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Composition of bacterial and archaeal communities during landfill refuse decomposition processes

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ABSTRACT

Little is known about the archaeal and the bacterial diversities in a landfill during different phases of decomposition. In this study, the archaeal and the bacterial diversities of Laogang landfill (Shanghai, China) at two different decomposition phases (i.e., initial methanogenic phase (IMP) and stable methanogenic phase (SMP)), were culture-independently examined using PCR-based 454 pyrosequencing. A total of 47,753 sequences of 16S rRNA genes were retrieved from 69,954 reads and analyzed to evaluate the diversities of the archaeal and bacterial communities. The most predominant types of archaea were hydrogenotrophic *Methanomicrobiales*, and of bacteria were *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. As might be expected, their abundances varied at decomposition phases. Archaea *Methanomicrobiales* accounts for 97.6% of total archaeal population abundance in IMP and about 57.6% in SMP. The abundance of archaeal genus *Halobacteriale* was 0.1% in IMP and was 20.3% in the SMP. The abundance of *Firmicutes* was 21.3% in IMP and was 4.3% in SMP. The abundance of *Bacteroidetes* represented 11.5% of total bacterial in IMP and was dominant (49.4%) in SMP. Both the IMP and SMP had unique cellulolytic bacteria compositions. IMP consisted of members of *Bacillus*, *Fibrobacter*, and *Eubacterium*, while SMP harbored groups of *Microbacterium*. Both phases had *Clostridium* with different abundance, 4–5 folds higher in SMP.

1. Introduction

Disposal of municipal solid waste (MSW) in sanitary landfills is globally practiced, cost-effective method, and is the main disposal method for roughly 70% of MSW in China (Zhao et al., 2007). Refuse contains a high proportion of biodegradable fractions such as cellulose and hemicelluloses that undergo a complex series of microbiological and chemical reactions as they reside in the landfill (Barlaz et al., 1989; Kjeldsen et al., 2002; Zhao et al., 2007). Most landfills are characterized by anaerobic zones, where microbial decomposition of refuse follows well-known sequence of decomposition processes, including an initial aerobic phase (IAP), an anaerobic acid phase (ACP), an initial methanogenic phase (IMP), and a stable methanogenic phase (IMP) (Barlaz et al., 1989; Kjeldsen et al., 2002).

Key parameters influencing the phase of decomposition are O_2, pH and BOD_5/COD (biological oxygen demand and chemical oxygen demand) ratios (Youcai et al., 2000; Kjeldsen et al., 2002). For example, during short (days) IAP, O_2 in the void space of the refuse is rapidly consumed producing CO_2. After the O_2 is depleted, refuse decomposition shifts to the ACP in which the hydrolytic, fermentative, and acetogenic bacteria decompose the major biodegradable components, resulting in an accumulation of carboxylic acids, a pH decrease (to below 5.0) and a high value of BOD_5/COD ratio (above 0.4). In the IMP, the accumulated acid is converted to CH_4 and CO_2, and the cellulose and hemicelluloses hydrolyzed, thus decreasing the concentrations of COD, BOD_5, and BOD_5/COD ratios. Accordingly, pH is increased. In the SMP, the amount of CH_4 produced depends on the hydrolysis rate of cellulose and hemicelluloses. During this phase the BOD_5/COD ratios generally falls below 0.1 as carboxylic acids are quickly consumed and pH continue to increase to some steady state value.

Although landfill refuse decomposition phases are well characterized by the changes in the physiochemical parameters described above, little is known about the changes in microbial community composition and function in different decomposition phases.

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Efforts to characterize microbial community in landfills have used culture and culture-independent techniques. Isolation efforts yielded cultures of bacteria in landfill and leachate such as cellulolytic bacteria. Study on cellulolytic bacteria distribution in fresh, 1 year old, and 5 years old refuse samples in a French landfill showed that *Cellulomonas*, *Microbacterium* and *Lactobacillus* were predominant (Pourcher et al., 2001). The genus *Microbacterium* has been isolated from a landfill in India (Krishnamurthi and Chakrabarti, 2013) and cellulolytic *Clostridium* and *Eubacterium* from landfill in England (Westlake et al., 1995). Although archaea is difficult to culture, strains of *Methanobacterium formicicum* and *Methanosarcina barkeri* have been isolated from landfills (Fielding et al., 1988). Because the majority of bacteria and archaea could not be cultured, culture-independent clone library technique based on small-subunit 16S rRNA have been widely used to determine the composition of microbial communities in landfills and leachate. For instance, investigation on the bacterial communities of landfill and leachates of China (Huang et al., 2004), England (Van Dyke and McCarthy, 2002), and India (Krishnamurthi and Chakrabarti, 2013) indicated that phylum *Firmicutes* was present predominantly in all landfills, while *Actinobacteria* and *Proteobacteria* were also present. Study on archaeal communities in landfills showed that the methanogen group was dominant including orders of *Methanomicrobiales*, *Methanosarcinales* and genera of *Methanosarcina*, *Methanoculleus*, *Methanosaeta*, *Methanothermobacter*, and *Methanosaeta* (Chen et al., 2003; Huang et al., 2003; Laloui-Carpentier et al., 2006; Bareither et al., 2013; Krishnamurthi and Chakrabarti, 2013; Huang et al., 2002).

The limited number of isolates and clones sequenced in the above studies precludes a complete profile of the diversity and complex interactions of the microbial population in landfill. Considering that global landfills account for the second largest source of anthropogenic methane (Staley et al., 2012), there is a need to better characterize microbiological processes and communities during refuse decomposition to better understand processes working to produce methane in these landfills The 454 pyrosequencing technique, which allows for high-throughput sequencing in a single sequencing run, offers the opportunity to better characterize the microbial ecology in the landfill and provide insights for understanding the microbial interactions in various phase of landfill refuse decomposition.

Therefore, the first object of this study was to investigate the bacterial and the archaeal community composition in landfill through the pyrosequencing technique. The second objective was to characterize the changes in the bacterial and archaeal compositions in two phases of landfill refuse decomposition. To achieve the objects, we took samples from Laogang landfill – the largest landfill – located in Shanghai, China and analyzed the bacterial and the archaeal community compositions by a bar-coded 454 pyrosequencing approach. We analyzed 11 chemical parameters of landfill leachate to identify the decomposition phase.

2. Materials and methods

2.1. Sampling site description

Shanghai Laogang refuse landfill, the largest landfill in China, was constructed along the shore of the East China Sea and started-up at the end of 1989 and has been subjected to long-term monitoring (Zhao et al., 2007). Currently, around 75% of the total refuse generated in Shanghai (7600 tons per day) is placed in the landfill. Shanghai Laogang refuse landfill has been extensively studied in terms of the refuse stabilization process (Youcai et al., 2000), aging of the refuse (Zhao et al., 2007), production of landfill leachate (Ziyang et al., 2009), and gas management in the landfill (Xiaoli et al., 2010).

Landfill leachate results from the percolation of liquid through the landfill site, it potentially provides a comprehensive sample of the landfill microbiota (Kjeldsen et al., 2002; McDonald et al., 2010). Therefore, we sampled the landfill leachate from landfill units close for 4 months and 3 years. The BOD_5/COD ratio (0.39) and pH value (7.79) in the leachate of the landfill closed for 4 months suggested that landfill was at the IMP. The detected pH (8.0) and BOD_5/COD ratio (0.02) in the leachate of the landfill closed for 3 years indicated this landfill is at the SMP.

2.2. Sampling

Landfill leachate was collected (28 September, 2012) from the inlet of leachate collection ponds into 1 L sterilized HDPE sample bottles (Fisher Scientific, USA) and stored on ice during transport back to the laboratory. Twenty milliliters of leachate for microbial community analysis were filtered through a sterilized 0.22 μm filter (Millipore, USA).

2.3. Chemicals analysis

Total organic carbon (TOC) and total nitrogen (TN) were measured by a high temperature catalytic oxidation on Multiy TN/TC Analyzer 3100 (Jena, Germany). Anions (Cl^-, NO_3^- and SO_4^{2-}) were measured by ion chromatography ICS 1100 (Agilent, USA) by 4110-B (APHA, 1998). Ammonia nitrogen (NH_3–N), BOD_5 and COD were determined according to the standard method of the National Environmental Protection Administration of China (China SEPAo, 2002). NH_3–N was measured by Nessler's reagent spectrophotometry method. BOD_5 was determined by inoculation method. COD was determined by potassium bichromate method. pH, turbidity, and conductivity were measured by Portable Multi Water Quality Parameters Analyzer (Hach, USA).

2.4. DNA extraction

DNA was extracted from one half of a filter with the Ultraclean soil DNA Kit (Mobio Laboratories Inc, USA) according to the vendor's protocol. The eluate was quantified with a NanoVue plus spectrophotometer (GE, USA).

2.5. High-throughput 16S rRNA gene pyrosequencing

The bacterial and archaeal community structures were assessed using 454 pyrosequencing. Bacterial amplicon libraries were constructed for 454 pyrosequencing using primers 341F (5′-CCTACGGGAGGCAGCAG-3′) and 1073 R (5′-ACGAGCTGACGACARCCATG-3′) for the V3-V6 region of the 16S rRNA gene (Uroz et al., 2012), while archaeal amplicon libraries were constructed by primers 344F (5′-ACGGGGYGCAGCAGGCGCGA-3′) and 915R (5′-GTGCTCCCCCGCCAATTCCT-3′) for the V3-V5 region of the 16S rRNA gene (Raskin et al., 1994). Ten-nucleotide and eight-nucleotide barcodes were designed for bacterial and archaeal sequencing to sort multiple samples in a single 454 GS-FLX run, respectively. Prior to emulsion PCR and sequencing, archaeal communities were amplified with high fidelity Taq polymerase (Invitrogen, USA) under the following conditions: initial denaturization at 95 °C for 2 min, followed by 30 cycles (30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C) and a final 5 min extension at 72 °C. Bacterial community amplification condition is similar to archaeal community except that the cycle's number was 25. Subsequently, the three PCR products per sample were pooled in equal amounts, purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and quantified using a NanoDrop spectrophotometer (NanoDrop Technologies,