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Microbial ecology of autothermal thermophilic aerobic digester (ATAD) systems for treating waste activated sludge

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ABSTRACT

Despite their widespread use, our understanding of the microbial ecology of the autothermal thermophilic aerobic digesters (ATAD) used to dispose of sludge from wastewater treatment plants is poor. Applying both culture-dependent and molecular methods to two ATAD systems in Victoria, Australia treating different wastewaters revealed that their communities were highly specialized. Denaturing gradient gel electrophoresis (DGGE) profiling suggested differences in their population compositions and both changed over time. However, both showed low level biodiversity, and contained several novel bacterial populations. 16S rRNA clone library data and FISH analyses showed that *Thermus thermophilus* dominated both communities and that of a third ATAD plant in NSW (more than 90% of the total bacterial biovolume in repeated samples taken from each of the three ATAD plants). Culture-dependent methods also showed *Geobacillus* spp. were present in both Victorian communities. Nevertheless, the ecophysiology of these populations and their putative roles in sludge digestion remain unclear. FISH/microautoradiographic studies did not provide conclusive data elucidating which substrate/s *T. thermophilus* might utilize in the ATAD reactors.

Introduction

Most activated sludge plants generate large volumes of secondary sludge, which is generally viewed as a waste product of the process, and it is expensive to treat [24,68]. Secondary sludge, or waste activated sludge, usually contains heavy metals, pathogenic viruses and bacteria, all at levels which may limit how it is eventually handled [27,82]. Different disposal methods have been used in attempts to maximize the economic value of this material and each has its advantages, but the most popular method in countries where land space is available is its application to land as a fertilizer [5,43]. However, land disposal requires some pre-treatment to stabilize the sludge to minimize odour production and reduce the level of pathogens before its application [46,62]. In addition to anaerobic digestion, other popular full-scale stabilization methods in current use include chemical treatment, thermal drying and composting [5,82], each of which varies in its costs, land requirements, and effectiveness in reducing the pathogen and toxic chemical levels [10,29].

One potentially attractive method is autothermal thermophilic aerobic digestion (ATAD). The systems using this method were designed originally from work in Europe and North America [37,83] and were commercially patented and implemented first in Germany in 1980 [26]. ATAD relies on the solubilisation of sufficient oxygen in a reactor so that the resulting high levels of aerobic bacterial metabolic activity generate a high operating temperature (>60 °C), leading to rapid inactivation of viral and mesophilic bacterial pathogens, such as *Salmonella* and other *Enterobacteriaceae*, in the sludge [29,66,86]. Thus, the benefits claimed for ATAD systems include production of a safe, high quality end product suitable for land application [42,86], and a substantial reduction in volatile solids levels [67]. Furthermore, much shorter sludge retention times compared to conventional composting protocols mean that the system has very low space requirements [77].

However, this system is not without its critics. Operating problems include difficulties in dewatering the sludge from ATAD reactors, incurring corresponding increases in costs [1,50]. Frequent episodes of foaming in the reactors can also occur [41,42], and difficulties in supplying enough oxygen to maintain aerobic conditions in bioreactors run at temperatures usually above 60 °C mean that these ATAD processes become anaerobic, leading to microbial formation of odorous compounds, such as mercaptans and dimethyl sulphides [11,33]. While these systems have attracted the interest of engineers [42], surprisingly little is known about the microbial composition of the ATAD communities [68]. Culture-dependent studies with laboratory [32], pilot [73], and full scale ATADs [56], treating domestic waste sludge have all detected

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Table 1
Operational features for Victorian ATAD plants studied.

Parameter	Castlemaine	Bendigo
Population equivalent (p.e)	40,000	120,000
Source feed sludge	On-site EBPR secondary sludge	On-site EBPR secondary sludge
Number of treatment stages; number of reactors; volume/reactor (m^3)	II; 3; 65 m^3	II; 3; 120 m^3
Hydraulic retention time (HRT)	6–10 days	6–10 days
Operating temperature (°C)	50–70	50–70
Storage facility for ATAD treated sludge	Transported to Bendigo Facility (30 km north)	120 m^3 concrete Tank
Biosolids disposal method	Land application	Land application

Geobacillus spp. and *Bacillus* spp., while *Thermus ruber* was isolated from one system [32]. In the only published culture-independent study of a full scale batch-fed ATAD system, the 16S rRNA clone library generated contained sequences closely related to *Bacillus* spp. and *Paenibacillus* spp. [58]. This study therefore undertook to use culture-dependent and culture-independent methods to identify which bacterial populations were present in two full scale ATAD systems and, by a combination of fluorescence *in situ* hybridization (FISH) and microautoradiography (MAR), attempted to understand better their *in situ* physiology in the hope that this information might eventually enable these processes to be better operated and controlled.

Materials and methods

Design and performance criteria of the ATAD systems

Two ATAD systems were used for most of this study. They were operating to dispose of the waste activated sludge (WAS) from two enhanced biological phosphate removal (EBPR) plants, both of which were configured as modified University of Cape Town (MUCT) systems. The ATAD at Castlemaine, Victoria treated a mixture of domestic wastes and effluent from a meat processing plant (approximately 80% domestic and 20% meat processing wastewater), while that at Bendigo, Victoria handled predominantly domestic sewage. Both ATAD facilities were configured to operate as two stage continuous, and when required, semi-continuous systems. In both plants, the waste activated sludge was first thickened using conventional gravity settling (CGS) followed by dissolved air flotation (DAF), assisted by addition of Ciba® Zetag® cationic polyelectrolyte [51], to give an influent total solids (TS) content of 6.3 (±0.5)%. The key operational features of these two plants are compared in Table 1. Regular monthly analyses for the faecal indicator organisms *E. coli* and *Enterococci* always failed to detect their presence, suggesting that the process successfully eliminated enteric pathogenic bacteria. The microbial community from a third two stage ATAD plant in Gerroa, NSW incorporating spiral aeration technology, where mixers can also act to draw air into the reactor, as opposed to the Victorian ATAD plants that maintained aerobic conditions by mechanical mixing of pure oxygen introduced through static aerators, was also examined.

Sample collection

Sludge samples were collected from ATAD stages I and II (reactors 2 and 3) from the plants at Bendigo, Castlemaine, and Gerroa, NSW. All were immediately placed on ice. Additional samples for later analysis using FISH, were collected at the same locations, and fixed immediately with ethanol or paraformaldehyde [84]. The pH, temperatures, and redox potentials of each reactor system were measured continuously *in situ*. Sludge samples for DNA extraction were stored at 4 °C for no more than 24 h before being analysed, while those fixed for FISH were kept at −20 °C. Samples for denaturing gradient gel electrophoresis (DGGE) analysis and ecophysiology studies were taken from ATAD reactor 2 (stage I) at Bendigo and Castlemaine. Samples collected from reactor 2 were chosen since regular microscopic examination of samples from reactor 1 always revealed the presence of many viable aerobic mesophiles and filamentous bacteria carried over in the secondary sludge from the final aerobic basin of the treatment plants.

Isolation and identification of pure cultures from ATAD sludge

Isolates were cultured from ATAD samples by streaking onto a complex medium previously used to isolate *Thermus* sp. [81], which was chosen because clone library and FISH analyses (see later) suggested that *Thermus* spp. were dominant community members. Plates were incubated at 65 °C for 24 h. Single, often spreading, colonies, differing in their appearance, were then re-streaked repeatedly onto the same medium until eventually Gram staining suggested they were pure. Their genomic DNA was extracted with a MoBio Laboratories UltraClean® Soil DNA Extraction Kit and 16S rRNA genes were PCR amplified with the universal primers 27F and 1525R [40], subjected to agarose gel electrophoresis and the bands of the appropriate size (about 1500 bp) were cut from the gel and purified with Qiagen QIAquick® Gel Extraction kits. The genes were cloned using the pGEM®-T Easy Vector system (Promega, USA). Inserts were then sequenced with the M13F and M13R plasmid specific primers [49], and 926F, 907R, 519R and 530F primers [40], and the Applied Biosystems PRISM BigDye 3.1 Terminator Chemistry Kit. Amplicons were sent to the Australian Genome Research Facility (AGRF), University of Queensland for sequencing, and the results were analysed with Biomatters Ltd, Geneious Pro 4.0.4 software and the BLAST [2] facility available from www.ncbi.nih.gov/blast. Phylogenetic trees were constructed with the maximum likelihood [21], maximum parsimony [38] and neighbour joining [63] algorithms. Using the Jukes and Cantor [36] distance model, an evolutionary distance tree was created from the neighbour joining method. The SEQBOOT and CONSENSE options from PHYLIP [22] were used to determine bootstrap values (as percentages of 1000 replications).

DNA extraction and amplification from ATAD biomass

ATAD sludge samples were pre-washed following the protocol of Purohit et al. [59], in order to remove humic substances. Genomic DNA was then extracted from each sample with a MoBio Laboratories UltraClean® Soil DNA Extraction Kit following the manufacturer's protocol and stored at −20 °C. This extraction method provided large amounts of high quality non-sheared DNA from all ATAD samples examined. Amplification of 16S rRNA genes was performed with Applied Biosystems Amplitaq Gold® DNA polymerase, PCR primers 27F and 1525R [40] and the following protocol: 94 °C (7 min), followed by 35 cycles of 94 °C (1 min), 50 °C (50 s), 72 °C (90 s) and a final extension step of 72 °C for 10 min. PCR amplicons were cloned with the Promega pGEM®-T Easy Vector System.

16S rRNA gene clone library construction

Plasmids were extracted with the Promega Wizard® SV Miniprep DNA purification kit and inserts (one hundred for each