CHAPTER 2

Design and Operation of Bench-Scale Landfill Bioreactors

CHAPTER 2.1.

Description of a Bench-Scale Landfill Bioreactor for Investigating the Production of Biogas from Solid Waste

ABSTRACT

A laboratory-scale landfill bioreactor was set up to simulate the microbiological processes in a field landfill. The bioreactor was constructed of two sections. Waste degradation was carried out using digested sewage in one section, the anaerobic bioreactor, while methane (CH_4) degradation occurred in the second section, the aerobic bioreactor, using garden compost as the methanotroph seeding culture. CH_4 gas produced in the anaerobic bioreactor diffused to the aerobic bioreactor via Tygon tubing. A manometer attached to the anaerobic bioreactor served to indicate maximum gas yields. The bioreactor system was run for a 21-week period to monitor the optimum operating conditions in both bioreactors. Leachate samples were spread onto Tryptone, Yeast, Glucose (TYG) media plates and observed for morphological colony differences. Soil samples were plated onto nitrate mineral salts (NMS) media for methanotroph enrichment. Morphological features of the anaerobic bioreactor showed a change in leachate colour and turbidity over time. Leachate recycling was shown to effectively accelerate the waste degradation process with a total waste height reduction of 90 mm and a final waste height of 310mm. Leachate pH was seen to change over time with the lowest pH of 5.5. At this pH value waste digestion and methane production stopped, highlighting the sensitivity of methanogenesis to pH shifts. The pH decrease was attributed to the accumulation of volatile fatty acids in the leachate. Morphological bacterial observations from leachate showed no differences in bacterial populations at each sampling point. No methanotrophs were successfully isolated on NMS media.

INTRODUCTION

Landfills serve as disposal sites for different types of wastes e.g. hazardous, sanitary or municipal solid waste (MSW). Landfills consist of a basal liner, waste bodies and soil cover layers, above the waste layers, which prevent erosion of the waste layer. Cellulose rich components, proteins, starch and sugars from waste serve as nutrient sources for microorganisms responsible for waste degradation (Zinder, 1999). Waste degradation is a controlled, multi-phased process initiated by different bacterial species at each phase (Swarbrick, 2001) where aerobic decomposition is the primary phase with anaerobic decomposition by methanogenic bacteria being the most important phase. Methanogenic bacteria are strict anaerobic, archaean bacteria that use acetate, hydrogen and carbon dioxide as growth substrates, and produce methane (CH_4) as a biogas (Zinder, 1999).

It is the methanogenic degradation phase that is the most important phase of waste degradation as the majority of waste decomposition is reliant on methanogens. In additions, methanogens are the slowest growers compared to other waste degrading bacteria, thus methanogenesis is rate-limiting step of waste degradation (Rohrs, *et al.* 1998). Furthermore, methanogenesis is highly sensitive to pH, oxygen and temperature shifts (Yu and Fang, 2003). Such disturbances lead to decreased methanogenesis, leading to decreased rates of waste degradation. Temperatures ranging from 37 - 41ºC and pH 7 are considered as optimum conditions for methanogenesis (Yu and Fang, 2003). It has been reported that a drop in pH from 7 to 6.6 halts methanogenesis and hence waste

degradation (Mosey and Fernandes, 1989). Changes in pH are attributed to an accumulation of short chain volatile fatty acids in the waste increasing overall acidity.

CH_4 is a potent greenhouse gas, contributing to global warming at an estimated 12 % of the enhanced rate of global warming with landfill CH_4 emissions contributing 20-70 Tg (1 Tg = 1 million tons) globally (De Visscher, *et al*. 1999). Methanotrophs are aerobic bacteria that are capable of oxidising CH_4 to carbon dioxide (CO_2) and cellular carbon, with oxygen (O_2) as the catalyst (Hanson, 1996). This unique characteristic renders methanotrophs useful for bioremediation of CH_4 from landfills. Methanotrophs possess the enzyme methane monooxygenase (MMO) which functions to initiate the methane oxidation process (Mancinelli, 1995; Hanson, 1996; Murrell and Radajewski, 2000). Methanotrophs are found in various habitats e.g. lakes, oceans, sediments and soil and exist as various forms (cocci, rods or vibroids) (Mancinelli, 1995; Hanson, 1996, Hanson and Hanson, 1999).

Methanotrophs require adequate moisture to efficiently carry out CH_4 oxidation (Mancinelli, 1995; Hanson, 1996). Johannesburg is a semi-arid area and CH_4 emissions from existing landfills are common. Arid soils do not support efficient CH_4 oxidation and methanotrophic bacteria may enter a dormant state by surviving as exospores or cysts which are heat and desiccation resistant (Mancinelli, 1995). Therefore, although methanotrophs may be present in cover soil, inadequate moisture prevents these bacteria from performing CH_4 oxidation resulting in increased CH_4 emissions from landfills. Leachate recycling is being considered as an addition to future South African landfills in

order to increase the moisture content of landfills, which will supplement waste degradation and CH_4 oxidation.

This study attempted to simulate a field landfill process by establishing and monitoring the anaerobic degradation phase and aerobic CH_4 oxidation phase in separate bench-scale column bioreactors.

MATERIALS AND METHODS

Bioreactor design and construction

A bench-scale landfill bioreactor was designed to simulate a field landfill. A two-phase bioreactor system consisting of an anaerobic bioreactor (to represent the waste degradation phase of landfills) and an aerobic soil bioreactor (to represent the soil cap on landfills) was constructed out of Perspex (Figure 2.1.1 and Figure 2.1.2). The bioreactors were connected by Tygon tubing (Figure 2.1.1 o) which also served as the gas transport mechanism from the anaerobic to aerobic bioreactor. CH_4, which would be produced in the anaerobic bioreactor by methanogens degrading the waste, flowed to the aerobic bioreactor where it would stimulate the methanotrophs in the soil to oxidise the CH_4.

Anaerobic system

An anaerobic bioreactor 500 mm in height was constructed with outer and inner diameters of 80 mm and 70 mm, respectively (Figure 2.1.1 (1) and Figure 2.1.2 (1)). Solid Perspex lid (10mm) with O-rings creating gas tight seals was used to seal the

bioreactor vessel or chamber. For leachate recycling with a peristaltic pump (Figure 2.1.1 a and b), liquid entry (Figure 2.1.1 f) and exit points (Figure 2.1.1. d) were installed in the centre of the solid Perspex lid to which Tygon tubing was attached. A funnel (Figure 2.1.1 d) was created at the bottom of the bioreactor to facilitate leachate recycling; clogging was prevented by the use of a perforated PVC plate. On the bioreactor lid two further ports were added, one for pressure (Figure 2.1.1 g) and the other serving as a gas exit port (Figure 2.1.1 i) to a gas trap filled with anaerobic distilled water (Figure 2.1.1 j). This gas trap served to hydrate the gas on its way to the supplementary aerobic bioreactor. Each bioreactor was surrounded with a water jacket (Figure 2.1.1 e) and bioreactor temperature was kept constant at 37º C by circulating water through the water jacket from a waterbath.

Aerobic system

The aerobic bioreactor was constructed as described for the anaerobic bioreactor (Figure 2.1.1. (2) and Figure 2.1.2 (2)) except that no funnel existed. Solid Perspex was again used the bioreactor lid (capping). A gas entry/exit port (Figure 2.1.1 l) was created on either side of the bioreactor column for O_2 entry/CO_2 exit. One of the gas ports was used for gas sampling purposes (Figure 2.1.1 n). The aerobic bioreactor was connected to the anaerobic gas trap via Tygon tubing to allow CH_4 diffusion (Figure 2.1.1 m). Bioreactor temperature was kept constant at 37º C recycling water from a waterbath through the water jacket (Figure 2.1.1 e).

Laboratory-scale landfill system

MSW was collected and processed according to the recipe in Table 2.1.1. Individual waste components were cut into 1-2 cm pieces to provide adequate surface area for digestion. The anaerobic bioreactor was filled with approximately 450 g of MSW, 70 % (w/v) anaerobic distilled water for leachate recycling purposes and 50 % (v/v) partially digested sewage sludge (Olifantsvlei Wastewater Treatment Plant, Johannesburg, South Africa) added as the seeding culture. This phase was 400 mm in height with a gas headspace of 100 mm (Figure 2.1.2 (1)). Resting on the PVC plate was a layer of sterile glass beads to further prevent clogging of the anaerobic bioreactor during leachate recycling. Fresh anaerobic water was added in 10-ml volumes at weekly intervals for leachate dilution purposes.

The aerobic bioreactor contained 300 mm of sterile sandy soil with a 200 mm-gas headspace (Figure 2.1.1 (2) and Figure 2.1.2 (2)). Soil was collected from the Simmer and Jack landfill site located at Germiston, South Africa. Landfill soil was left overnight at room temperature to dehydrate. The next day the soil was sieved using a 2 – 3.6 mm sieve. The remaining larger soil grains were crushed with a pestle and mortar and then re–sieved. All stone particles more than 4 mm were removed by hand. The soil was then autoclaved for 2 hours to inactivate fungal spores. The sterile soil was re-hydrated to 9 % using sterile distilled water and overnight incubation at room temperature. The rehydrated soil was then mixed with 15 % (w/w) garden compost, which served as the seeding culture for methanotrophs in the aerobic bioreactor.

Pressure measurements

Gas pressure measurements for the anaerobic bioreactor were determined using a manometer (Figure 2.1.3 A and B) filled with linseed oil. A manometer fitting was created on the upper solid Perspex lid (Figure 2.1.3 i), and the manometer was held in place using metal springs (Figure 2.1.3 f). A pilot study was performed to assess the time period taken for biogas accumulation in the manometer indicated by movement in the manometer fluid (linseed oil) to the highest level (Figure 2.1.3 a and c) , indicating that maximum gas production had occurred. This required monitoring the manometer liquid level for 5 hours, at 1-minute intervals. Post pressure measurements, gas was left to build-up for the same time period as that taken for the manometer fluid to reach its highest point, for further gas chromatographic analysis.

Gas chromatography sampling and analysis

Gas sampling, in parallel with pressure measurements, occurred daily. All exit ports were sealed with rubber stoppers to allow for gas accumulation in the anaerobic and aerobic bioreactors. Diffusion of anaerobic bioreactor gas into the aerobic bioreactor was prevented by clamping the interconnecting Tygon tubing between bioreactors. Anaerobic gas was allowed to accumulate for two hours and then sampled at the gas trap (Figure 2.1.1 j) using a gas collection apparatus. The gas trap was thereafter re-sealed to prevent oxygen re-entry into the anaerobic bioreactor. Gas sampling for the aerobic bioreactor occurred in the same manner as for the anaerobic gas and occurred at a single gas exit point (Figure 2.1.1 n).

Gas concentrations were measured using a Shimadzu-17A gas chromatograph (Japan). Gas was injected into the gas chromatograph using a 500-µl gas tight syringe (Vici). Unknown samples were compared to standards of CH_4, CO_2 and air. Results obtained were calibrated against the standards to obtain the concentrations of samples.

Bacterial sampling and culture methods

Ten millilitres of leachate from the anaerobic bioreactor was sampled every 3 days and spread plated onto aerobic tryptone, yeast, glucose (TYG) plates (tryptone 5 g/l, yeast extract 3 g/l and glucose 1 g/l) to assess the preliminary aerobic bacteria that may occur in the leachate. Media was diluted 5x to represent a lower nutrient level that is representative of field conditions (Amann, *et al.* 1998). Visual observations of colonies were made after each incubation at 37 ºC to ascertain the time intervals for sampling. At each time interval for sampling leachate pH was measured using pH strips (Merck).

Soil sampling was performed to analyze the aerobic microbial population present in the soil. Five gram soil samples were removed from the aerobic bioreactor every 7 days for analysis using a sterile cork borer. Bacteria were dislodged from the soil particles by shaking with 20 g glass beads in 20 ml sterile distilled water and thereafter serially diluted (Lindsay and von Holy, 1997). Spread plates were performed on 5x diluted aerobic TYG for the isolation of a general soil bacterial population. Nitrate Mineral Salts (NMS) medium was used for the growth of methanotrophs [($NaNO_3$ 850 mg/l, K_2SO_4 170 mg/l, $MgSO_4.7H_2O$ 37 mg/l, $CaCl_2.6H_2O$ 7 mg/l; 10 ml **phosphate buffer** solution/l

NMS: 1.4g KH_2PO_4, 3.6 g Na_2HPO_4 – pH 6.8; 0.5ml **trace element** solution/l NMS: $ZnSO_4.7H_2O$ 10mg/l, $MgCl_2.4H_2.4H_2O$ 3 mg/l, H_3BO_4 30 mg/l, $Na_2MoO_4.2H_2O$ 3 mg/l, $CaCl_2.6H_2O$ 20 mg/l, $NiCl_2.6H_2O$ 2 mg/l, $CuCl_2.2H_2O$ 1 mg/l; 1 ml **iron solution/**l NMS: 0.5 ml o.25M H_2SO_4, 1.12g $FeSO_4.7H_2O$ (Kussmaul *et al.* 1998)]. All removed soil was replaced with sterile stock soil.

All aerobic plates (in duplicate) were incubated at 37º C. NMS media plates were left for 10 days in a mixture of air supplemented with a 90% CH_4: 10% CO_2 atmosphere to allow for methanotroph selection. To eliminate non-methanotroph populations, colonies grown in this manner were then plated onto Standard One Nutrient Agar, (SONA), (Biolab, Midrand, South Africa) using the streak plate method. It has previously been reported that methanotrophs are not cultivable on multicarbon compounds (Hanson, 1996) such as glucose, contained in SONA.

RESULTS

Waste morphology

Leachate colour in the anaerobic bioreactor changed from murky in week 1 to yellow in week 2, which remained till the end of the run (week 21) (Table 2.1.2). There was a total decrease of 9 cm in the waste height in the anaerobic bioreactor and residue attached to the bioreactor walls was also seen on the inner walls of the anaerobic bioreactor as early as week 1. The anaerobic indicator strip remained white throughout the run, indicating the maintenance of anaerobic conditions in the anaerobic bioreactor.

Manometer operation

Manometer fluid was seen to steadily rise. The time period for the manometer fluid to rise to its highest point was 2, 5 hours. Manometer fluid then dropped steadily after this period. The steady rise and fall of the manometer fluid was indicative that no leaks were present in the anaerobic gas system.

Leachate bacterial colony observations

The colonies which grew from leachate samples did not differ in size or colour for each week sampled. The majority of colonies were cream, yellow, orange or white and these were observed to occur on all the dilution plates at each time interval for sampling.

Methanotroph bacterial isolations

Fifty-seven purified isolates from NMS plates were streaked onto SONA. All isolates grew SONA and so were not studied further.

pH and gas profile of anaerobic leachate

A decrease in pH from 7 to 6 occurred in week 8, followed by a further decrease to pH 5.5 in week 14 (Figure 2.1.4). Gas chromatography at weeks 13, 14 and 15 showed low levels of CH_4 and CO_2 production (Figure 2.1.5). In week 21 the pH rose to 7 again, and an increase in the levels of CH_4 and CO_2 was observed (Figure 2.1.4. and Figure 2.1.5).

DISCUSSION

In this study, leachate colour changed from opaque to murky brown in the first week of recycling (Table 2.1.2). In week 2 this leachate turned to a yellow colour which remained till the study was terminated. Similar leachate characteristics were observed in a 5-year-old landfill leachate sample obtained from a field landfill (Weltevreden Park, Johannesburg, South Africa). Qualitatively, therefore our laboratory-scale landfill bioreactor functioned at near optimum conditions to produce leachate of similar appearance to that of the field landfill leachate. Black slime was observed on the upper, inner walls of the anaerobic bioreactor, which may be attributed to layer formation during the leachate recycling procedure.

During the 21-week period the waste height decreased by a total of 90 mm (Table 2.1.2). Initial decreases in the waste height are due to the action of leachate recycling on waste where air spaces are eliminated (Strachan *et al.* 2000). This result confirms the efficiency of leachate recycling in waste height reduction. Furthermore, waste height decreases are reportedly due to anaerobic decomposition of the wastes whereby the weakened waste structure falls back on itself (Wall and Zeiss, 1995). In this study, a reduction in waste height occurred due to leachate recycling which we propose in turn provided adequate moisture for anaerobic bacteria to efficiently carry out the digestion process. Other studies have confirmed the effectiveness of leachate recycling (Lay and Noike, 1998; Strachan *et al.* 2000). Leachate recycling in a closed bioreactor system also serves to re-introduce degrading populations into the anaerobic bioreactor ensuring that there is no

washout of methanogens. A washout of methanogens could lead to a retardation of degradation, as methanogenic populations need to re-establish in order to efficiently perform the degradation reactions (Rohrs *et al.* 1998).

The manometer connected to the anaerobic bioreactor showed that the highest fluid level (2 cm rise) occurred within 2.5 hours. All other gas ports were closed so as to prevent gas leakage. Two hours and forty minutes after the observation was initiated the manometer fluid started to drop. This is thought to be due to gas escaping through the manometer after observations, and air pressure causing the reverse effect. From this data it was therefore decided to leave the anaerobic bioreactor for two hours to allow for gas to accumulate to its highest potential level, for gas chromatography analyses.

Leachate pH was at a constant pH 7 from week 0 to 4. At week 5 it dropped to 6 and finally in week 14 it dropped to its lowest level of pH 5.5 (Figure 2.1.4). The optimum pH for methanogenesis is 7 (Yu and Fang, 2003) and methane formation was seen at pH 7 and 6 in this study. However, at pH 5.5 CH_4 production was retarded with low levels of CH_4 and CO_2 registering on the gas chromatograph in weeks, 13, 14 and 15 of bioreactor operation (Figure 2.1.5). This pH drop to acidic levels is due to the accumulation of volatile fatty acids (butyric, propionic and acetic acids) that are the by-products of acetogenesis (Leuders *et al*. 2004). Although the leachate was diluted weekly with 10 ml of anaerobic distilled water, results suggested that a higher dilution was needed to counteract these pH changes. The waste height did not decrease further during the acidic pH phase and did not re-initiate once the pH rose again to pH 7 in week 21. Gas

chromatography in week 21 showed a slight increase in CH_4 and CO_2 levels, which we attributed to the increased pH levels. Due to the souring of digester conditions, previously inhibited methanogenic populations could not re-establish quickly and so low levels of CH_4 and CO_2 were produced. Waste digestion had therefore been retarded.

Morphological observations of colonies from leachate on TYG medium revealed no differences in colonies between each sampling interval where the same colony types were frequently isolated. This result suggested that weekly sampling intervals were sufficient to trace the bacterial populations within the bioreactor system.

No methanotrophic bacteria were isolated from soil as all strains grew on SINA. It was therefore decided to include a pre-enrichment step in liquid NMS media prior to plating in order to enrich for methanotrophs.

CONCLUSION

Results from this study suggested that it was possible to mimic the anaerobic component of a field landfill using the laboratory-scale landfill bioreactor. In this study, pH maintenance at 7 was critical for maintaining methanogenesis at higher formation rates. As shown, any deviation from a pH of 7 resulted in retarded methanogenesis. In this study leachate recirculation was continuous and this could possibly have led to souring conditions and maintenance of the acidic state in the anaerobic bioreactor. It was therefore decided that leachate should be recycled daily as compared to continuously in

further studies. This is to serve as a ‘rest and rinse’ period, where hydration of the anaerobic bioreactor occurs only once per day by leachate recycling, thereafter followed by a ‘rest’ period, allowing for biochemical reactions to occur. Manometer readings showed that maximum gas yields occurred after 2 hours for gas sampling and chromatography.