Ocean bacteria: performance on COD\textsubscript{Cr} and NH\textsubscript{4}\textsuperscript{+}-N removal in landfill leachate treatment

Yali Feng, Aifei Yi, Haoran Li, Weida Wang and Yunlong Du

ABSTRACT

An experiment was carried out to investigate the performance of mixed ocean bacteria, isolated from the ocean sediment, on landfill leachate treatment. In this treatment, ocean bacteria were the only constituent added to remove organics and NH\textsubscript{4}\textsuperscript{+}. Given their considerable influence on wastewater purification, factors such as inoculum, initial pH, processing time and oxygen condition, were directly involved in this research. As indicated by laboratory test results, chemical oxygen demand (COD\textsubscript{Cr}) and NH\textsubscript{4}\textsuperscript{+} removal could reach 94.45% and 67.87%, respectively, after 3 days of treatment, in conditions of natural pH 6.3 and with the application of oxygen. The volt–ampere characteristics of the bacteria solution verified the redox-active ability of the bacteria in landfill leachate treatment.

Key words | COD removal, landfill leachate, NH\textsubscript{4}\textsuperscript{+} removal, ocean bacteria, volt–ampere characteristics curve

INTRODUCTION

Landfill leachate, one of the main problems resulting from sanitary landfill, has quickly produced a considerable increase in quantities of solid waste in China (Bing et al. 2008). Being high-strength wastewater, it is characterized by extremes of high chemical oxygen demand (COD\textsubscript{Cr}), inorganic salts, heavy metals and toxicity. The composition of the leachate is based on the age of the landfill, local rainfall, composition and degree of contouring, compacting of solid wastes and physicochemical conditions at the landfill (Gotvajn et al. 2009). Therefore, young landfill leachates present distinct features during treatment compared to that of older landfill leachates. Many different organic and inorganic compounds are contained in the leachate; these may be either dissolved or suspended and biodegradable and non-biodegradable (Bilgili et al. 2008). If not disposed of safely, landfill leachate can provide a major source of water contamination, because it can cause high pollution in the receiving water as it percolates through soil and subsoil (Oman & Junestedt 2008). Thus, to minimize water resources pollution and to avoid acute and chronic toxicities, leachate treatment before discharge should be enforced by legal requirement.

To overcome the considerable negative impacts of landfill leachate on the environment, several techniques have been reported for treating this wastewater (Renou et al. 2008), which include biological treatment (Lim et al. 2012; Capodici et al. 2014), physical treatment, such as flotation (Zouboulis et al. 2005) and absorption (Wang et al. 2002), chemical treatment, such as precipitation (Kim et al. 2007) and new treatments, such as membrane processes (Piatkiewicz et al. 2001). Biological treatment is an environmentally friendly technique for wastewater treatment. Orupoad et al. (2000) studied the biological treatment of leachate from oil shale semicoke ash heaps in a wastewater lagoon using laboratory-scale batch processes; the best COD removal was up to 70.45% for 10 days in the intermittently aerated batch. It indicated that leachate could be treated in an aerated lagoon system, and that even for high pH leachate, the treatment is feasible without pH adjustment.

In this study, aerobic biological treatment of landfill leachate was investigated in batch. Since ocean marine sediment includes diverse bacteria, which can survive even in extreme deep-sea environments, they were chosen as inocula and used innovatively to treat landfill leachate. A flask reactor was filled with landfill leachate and fixed in a digital biochemical incubator to remove contaminant. The objective of the study was to investigate performance on organics and NH\textsubscript{4}\textsuperscript{+} removal in landfill leachate treatment, without the addition of extra inorganic or organic nutrients.
MATERIALS AND METHODS

Landfill leachate

The landfill leachate, without any efficient bacteria, was collected from a local landfill site in Dalian, Liaoning Province of China, and stored at 4 °C in a plastic container. Analysis results showed that the landfill leachate before treatment had a pH value of 6.3, and the concentration of each composition is listed in Table 1.

Data reported in Table 1 provide a biochemical oxygen demand/chemical oxygen demand (BOD/COD) ratio equal to 0.44, which in respect of landfill leachate is representative of a leachate derived from a young landfill; it also shows that the leachate treated in this study is relatively highly biodegradable.

Bacterial suspension

Different ocean sediment samples (from the China Ocean Biologic Sample Repository, Qingdao, Shandong Province, China) were added to landfill leachate without adding any other elements. When bacteria in the sediment sample cultured, by shaking for 5 days, it was transferred to fresh landfill leachate medium for another 5 days to screen effective bacteria with an excellent ability to remove organics and NH₄⁺-N from the landfill leachate. The effective ocean bacteria were inoculated to fresh leachate medium for 3 days for bacteria enrichment. Afterwards, the leachate medium was centrifuged at a speed of 12,000 rpm for 10 min, and was then filtered through a membrane. The cells were washed off with sterile physiological NaCl solution (mass fraction 0.7%). Moreover, the cells were diluted in sterile phosphate buffer (pH = 7.0), where the OD₅₁₀ density of bacterial suspension was 2.21. The bacterial suspension was then stored at 4 °C for later utilization in the batch experiments. As a control group, the same inoculum was cultivated in saline water for 3 days. The effects of water type on the growth of the ocean bacteria are shown in Table 2.

Experimental procedures

The experiments were carried out in batch mode. First, the desired amount of bacteria suspension was added to the 100.0 mL sample leachate in the flask fixed to the digital biochemical incubator (HZQ-F160, ETD Co., Ltd, Ningbo, China). After the desired duration, 10-mL post-processing leachate samples were centrifuged (GT16-3, TBL Co., Ltd, Beijing, China). Then, the COD₅₅, NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N concentrations of the supernatant were analyzed. Repeated experiments were carried out, and the results were obtained in triplicate within (±5%) data deviation. The initial pH of the landfill leachate was adjusted with diluted HCl or NaOH solution.

Analyses and calculations

Total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N) and nitrite nitrogen (NO₂⁻-N) were measured as described previously (APHA 1998). The pH of the leachate was recorded by pH meter (pHXS, REC, Shanghai, China). Optical density at wavelength 510 nm (OD₅₁₀), detected by ultraviolet-visible spectrophotometer (UV-1,750, Mfg Co., Ltd, Tokyo, Japan) was used to characterize the cell concentration. The COD₅₅ was measured by COD Digital Reactor Block (South China Institute of Environment Sciences, MEP, Guangzhou, Guangdong Province, China). Contaminant removal ratios post-treatment were calculated by Equation (1)

\[
\text{Removal ratio} (\%) = \left\{ \frac{(C_1 \times V_1 - C_2 \times V_2)}{(C_1 \times V_1)} \right\} \times 100. \tag{1}
\]

C₁ and C₂ are the initial and the post-processing COD₅₅ concentrations of the supernatant collected after centrifugation (mg/L), and V₁ and V₂ are the volume (litre) of
landfill leachate and that of bacterial suspension mixed with leachate, the values of which are 0.1 and 0.12, respectively.

Voltammetry incorporates three electrodes: a working electrode (Pt/C electrode), a reference electrode (calomel electrode) and a counter electrode (Pt electrode) at 25°C with a scanning rate of 50 mV per min, pH = 7.

RESULTS AND DISCUSSION

Inoculum dose

To investigate the effect of inoculum dose on landfill leachate treatment, experiments were carried out. As can be seen in Figure 1, after 3 days, with inoculum dose in a range 0–10% (V/V), cell concentration (OD 510), the CODCr and NH₄⁺-N removal ratio dramatically increase with increasing inoculum. With an inoculum boost of more than 10%, CODCr and NH₄⁺-N removal ratios change very little. These results indicate that ocean bacteria utilize the organic carbon and NH₄⁺-N in the leachate for bacterial reproduction, while removing contaminants. This considerable efficiency in removing organics and NH₄⁺-N may be mainly due to efficient microorganisms (Shalaby 2011), which probably occur in the ocean bacteria. For the leachate sample without ocean bacteria inoculation, the CODCr removal reached 30% after 3 days of processing, which was much lower than that of any other groups with ocean bacteria inoculation. This proves that there are no efficient bacteria in the untreated landfill leachate.

Optimal pH

The effect of the initial pH within the range of 3.0–9.0 on landfill leachate treatment was also studied. As can be observed in Figure 2, the pH of leachates ranges from 3.0–4.0, with little difference on increasing OD₅₁₀ and COD Cr removal efficiency, which reveals that the heterotrophic bacteria are inhibited by heavily acidic conditions, and chemolithotrophic bacteria can use NH₄⁺-N as a nutrient to remove NH₄⁺-N. However, with increasing pH, ocean bacteria can actively reproduce and degrade contaminants. The high COD Cr and NH₄⁺-N removal reached 94.45% and 65.36%, respectively, at a pH of 5.0–10.0 after 3 days. It indicates that bacteria can work efficiently at a pH = 7 value which benefits water discharge. The contaminant removal ratio through this biological method is much more desirable compared to precipitation of landfill leachate. With precipitated ammonium ions, for example magnesium ammonium phosphate (Yangin et al. 2002), its maximum NH₄⁺-N removal of 66–85% was achieved at pH 9.3–11.0. A larger alkali dose consumption is demanded, and the treated leachate pH needs to be adjusted before discharge.

Processing time

Figure 3 shows the effect of different processing times (h) on CODCr removal. As can be seen in Figure 3, in the first 24 h, COD Cr removal reaches 76.23%. After another 24 h, it increases to 93.71%. It is interesting to note that processing time after 48 h makes little contribution to removing contaminants. The reason may be due to lack of carbon source or refractory organic residue. In terms of COD Cr and NH₄⁺-N removal ratio, this method of biodegradation by shaking is better than chemical oxidation (Qureshi et al. 2002) and adsorption (Kargi & Pamukoglu 2004). However, the CODCr and NH₄⁺-N removal rate is much lower than with chemical oxidation.

Oxygen conditions

To study the ocean bacteria, the effect of oxygen being supplied or not on landfill leachate treatment was studied, and results are shown in Figure 4. Figure 4 shows that, under the condition of oxygen supplied, the bacteria density (OD₅₁₀), COD removal and NH₄⁺-N removal ratios of the treated leachate dramatically increase, but under the condition without oxygen supplied, ratios hardly increased. This reveals that the mixed ocean bacteria are aerobic microorganisms.

Figure 1 | Effect of bacteria inoculum on landfill leachate treatment, where conditions were pH = 6.3 with oxygen for 3 days.
Therefore aerobic processing of landfill leachate favours biodegradable organic pollutants and also ammonium nitrogen (Renou et al. 2008).

The mechanism of NH$_4^+$-N depletion

As can be seen in Figure 5, under the condition of aeration, NH$_4^+$-N concentration decreases, NO$_2^-$-N concentration first increases then decreases, and NO$_3^-$-N concentration increases with increasing time. These results are probably due to the mixed bacteria containing nitrite oxidizing bacteria and nitrobacteria. With nitrite oxidizing bacteria in the leachate, ammonia was oxidized to nitrite, which was quickly oxidized to nitrate by nitrobacteria. It can therefore be inferred that the mechanism of NH$_4^+$-N depletion is ammonia oxidation (Han et al. 2013).

Volt-ampere characteristics curve of the bacteria

An experiment was carried out to observe the volt-ampere characteristics of the bacteria solution. As can be seen in Figure 6 for curve 1, there are reductive peaks where the
potential is \(-0.599\) V and an oxidation peak where the potential is \(0.148\) V. The ratio of \(I_p\) (current of reductive peak) to \(I_p\) (current of oxidation peak) is about 1, which indicates that the group with bacteria, compared to the control group without bacteria, has an apparent redox peak. This proves that the electrochemical activity of the ocean bacteria can play a major role in landfill leachate treatment.

**CONCLUSIONS**

In landfill leachate treatment without any external carbon addition, it is feasible that ocean bacteria utilize organics as the only carbon source to efficiently remove carbon and \(\text{NH}_4^+\). In the experiments, the steady ammonia removal performance may be attributed to the enrichment of bacteria in mixed ocean bacteria, which showed high nitrogen-removal capacity.

Utilization of marine bio-resource to treat landfill leachate is not only a significant outcome, but also the most fundamental initial stage of an effective method for wastewater treatment, as the success of the latter is based on the former. The paper has established this by defining the baseline study in the landfill leachate context, and also by indicating the implications of marine bio-resource exploitation.

**ACKNOWLEDGEMENTS**

This research was supported by the China Ocean Mineral Resource R&D Association (No. DY125-15-T-08), the National Natural Science Foundation of China (No. 21176242 and No. 21176026), and the National High Technology Research & Development Program (863 program) of China (No. 2012AA062401).

**REFERENCES**


First received 29 June 2014; accepted in revised form 19 December 2014. Available online 3 January 2015