Science of the Total Environment 637–638 (2018) 174–181

Contents lists available at ScienceDirect

Science of the Total Environment

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

# A novel aerobic sulfate reduction process in landfill mineralized refuse

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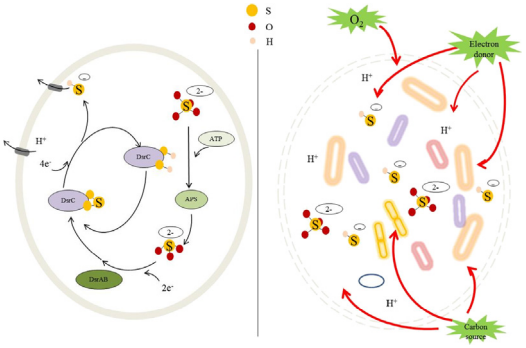
## HIGHLIGHTS

- $H_2S$ from mineralized refuse could be greatly promoted when exposing to $O_2$.
- MFB without SRB has high aerobic sulfate reduction capacity.
- Lactate and $S_2O_3^{2-}$ were the preferred electron donor and acceptor for MFB.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 27 February 2018
Received in revised form 5 April 2018
Accepted 23 April 2018
Available online xxxx

Editor: Baoliang Chen

*Keywords:*
Landfill
Aerobic
Sulfate reduction
Hydrogen sulfide
Electron

## ABSTRACT

It is thought that mineralized refuse could be excavated from almost-full landfill sites to provide space for the increasing burden of municipal solid waste. When excavating, however, the $H_2S$ emissions from the mineralized waste need to be considered carefully. In an attempt to understand how $H_2S$ emissions might change during this excavation process, we carried out a series of tests, including exposing anaerobic mineralized refuse to oxygen, isolating and determining possible functional bacteria, and characterizing the electron donors and/or acceptors. The results showed that $H_2S$ would be released when landfill mineralized refuse was exposed to oxygen ($O_2$), and could reach concentrations of 6 mg $m^{-3}$, which was 3 times the concentrations of $H_2S$ released from anaerobic mineralized refuse. Sulfur-metabolized microorganisms accounted for only 0.5% of the microbial functional bacteria (MFB) derived from the mineralized refuse when exposed to $O_2$ for 60 days, and SRB were not present. The MFB maintained $H_2S$ production by aerobic sulfate reduction using $SO_4^{2-}$ and $S_2O_3^{2-}$ as electron acceptors, and sulfate-reducing rates of 16% and 55%, respectively, were achieved. Lactate and $S_2O_3^{2-}$ were the preferred electron donor and acceptor, respectively. By enhancing the carbon source and electron transfer, MFB may undergo strong aerobic sulfate reduction even at low abundances of sulfur-metabolized microorganisms.



## 1. Introduction

The rapid growth of the economy and the improvement in living standards has led to increased production of municipal solid waste (MSW). However, the rapid expansion of urban areas also means that landfills are increasingly close to urban residential areas, and there is progressively more concern about pollution from landfill odor (Capelli et al., 2008; Masamoto et al., 2012). Hydrogen sulfide ($H_2S$), one of the strongest odors with a low olfactory threshold and high toxicity (Sun et al., 2017), is attracting a lot of attention because of its ability to corrode and potential to harm human health.

Hydrogen sulfide is mainly produced by sulfate-reducing bacteria (SRB) (Alzuhair et al., 2008; Muyzer and Stams, 2008), which have traditionally been considered a type of anaerobic microbe (Burow et al., 2014). However, an increasing number of recent studies have indicated

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https://doi.org/10.1016/j.scitotenv.2018.04.304
0048-9697/

that this metabolic process can survive in the presence of oxygen ($O_2$). Because of the toxic effects of $O_2$, SRB have developed strategies to protect themselves, namely behavioral strategies and molecular strategies (Cypionka, 2000). The former include aerotaxis and aggregate formation, while the latter allow SRB to remove $O_2$ and protect themselves from harmful effects (Dolla et al., 2006). Through forming aggregates, some SRB may be able to survive periodic exposure to oxic conditions in their natural habitats. Sulfate-reducing bacteria form clusters of cells. The cell numbers of SRB in the upper layer of these clusters vary with the time of day and the $O_2$ concentration. Selected species of SRB have high tolerances to $O_2$, even when grown in pure cultures, and can survive short-term exposure to $O_2$ (Cypionka and Widdel, 1985). *Desulfovibrio desulfuricans* NCIB8301 that was grown in the presence of $O_2$ survived well over the first 24 h but the cell viability decreased rapidly for longer exposure times. Another study reported that the cell yield fell drastically as the partial pressure of $O_2$ increased, and was correlated with a decrease in both lactate use and $H_2S$ production (Abdollahi and Wimpenny, 1990). In continuous cultures of *Desulfovibrio oxyclinae*, sulfate reduction was inhibited at oxygen concentrations of 1% in the gas phase (Krekeler et al., 1997). It is generally accepted that these processes are the result of sequential redox processes that are determined by the redox potential, which occurs in sediment and groundwater aquifers everywhere. However, not all phenomena can be attributed to the redox potential. For example, Canfield and Des Marais (1991) reported that sulfate reduction occurred consistently within the photosynthetic zone of microbial mats. Teske et al. (1998) found that SRB on the surface of high-salinity cyanobacteria had day and night sulfate reduction rates of 1000 and 2200 nmol $mL^{-1}$ $d^{-1}$, respectively, which were higher than the reduction rates reported in anaerobic biomats. Sigalevich and Cohen (2000) discovered that sulfate reduction occurred during co-culture of *Desulfovibrio oxyclinae* with aerobic bacteria (*Marinobacter* sp.) when exposed to 5% $O_2$ at a flux rate of 223 μmol $min^{-1}$. This evidence indicates that dissimilatory sulfate reduction can occur in the presence of $O_2$ and not just in sequential redox processes, and challenges the traditional view that sulfate reduction is an obiligate anaerobic process.

Excavation of aged landfill has been suggested as a sustainable strategy that will help to resolve the conflict between the rapid increase in the volume of MSW and the limited choice of new landfill sites. If this strategy is adopted, we need to pay attention to the $H_2S$ emissions during the excavation. In our previous studies, we found that $H_2S$ emissions could be enhanced rather than inhibited when mineralized anaerobic landfill refuse was exposed to air (Shen et al., 2015) and, from our experiments of a simulated landfill, we concluded that this enhancement was mediated by aerobic sulfate-reducing processes (Long et al., 2016). The rapid rates of sulfate reduction that we observed in the presence of high $O_2$ may indicate a novel biochemical pathway for sulfate reduction or that there are complex microbial interactions, the details of which are not yet known. The aim of this study was to gain an understanding of aerobic sulfate reduction in landfill waste that was exposed to $O_2$. To do this, we carried out a series of tests in which we exposed anaerobic mineralized refuse to $O_2$, isolated and determined the possible functional bacteria, and characterized the electron donors and/or acceptors.

## 2. Materials and methods

### 2.1. Mineralized refuse with $O_2$ exposure batch experiment

We took a sample of mineralized refuse from an anaerobic simulated landfill (Fang et al., 2016, b). We removed the inert fractions of mineralized refuse, including glass, metal, plastic, and stone and then crushed it and passed it through a 0.42 mm sieve to remove more small stones and pieces of glass. The sample was then mixed thoroughly and stored in an airtight plastic bag in a refrigerator. The main characteristics of the mineralized refuse sample are shown in Supplementary Table S1.

We carried out a series of $O_2$ exposure batch experiments in 150-mL serum bottles. A 30-g aliquot of mineralized refuse was weighed and placed into each serum bottle, and then each bottle was sealed with a butyl rubber stopper. The headspace $O_2$ concentrations were set as 0%, 5%, 10%, 15%, or 21% using the gas replacement method. Firstly, we used $N_2$ replaced air in serum bottles by stainless steel needle. Then the corresponding volume of $N_2$ was replaced by pure $O_2$, to achieve different $O_2$ concentration. We used 14 parallel serum bottles in our tests. All the serum bottles were incubated in a temperature-controlled room at 30 °C. To avoid any possible effects of opening and closing the bottles, all samples were collected by the destructive method; that is, a bottle from each group was sampled randomly at the designated sampling time and then was excluded from the experiment after the sampling. Mineralized refuse samples were analyzed for pH, moisture contents, and concentrations of dissolved organic carbon, sulfate ($SO_4^{2-}$), and forms of sulfide ($H_2S$, $HS^-$, and $S^{2-}$). Gas samples were analyzed for $H_2S$ and $O_2$.

### 2.2. Enrichment of mixed functional bacteria (MFB)

In batch experiment, we exposed the mineralized refuse to $O_2$ for 60 days. During the domestication process, the sulfate content decreased from 5116 mg $kg^{-1}$ to 3652 mg $kg^{-1}$ when the conditions were aerobic and the $O_2$ concentration was 15% (v/v). We considered the decrease in the sulfate concentration was associated with microbes. We collected the mineralized refuse from the reactor to acclimate the possible functional sulfate-reducing microbes. We then domesticated the refuse in a solution with a sulfate concentration of 170 mg $L^{-1}$ at a liquid-to-solid ratio of 10:1 and aerated the mixture for about 1 month to enrich the possible functional sulfate-reducing microbes. The sulfate content decreased by 43% during this process. The matrix was then separated and the supernatant was collected as MFB.

### 2.3. Metabolic characteristics of MFBs that were exposed of $O_2$

#### 2.3.1. Sulfate-reducing behavior of MFB

We set up a test (A) that comprised the MFB, basal medium, and sodium sulfate to investigate the sulfate-reducing behavior. We also set up a sterilized control (CK-A) with the same configuration as group A.

We carried out the tests in a series of sealed glass bottles with a capacity of about 580 mL under aerobic conditions. The headspace, which was about 120 mL, of each glass bottle was filled with sterilized air with an $O_2$ concentration of 21% (v/v) after the matrix was loaded with the basal medium and sodium sulfate. The initial sulfate concentration in each bottle was around 230 mg $L^{-1}$. Each treatment was performed in duplicate. The bottles were kept at 30 °C on an orbital shaker set to 100 r $min^{-1}$.

A modified mineral salt medium (Huang et al., 2014) was used in all the continuous culture experiments. The medium contained 4.5 g $L^{-1}$ of $Na_2HPO_4 12H_2O$, 1.0 g $L^{-1}$ of $KH_2PO_4$, 1.5 g of $L^{-1}$ $NH_4Cl$, 0.2 g $L^{-1}$ of $MgSO_4 7H_2O$, and 0.3 g $L^{-1}$ of yeast extract. The medium also contained trace elements (0.07 g $L^{-1}$ of $ZnCl_2$, 0.1 g $L^{-1}$ of $MnCl_2 4H_2O$, 0.006 g $L^{-1}$ of $H_3BO_3$, 0.13 g $L^{-1}$ of $CaCl_2 2H_2O$, 0.002 g $L^{-1}$ of $CuCl_2 2H_2O$, 0.024 g $L^{-1}$ of $NiCl_2 6H_2O$, 0.036 g $L^{-1}$ of $Na_2MO_4 2H_2O$, 0.238 g $L^{-1}$ of $CoCl_2 6H_2O$,

**Table 1**
The experiment design of electron acceptors.

| Treatments | Electron acceptor | Electron donor | Inoculum |
|---|---|---|---|
| B1 | $SO_4^{2-}$ | + | + |
| B2 | $S_2O_3^{2-}$ | + | + |
| B3 | | + | + |
| CK-B1 | $SO_4^{2-}$ | + | +,sterilization |
| CK-B2 | $S_2O_3^{2-}$ | + | +, sterilization |
| CK-B3 | | + | +, sterilization |

Note: $SO_4^{2-}$:960 mg $L^{-1}$ $S_2O_3^{2-}$:1120 mg $L^{-1}$ theelectron donor: glucose (10.8 g $L^{-1}$),Inoculum: 0.5 mL bacteria liquid.