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Phylogenetic analysis of the bacterial community in a full scale autothermal thermophilic aerobic digester (ATAD) treating mixed domestic wastewater sludge for land spread

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ABSTRACT

The bacterial community associated with a full scale autothermal thermophilic aerobic digester (ATAD) treating sludge, originating from domestic wastewater and destined for land spread, was analysed using a number of molecular approaches optimised specifically for this high temperature environment. 16S rDNA genes were amplified directly from sludge with universally conserved and Bacteria-specific rDNA gene primers and a clone library constructed that corresponded to the late thermophilic stage (t = 23 h) of the ATAD process. Sequence analyses revealed various 16S rDNA gene sequence types reflective of high bacterial community diversity. Members of the bacterial community included α- and β-Proteobacteria, Actinobacteria with High G + C content and Gram-Positive bacteria with a prevalence of the Firmicutes (Low G + C) division (class Clostridia and Bacillus). Most of the ATAD clones showed affiliation with bacterial species previously isolated or detected in other elevated temperature environments, at alkaline pH, or in cellulose rich environments. Several phylotypes associated with Fe(III)- and Mn(IV)-reducing anaerobes were also detected. The presence of anaerobes was of interest in such large scale systems where sub-optimal aeration and mixing is often the norm while the presence of large amounts of capnophiles suggest the possibility of limited convection and entrapment of CO_2 within the sludge matrix during digestion. Comparative analysis with organism identified in other ATAD systems revealed significant differences based on optimised techniques. The abundance of thermophilic, alkalophilic and cellulose-degrading phylotypes suggests that these organisms are responsible for maintaining the elevated temperature at the later stages of the ATAD process.

1. Introduction

Autothermal thermophilic aerobic digestion (ATAD) is a tertiary wastewater/sludge treatment system that utilises the heat generated by microbial metabolism in insulated reactors to stabilise and pasteurise sludge (Kelly et al., 1993; Layden et al., 2007) generating a class A biosolids. Initially secondary sludge is thickened and then aerated with stirring in insulated

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bioreactors allowing the temperature to rise above 60 °C within the closed system. The sludge itself undergoes changes as biodegradation proceeds and the energy is converted to heat and microbial biomass. Effective thermal processing ensures that the resultant biosolids are essentially free from pathogens (Piterina et al., 2010a,b) carried over from the original feed and is suitable for land spread. Although the microbial community contributes to sludge ecology (Chiang et al., 2001), sludge degradation (Piterina et al., 2009) and heat production (Lapara and Alleman, 1999), the bacterial communities present within the ATAD process have received little attention. Culture based analysis (Sonnleitner and Fiechter, 1983), revealed little diversity with a predominance of *Bacillus* family members which were difficult to identify to the species level based on standard methods. Indeed it has been hypothesized that the ATAD environment may represent a highly selective niche with limited species diversity and thermophilic populations with specific nutrient requirements limiting the use of culture based methods (Sonnleitner, 1983; Gilbride et al., 2006). Developments in cultivation-independent techniques (Gilbride et al., 2006), analysing 16S rDNA from native communities with extensive bioinformatic databases (Maidak et al., 2001) and software (Hall, 1999; Altschul et al., 1997) now permit detailed analysis of mixed microbial communities, and have become the method of choice to characterize microbial communities. Previous reports on ATAD diversity have differed with respect to the nature of the feed and the size of reactor analysed. These have included a study of a full scale ATAD system in Canada treating swine manure (Juteau et al., 2004), a US study of an ATAD treating pharmaceutical waste (LaPara et al., 2002), a small lab-scale ATAD study of domestic sludge (Liu et al., 2010), and an Australian full scale ATAD study treating domestic sludge (Hayes et al., 2011), however there has been little comparison of the recovered diversity in the different systems. In these studies aerobic organisms were identified with small numbers of anaerobes, mainly *Clostridium* spp., thought to be present as artefacts while cellulose decomposers, actinomycetes, sulphur-reducing bacteria, denitrifiers and granule-formers were reported to be negligible.

In attempting to analyse the ATAD sludge community associated with a full scale ATAD reactor system treating mixed domestic sludge (Piterina et al., 2006; Layden, 2007), we initially noted a number of limitations in the sampling and processing of DNA from ATAD sludge which has received little attention. Extensive analysis on the factors that limited diversity recovery from ATAD sludge revealed that the presence of thermophilic nucleases released upon lysis at elevated temperatures, the nature of the extraction procedures used, the extent of humic substances present, the choice of primers used in amplification of extracted templates, the type of polymerase used, its inhibition and the need for additives while processing were all key factors that needed to be optimised to recover optimal and reproducible microbial diversity specifically from ATAD sludge (Piterina et al., 2010a,b). Thus specific optimisation is needed to reveal the true community diversity in ATAD systems. Here we report a phylogenetic analysis of a full scale ATAD plant treating mixed domestic sludge using these novel optimisation techniques. The aim of this study was to identify the nature of organisms associated with the thermophilic stage without bias from the methodology and to observe the nature of the organisms present in an attempt to understand the processes occurring at the thermophilic stage. Our results indicate that although aerobic-thermophilic populations are dominant, the presence of microaerophilic and anaerobic species may serve as important trophic groups within the sludge and contribute significantly to the digestion process.

2. Material and methods

2.1. Site description and sample collection procedure

Unclarified sludges were collected aseptically from the Killarney Sewage Treatment Works using a deep-water sampler to sample from the middle of the thermophilic reactor 2A (t = 23 h of thermal treatment). This ATAD processes the majority of the domestic wastewater for a population of 20,000–51,000 people (mean solids production of 500 tones per annum). The ATAD feed consists of a combination of primary and secondary treated sludges, thickened to 4–6% TS on a belt filter and processed in a semi-batch process via two reactors. Reactors 1 and 2 (110 m^3) are operated in series, with partially digested sludge being fed from the first mesophilic ATAD reactor 1 (operation temperature range 35–49 °C) to the second thermophilic ATAD reactor 2 (operation temperature range 58–65 °C). Details of the system design, process performance and process variations have been reported (Piterina et al., 2006; Layden, 2007). ATAD sludges sampled following 23 h of thermal treatment in reactor 2 were processed immediately for DNA extraction without storage to minimise undesirable changes and processing conditions applied to give optimal diversity recovery (Piterina et al., 2010a,b).

2.2. Nucleic acid recovery from sludge matrix by two direct extraction methods

Bulk environmental DNA from ATAD thermophilic sludge was extracted by two extraction methods (Piterina et al., 2010a,b) to maximize the recovery and diversity of nucleic acid from ATAD sludge.

2.3. Analysis of integrity and size of extracted genomic DNA

Agarose gels (0.8% wt/v) were prepared in 1x TAE buffer and 1 μl of DNA extract were electrophoresed at 80 V in 1x TAE buffer and post-stained in 1x TAE buffer containing 0.1% ethidium bromide (Sambrook and Russell, 2000).

2.4. Molecular analysis of ATAD DNA

2.4.1. PCR amplification of 16S rDNA

To access 16S rDNA bacterial sequences in the extracted DNA, PCR amplification was carried out using universal reverse primers 1492r (5′-GGTTACCTTGTTACGACTT-3′) and Bacteria-specific 27f (5′-AGAGTTTGAT CCTGGCTCAG-3′) (Lane, 1991) to achieve amplification of almost the complete 16S rDNA gene. To avoid PCR bias, previously described in the literature