BIOREMEDIATION OF SODA ASH CONTAMINATED WITH GREASE : THE APPLICATION OF COMPOSTING TECHNOLOGY

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BIOREMEDIATION OF SODA ASH CONTAMINATED WITH GREASE : THE APPLICATION OF COMPOSTING TECHNOLOGY

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Summary. Physical and chemical changes in biopiles were examined during composting of soda ash contaminated with grease. All compost trial temperature profiles illustrated initial elevated temperature levels followed by a slow gradual temperature decreasing phase. The C:N ratio and bulking agent seem to have the greatest impact on producing thermophilic temperatures. The composting process did not remove the contaminants to the agreed end-points after 5 months. Therefore more time is needed to reduce total petroleum hydrocarbon (TPH) to disposal limits. With the experiments being a success in producing self-heating elevated temperatures, it is obvious that the precise parameters necessary are still vague.

Key words: Biopiles — Composting — Soda ash — Temperature — C:N ratio – Bulking agent — Thermophilic – TPH

Introduction

Composting has been used to degrade solid waste materials, such as agricultural wastes, sewage sludge, and food wastes. More recently, composting has been investigated as a remediation technology for hazardous waste (Semple *et al.* 2001; Potter *et al.* 1999; Schonberner 1998; Ziegenfuss and Williams 1991).

One of the technologies currently being developed for use in remediation of soil and sediment is composting (Williams & Keehan 1993). Composting has become increasingly popular during the past decade as an alternative to incineration or tipping of decomposable organic household waste. However, composting may also be a useful treatment process for hazardous biodegradable waste. Whereas the primary benefit from composting of household waste is the reduction of volume, saniterization and stabilisation for recycling or ultimate disposal, the objective of composting hazardous materials is solely to convert these substances into an innocuous end-product.

This paper represents a part of a series of investigations conducted to determine the mechanisms involved in bioremediation of soda ash contaminated with grease. The succession of microorganisms during composting and its physical and chemical properties are described and compared among biopiles. The overall aim of this research is to assess the efficacy of bioremediation to reduce total petroleum hydrocarbon concentrations of greasy soda ash to acceptable regulatory levels.

Materials and Methods

Composting: The sieved soda ash was stacked with the bulking agent in separate piles of approximately 2.0 m height, and 2.0 m width. Four piles of 5 m length were constructed with the greasy soda ash-contaminated soil in 12 April 2006. The ambient temperature during the preparation work was 15 ± 20°C. Gum tree (*Eucalypthus* sp.) leaves were used as the bulking agent. The total amount of bulking agents in the piles were added according to Table 1.

Table 1. Ratio soda ash, biosolids and green waste of biopiles

FEEDSTOCKS	**Mass**	**FEEDSTOCKS**	**Mass**	**Volume**
(Pile 1)	(t)	(Pile 2)	(t)	(m^3)
Soda Ash	7.2	Soda Ash	9	10.0
Biosolids	0	Biosolids	3.2	4.0
Green Waste	4.8	Green Waste	3.6	12.0
Total	12	Total	15.8	26.0
C:N	34	C:N	34	
FEEDSTOCKS	**Mass**	**FEEDSTOCKS**	**Mass**	**Volume**
(Pile 3)	(t)	(Pile 4)	(t)	(m3)
Soda Ash	9	Soda Ash	11	12.2
Biosolids	0	Biosolids	1.7	2.1
Green Waste	3.6	Green Waste	3	10.0
Total	12.6	Total	15.7	24.3
C:N	51	C:N	51	

When temperatures began to decline, the compost heap was turned, using a front-end loader, on days 41 and 55. The pile was turned at these times, in order to maintain the optimum temperature for decomposition.

Temperature monitoring. Temperatures throughout the compost heap were monitored with a Temperature data loggers (T-TEC7-3E, Temperature Technology Adelaide, South Australia). Thermocouples were placed in the center of piles. The temperatures were recorded every 1/2 h for the duration of the composting process. To start the logging and to download the result, the data loggers are connected to PCs via interface cables. Graphic software provides options for initial setting and for extracting information.

Moisture content. The moisture content was determined in three 100-g portions from compost sampled for microbial counts. These portions were dried at 105 °C for 24 h and then weighed.

pH. pH determination was performed weekly from sub-samples collected from the biopiles. The samples were pooled from the various parts and suspended in water (w/v, 1:10), shaken for 1 min on a rotary shaker and the pH of the supernatant was determined using a pH meter (Scientific Instruments Co.).

Determination of microbial numbers. Tryptone Soy Agar was used for the medium for bacteria. Manual counting with a loupe and MACE 100 software was employed. Only those plates having 30 to 300 colonies were considered in determining the plate count.

DGGE analysis. Changes in the microbial community during biodegradation of greasy soda ash in the controlled composting test were studied using the PCR-based technique DGGE (denaturing gradient gel electrophoresis). The samples for DGGE analysis were taken from the controlled composting test after 0, 41, 55, 69 and 100 days of incubation and frozen. DNA extraction was performed using an Ultra Clean Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, California). The PCR products were analysed by DGGE. All DGGE analyses were performed with the D-Gene system (Bio-Rad Laboratories, Hercules, CA, USA), with gradients of 0 to 100% denaturant and running conditions of 75 V for 16 hours. Gels were run in 0.5× TAE (Tris-acetate-EDTA) buffer at a constant temperature of 60°C. Gels were stained for 15 min in SYBR Green I (FMC Corporation) and destained for 1 min in MilliQ water prior to UV transillumination. Gel images were digitally captured with the VersaDoc system.

Measuring total petroleum hydrocarbon. Leeder Consulting (4-5, 18 Redland Drive Mitcham, Victoria, Australia) determined the amount of total petroleum hydrocarbon and extractable biogenic material in compost samples. An aliquot of the sample extract was analysed by GC FID to determine the Total Recoverable Hydrocarbon (TRH). A second aliquot was analysed by GC-MS to determine the amount of recoverable biogenic material.

Results

TPH contamination of greasy soda ash

Based on the analysis of Leeder Consulting (2006), the soda ash contains a large "hump" of un-resolved hydrocarbon which is typical of lube oil. The presences of hopanes in the sample also indicate the presences of lube oil. The green organic also contains a small amount (<25mg kg^{-1}) of Polynuclear Aromatic Hydrocarbons (PAH) but it contains a much larger amount of biogenic sources (3,600 mg/kg).

Table 2. Initial assessment TPH and total biogenic extractable of green organic and soda ash

Materials	TPH (mg/kg)	Total Biogenic Extractable (mg/kg)
Green Organic	<50	3,600
Soda Ash	78,000	<100

Biopiles formation

The pilot-scale composting resulted in the soil changing from a light yellow-brown clay, containing obvious yellow greasy residues, to a dark organic appearance soon after the composting commenced. No objectionable odours were generated from the process or were noticeable in the treated greasy soda ash. Moisture additions were managed so that leachate generation was minimized.

TPH Degradation during co-composting of soda ash

Because co-composting involved the biodegradation of the total petroleum hydrocarbons is a biodegradative process, the C:N ratio of the compost materials is a very important factor for optimum hydrocarbon degradation. In biopiles 1 and 2 with C:N ratios of 34, the rate of TPH degradation was better than for C:N ratios of 51. In biopiles 1 and 3 the TPH degradation rate reached approximately 50% for period 3 months from 78,000 mg kg^{-1} to approximately 36.250 mg kg^{-1} - 37,000 mg kg^{-1}.

Changes in temperature during the co-composting of greasy soda ash

In general, the compost pile temperatures rose rapidly after mixing, the temperatures then declined slowly as the biodegradable material was decomposed. The piles were regularly mixed to provide aeration using mobile earthmoving equipment. The temperature profiles of the developing biopiles were significantly different; the biopiles without biosolids reached a temperature of 50°C after 14 days, while the biopiles with biosolids required 3 weeks to reach this temperature. Furthermore, biopiles 1 and 3 maintained a temperature over 40°C throughout the remainder of the composting period monitored (90 d), while biopiles 2 and 4 maintained peak heating for a significantly shorter time period (approximately 30 d).

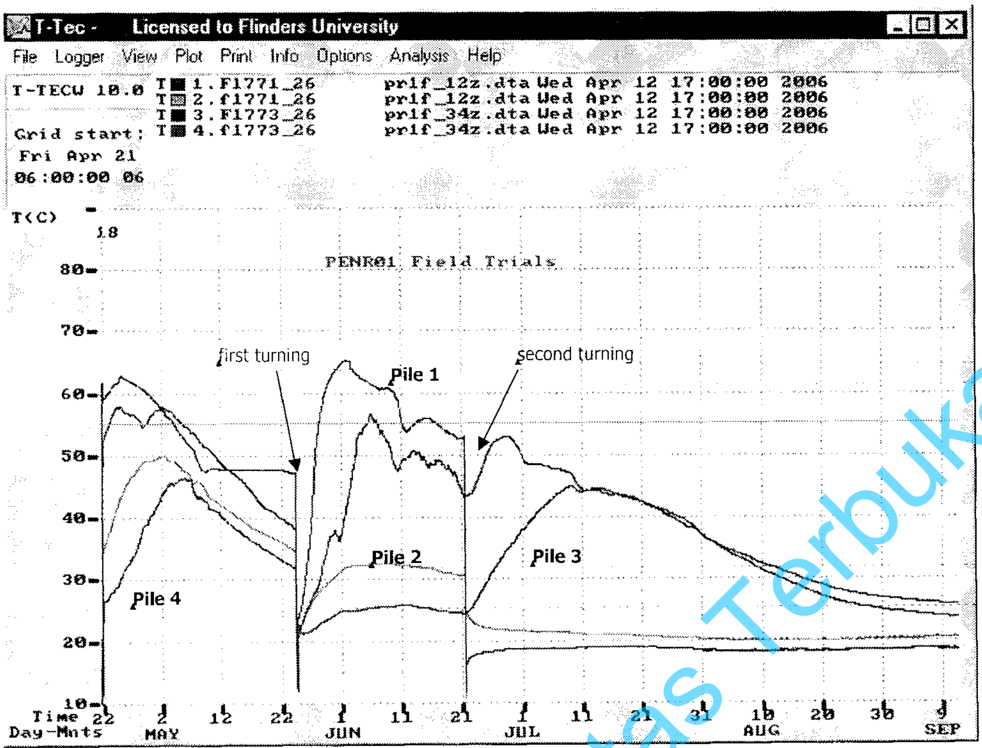

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Figure 1. Temperature of biopiles

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Changes in pH during the co-composting of greasy soda ash

The variation of pH for the four piles is shown in Figure 2. During the first phase of composting, the pH varied from 9.0 to 9.5. In the latter phases, as the temperatures rose, so did the pH. In general, initially the pH rapidly increased and then decreased gradually after 70 d, with the exception of biopile 4. The pH of biopiles 3 and 4 (with C:N ratio 51) increased after the first month reaching 9.5 in the second month. The decrease after the third month was gradual and reached 9.0 at the end of the experiment for biopile 3. The pH of the biopiles 1 and 2 (C:N ratio 34) followed the same pattern of increase and reached 9.2 at the end of the experiment for biopile 2. In particular, the pattern of pH of biopile 1 is unique because it has the lowest pH during composting and it tends to decrease continuously.

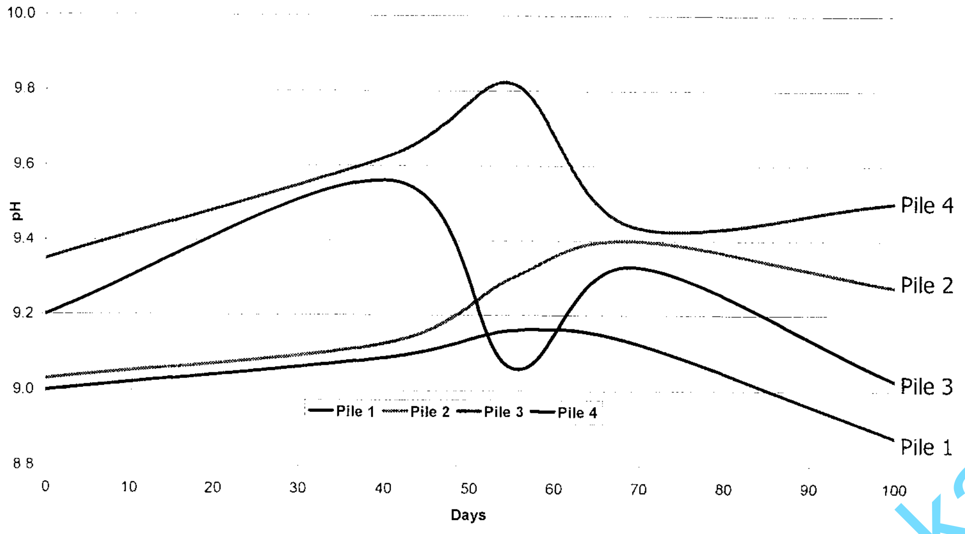

Figure 2. pH of biopiles

Changes in moisture content of greasy soda ash during co-composting

Samples were taken 5 times for moisture evaluation, and water was added to the appropriate biopiles five times. The moisture content of the composting materials on each sampling day, including when the biopiles were created is shown in Figure 3. An appropriate amount of water was added to each biopile after the samples were collected to maintain the desired moisture contents (> 40%). The rainfall of 2006 at the Southern Waste Depot was very low during this period. The moisture content of the compost decreased from day 0 to day 50, thereafter a decline state was established until day 70; after that the moisture content increased rapidly. Mean moisture contents decreased from 60% at day 0 to 35% on day 50.

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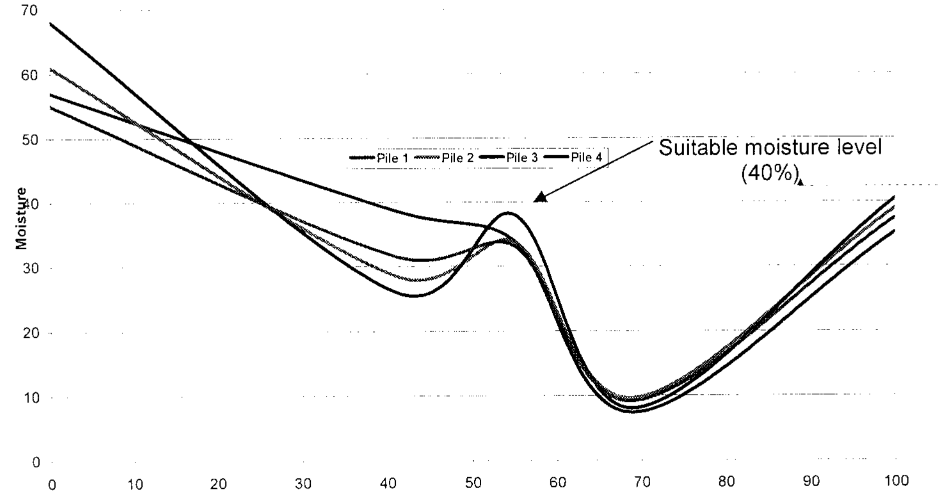

Figure 3. Soil moisture contents of biopiles

Microbial population

The increase in the bacterial population during the composting process is shown in Figure 4. Bacterial numbers increased over the first 7 weeks of treatment, then rapidly declined and increased steadily as the compost matured. The overall population of bacteria declined as the concentration of readily available nutrients, moisture content, and the temperature of the compost pile declined with maturity.

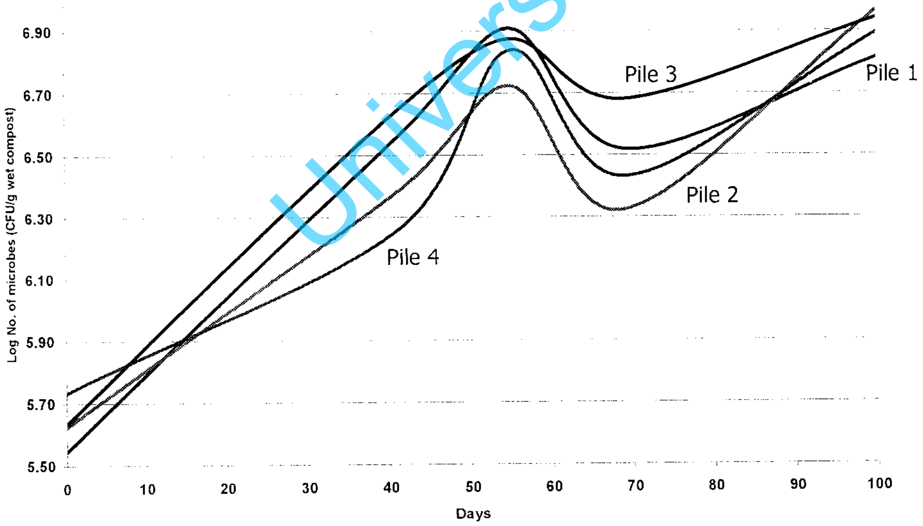

Figure 4. Changes in the bacterial population (cfu ml^{-1}) during co-composting of greasy soda ash

DGGE

The aim of this method was to reveal the microbial succession that was occurring during the composting of greasy soda ash under various biopile conditions, from initial to later phases. Figure 5 shows an ethidium bromide-stained agarose gel used to visualize the DNA extracted from biopiles. The largest DNA was greater than 600 base pairs, while most DNA was in the size range of 400 to 600 base pairs. DNA shearing during the extraction procedure was less evident when the Mo Bio Kit was used when compared to the technique of bead beating followed by phenol-chloroform extraction, as evidenced by shearing of bands.

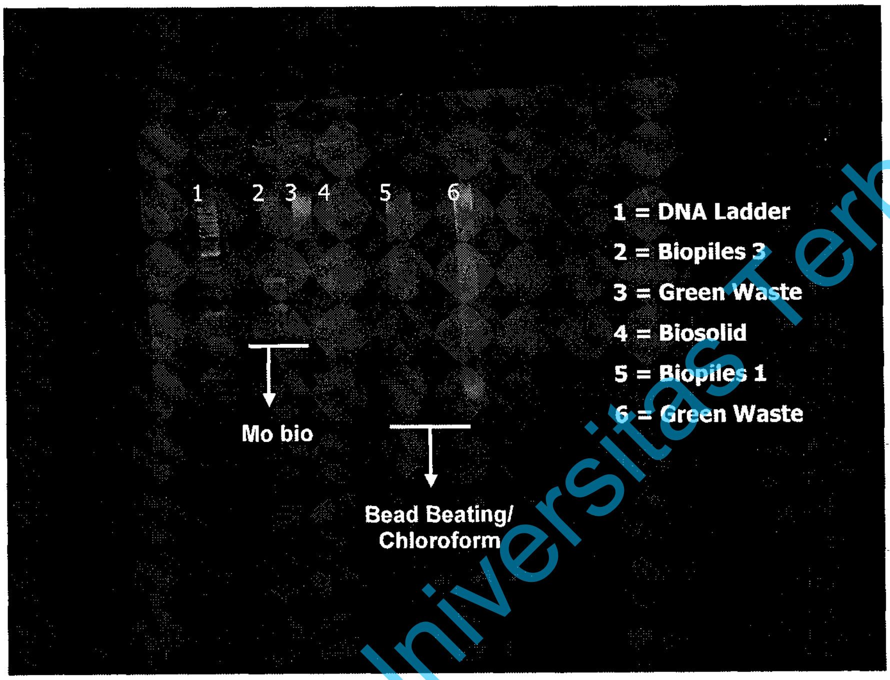

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Figure 5. DNA of microorganisms in biopiles extracted by Mo Bio Kit method compare to chloroform method

Discussion

In this study Biopiling was used as a methodology for Co-composting. Generally, land requirement is determined by the amount of contaminated soil to be treated. Additional land area around the biopiles is required for sloping the sides of the pile, and for access. The length and width of biopiles is generally not restricted unless aeration is to occur by manually turning the soils. In general, biopiles which will be turned should not exceed 6 to 8 feet in width. Biopile layout is determined by the configuration of and access to the land available for the biopiles. The biopile system in this study used multiple piles.

The type of biosolids used may have an effect on the composting process. In this study, the biosolids were originally operation sludge sourced from a local waste water treatment plant that had been air dried in shallow lagoons. Once the material was dry enough, it was brought to the landfill for reuse as biosolids. The biosolids we used would be at four years old and possibly older. Composting can be accomplished with unstabilized biosolids, as well as anaerobically and aerobically digested biosolids. Raw sludge has a greater potential to cause odours because they have more energy available and will, therefore, degrade more readily. This may cause the compost pile to achieve higher temperatures faster if sufficient oxygen is provided and may also cause odors (EPA, 1985).

Wood chips and leaves of gum tree were added as “bulking agents” or “amendments” to the compost mixture to provide an additional source of carbon and to control the moisture content of the mixture. Other common bulking agents used by facilities around the country include wood waste, leaves, brush, manure, grass, straw, and paper (Goldstein, 1994). Because of their cost, wood chips are often screened out from the matured compost, for reuse. Although sawdust is frequently used for in-vessel composting, coarser materials such as wood chips, wood shavings, and ground-up wood are often preferred because they permit better air penetration and are easier to remove. Recycled compost is often used as a bulking agent in windrows, especially if bulking agents must be purchased. However, its use is limited because the porosity decreases as the recycle ages (EPA, 1989). The amount of biosolids and bulking agent which must be combined to make a successful compost is based on a mass balance process considering the moisture contents, C:N ratio, and volatile solids content.

In general, biopiles with C:N ratio 34 (piles 1 and 2) performed better, in terms of TPH degradation than biopiles with C:N ratio 51 (piles 3 and 4). Also, biopiles without biosolids (piles 1 and 3) performed better than biopiles with biosolids (piles 2 and 4). These results suggested that the C:N ratio between 34 and 51 in the raw materials is optimum for biodegradation of greasy soda ash. However the fact that TPH degradation occurred over a range of conditions may be advantageous for constructing a simple system without precise control.

Microorganisms need carbon for growth and nitrogen for protein synthesis. Microorganisms use carbon for both energy and growth, while nitrogen is essential for protein production and reproduction. The ratio of carbon to nitrogen is referred to as the C:N ratio. An appropriate C:N ratio usually ensures that the other required nutrients are present in adequate amounts. For an active aerobic metabolism, a C:N ratio of 15 to 30 is suggested (Haug 1993). Zucconi & Bertoldi (1986) suggested that the C:N ratio of the microbial cell be about 10. However, due to energy requirements, initial C:N values of 28-30 maximize metabolic rates. According to Pace et al (1995), raw materials blended to provide a C:N ratios of 25 to 30 are ideal for active composting although initial C:N ratios of 20 to 40 consistently give good composting results. A C:N ratio below 20 produces excess ammonia and unpleasant odours while a C:N ratio above 40 does not provide enough N for microbial growth and a fast composting process. Once completely composted, the treated waste should offer a C:N ratio ranging between 15 and 20, to be used as a balanced soil amendment. If the C:N ratio exceeds 20, N becomes deficient in the soil, and if the ratio is significantly below 15, N can be lost by volatilization from the soil and can have a toxic effect on plants (Bilitewski et al 1994).

During the composting process control of volatile and aqueous emissions must be addressed. In this study, soil erosion from wind or water was controlled by diversion within the bunded treatment area. In terms of odours, which are a by-product of the microbial degradation, initially a foul smell was detected, characteristic of waste sludge. Later, the odour had a hydrocarbon-type characteristic, as expected from the microbial transformation TPH.

The large increase in temperature observed in the first 2 weeks may have been due to the high initial nitrogen content of the green waste, which resulted in high metabolic activities. However, the high temperature became inhibitory to continued microbial growth after 2 weeks, resulting in a decrease in microbial activity and subsequently a decrease in temperature in the third week. This decrease from the thermophilic temperature to a mesophilic temperature resulted in a renewed increase in microbial activity in the sixth week. After an initial high temperature period (of a few days to several weeks), compost pile temperatures dropped. Turning the compost rejuvenates the oxygen supply and exposes new surfaces to decomposition, causing temperatures to rise.

The changes in temperature during these trials were similar to those seen in a typical composting process (EPA, 2003). In this thesis, the composting process is classified into three phases based on process dynamics and inspired by the works of Smårs (2002). These phases are (A) the initial phase; (B) the high-rate phase; and (C) the curing phase.

The decrease in pH was observed in the compost over time may be related to a decrease in substrate concentration. This is believed to due to the production of microbial metabolites which resulted in a decrease in pH as the microorganisms utilize the substrate. Generally, the pH level drops at the beginning of the composting process as a result of the acids formed by the acid-forming bacteria which initialize the process by breaking down complex carbonaceous materials. The later break down of proteins and liberation of ammonia account for the subsequent rise in pH (Zucconi & Bertoldi 1986, Bilitewski et al 1994). According to Pace et al (1995), the preferred range of pH is 6.5 to 8.0. Higher pH mixtures may result if lime stabilized biosolids are used. They can be composted; however, it may take longer for the composting process to achieve the temperatures needed to reduce pathogens. During composting of greasy soda ash the pH remained between 8.8 and 9.8, which is outside of the recommended range for composting organic materials. The increase in the first 7 weeks could be due to the high ammonium content of the biosolids. The decrease observed in subsequent weeks are attributed to the degradation of the compost and the hydrocarbons, which resulted in the release of intermediate and final products that probably had lowering effects on the pH of the biopiles.

The observed decreases in the moisture content in the first 50 days of composting greasy soda ash are likely to be due to evaporative effects as the biopiles are exposed to environmental conditions leading to drying. To counteract the effects of drying water was added each turning time to each biopiles. Thus the increase in biopile moisture content after 50 days was due to water addition. However, it was difficult to maintain biopiles at 40% moisture content. A pyramidal shape is the best for allowing natural convection to occur, where air flows in from the bottom and up through the material exiting out the top of the biopiles. However, biopiles maintained at 40% moisture content, widen at the base of the biopile making it difficult to maintenance the correct shape.

There was only a slightly difference among the number of organisms between biopiles. It is important to note that the dilution-plate method gives only an estimate of the populations. It records the number of viable cells, spores, and mycelial fragments that are capable of growing on the agar media used. Therefore, dilution plating provides some information on trends in groups of organisms, but little in regard to their activity. In the present trial, the population trends of microorganisms were very similar throughout the composting process, especially for the first 100 days of composting. These results imply that although the composition of the initial biopiles are different because of variations in C:N ratio and composition, when biopiles become stabile and mature, their microbial properties reach similar values.

We found that shearing occurred during longer homogenization times and at higher speeds of bead beating. Furthermore, including chloroform in the extraction mixture, particularly when the bead beater was employed, also enhanced shearing through an unknown mechanism. To avoid DNA shearing, we recommend to use bead beating time 20 seconds at maximum speed.

It is important to reiterate the remarkable complexity of compost types and the fact that there are multiple factors that may affect the performance of a DNA extraction method. Generalizations from this result may be limited to compost from greasy soda ash, biosolids and green waste (gum tree) having different organic matter contents; however, our results also provide useful guidelines that may be applied to developing protocols for other types of samples as well.

Amplification by PCR has been reported to be inhibited by a number of substances usually found in environmental samples (Tsai and Olson, 1991; Jacobsen, 1995; Zhou *et al.*, 1996), generally coextracted with DNA during standard extraction procedures and difficult to eliminate during purification protocols. Humic acids and exopolysaccharides are likely the most commonly reported PCR inhibiting substances (Wilson, 1997; Moreira, 1998). In this study, no bands could be visualized using DGGE.

Conclusions

All compost trial temperature profiles illustrated initial elevated temperature levels followed by a slow gradual temperature decreasing phase. The C:N ratio and bulking agent seem to have the greatest impact on producing thermophilic temperatures. The composting process did not remove the contaminants to the agreed end-points after 5 months. Therefore more time is needed to reduce TPH to disposal limits. With the experiments being a success in producing self-heating elevated temperatures, it is obvious that the precise parameters necessary are still vague.

Apart from the obvious importance of pH and moisture contents of biopiles, this study has shown that the amount of bulking agents and the C:N ratio of biopiles are key factors affecting composting of this particular greasy soda ash. Future work should focus on the mechanisms responsible for the positive effects of temperature and organic amendment in the hope that general principles may be developed for composting of greasy soda ash. In particular, physical factors such as increased bioavailability due to changes in biopiles structure must be studied. These factors need to be separated from the effects of the amendments on biological components such as the composition, size and activity of the microbial population. This knowledge will significantly increase our understanding of the in situ degradation processes taking place and the organisms involved at contaminated sites and can be useful in the design and evaluation (monitoring) of new bioremediation strategies.

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