen novel adsorption materials (Feng et al., 2013). As emerging contaminants, there are so far not many published methods to remove pathogenic bacteria and ARGs from the landfill leachate. The using of active carbon\biochar\limestone\bentonite to remove heavy metals\organic pollutants\nutrients from landfill leachate has been reported previously (Li et al., 2015; Westholm et al., 2014). However, the current adsorbents were mostly applied to the traditional organic/inorganic pollutants, instead of novel contaminants, such as pathogenic bacteria and ARGs. In addition, among these adsorbents eggshells as green adsorbent bioresource have attracted extensive attention due to their inert porous structure, large ratio surface area, temperate chemical property, low cost and non-toxicity (Baláž et al., 2015; Choi and Lee, 2015). Furthermore, as the highest egg production in the world. It should be readily accessible for the eggshell obtaining in China (Ye et al., 2016). Previous studies have reported the application of conventional/modified eggshells to remove heavy metals (Pb^{2+}, As^{5+}, and Cd^{2+}) (Flores-Canoa et al., 2013; Liao et al., 2010; Markovski et al., 2014), organic dyes (Tsai et al., 2006, 2008), and fluoride from the aqueous environment (Lunge et al., 2012). However, the possibility of applying such material to remove newly emerged contaminants like pathogenic bacteria and ARGs from landfill leachates remains investigated.

The objectives of this work were: (i) preparing four kinds of modified eggshell materials, and screening out the optimal adsorbent to remove gentamycin-resistant *E.* coli and *gmrA* gene from artificial contaminated landfill leachate; (ii) testing its further application in three different landfill leachates in Nanjing, China. The results obtained here extended the eggshell application range in environmental remediation area, and provided a practical solution to the management of reducing proliferation risk of pathogenic bacteria and ARGs in landfill leachate.

2. Materials and methods

2.1. Site description

Landfill leachates were collected from three landfill sites [Shuige landfill (SG, built in 1993, 31°53′45″ N, 118°45′4′ E), Jiao Zishan landfill (JZS, built in 1992, 31°59′ 44″ N, 118°53′56″ E), Tian Jinwa landfill (TJW, in operation between 1985 and 2015, 32°8′53″ N, 118°41′2″ E)] in Nanjing, eastern China. According to our preliminary survey, seasonal regular patterns for the fluctuation of ARGs could be detected in these sites (Sun et al., 2016). The abundance of ARGs in the landfill soils and leachates generally increased from September through December caused by the sharp increase in the waste dumping (Sun et al., 2016). Due to the long operating time, the wastes in these sites have formed the shape of waste mountains ranging in height from 50 to 100 m. The impervious layer of the landfills has been aged for many years without proper maintenance. Therefore, ten samples of leachates in each landfill were collected directly from the natural ditch at the foot of the mountains along the direction of the water between April and May 2016. Leachate samples were kept in brown glass bottles and put on dry ice during transport. The leachates were stored at −20 °C before DNA extraction and chemical analysis. Detailed information about the physiochemical properties of leachates is outlined in Table S1.

2.2. Eggshell preparation and characterization

Waste eggshells were collected from a local poultry market. Eggshells were immediately washed thoroughly with deionized water and dried at 105 °C for 24 h. All eggshells were grounded and passed through a 2.0-mm sieve. Four treatments were designated as follows: (1) Dry eggshells: dried mass without modification; (2) Calcined eggshells: dry eggshells modified by calcination at 500 °C for 12 h in muffle furnace; (3) Sorbent A:0.24 kg eggshells were added into ferric sulfate solution prepared by dissolving 1.87 kg of $Fe_2(SO_4)_3$ in 10 L deionized water. The mixture was adjusted to pH 3.0 using H_2SO_4 and continuously stirred for 12 h on shaker. After shaking, the mixture was transferred to Petri dish and dried at 105 °C for 12 h. (4) Sorbent B: Sorbent A modified by calcinations at 500 °C for 12 h in muffle furnace. The textural properties and elemental contents for different eggshells were provided in Table 1. Fourier transform infrared spectroscopy (FTIR) (Thermo Nicolet 670 FTIR) studies were carried out to identify the functional groups of the used eggshells (Lunge et al., 2012). Identification of the crystalline species present indifferent eggshells was recorded using X-ray diffraction (XRD) analysis (Rigaku, DMAX 200, diffraction meter) (Markovski et al., 2014). Scanning electron microscopy (SEM) was conducted with a Hitachi JSM-6700F SEM to observe the surface microstructures (Choi and Lee, 2015).

2.3. Adsorption experiment for E. coli

In this study, a gentamycin-resistant strain of the Gram negative bacterium *E. coli* (DH5α, 1pE × Gm Plasmid) was used as model pathogenic bacteria. The strain was incubated at a temperature of 37 °C until stationary phase, centrifuged to remove growth media, and washed three times with equal volume of sterile water. The biomass of *E. coli* was resuspended in a 100 mL of modified mineral salt medium (1.0 g L^{-1} NaCl, 1.0 g L^{-1} $(NH_4)_2SO_4$, 1.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KH_2PO_4, 0.1 g L^{-1} $MgSO_4{\cdot}7H_2O$, pH 7.0) to achieve an initial concentration of 1.5×10^7 colony forming units per mL (CFU mL^{-1}). The salt medium was sterilized before use. The concentration was achieved by using OD600. No carbon resource was added to the medium to maintain the bacterial physiological state of neither quick dying nor reproduction. Therefore, the gentamycin-resistant *E. coli* in the present work was considered as a single molecule pollutant.

To determine the optimum *E. coli* adsorption conditions for eggshells. One gram of different eggshells was added into 100 mL of medium respectively. Triplicate samples were performed. The batch adsorption experiments were conducted at room temperature at 100 rpm for 60 h in 250 mL Erlenmeyer flasks. Every 12 h, the supernatant was recovered by centrifugation at 4000 rpm for 1 min. The counts of *E. coli* in liquid phase were measured at different sampling time using Luria-Bertani (LB) agar (RHCA, Himedia, Shanghai) mixed with 50 mg L^{-1} gentamycin. Changes in16S rRNA and gentamicin resistance gene (*gmrA*) copies of the *E. coli* in the supernatant were quantified by quantitative polymerase chain reaction (qPCR) using a SYBR Green approach before and after different sampling time (Zhu et al., 2013). The primer design can be found in Supporting Information Table S2.

As shown in supplementary materials (Tables S4 and S5), the counts of pathogenic bacteria and the abundance of ARGs did not decrease significantly in the liquid without carbon source. In addition, all the 16S rRNA and *gmrA* genes were presented inside the *E. coil*. There were no free 16S rRNA or *gmrA* genes in the liquid phase in this study. This directly proved that the removal of pathogenic bacteria and ARGs was indeed caused by the eggshell adsorption. Meanwhile, the amounts of eggshell adsorbed *E. coil*. at certain sampling time could be calculated by that the initial counts minus the specific time counts in the liquid phase. Pseudo-first-order kinetic model calculation was used to fit the experimental raw data to describe the isothermal adsorption of *E. coli* amounts onto the different eggshells. The abundance of eggshell adsorbed 16S rRNA and *gmrA* genes was also calculated according to the above method:

Please cite this article in press as: Ye, M., et al. Feasibility of sulfate-calcined eggshells for removing pathogenic bacteria and antibiotic resistance genes from landfill leachates. Waste Management (2017), http://dx.doi.org/10.1016/j.wasman.2017.03.005