Acetate and propionate impact on the methanogenesis of landfill leachate and the reduction of clogging components

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**A B S T R A C T**

Synthetic leachate with different initial concentrations of acetate (500–2500 mg HAc/L) and propionate (500–3500 mg HPr/L) was treated with active biomass acclimated to landfill leachate under anaerobic mesophilic conditions for 72 h. Methanogenesis was observed within all samples during the first 48 h. The greatest removal of acetate (80–100%) and propionate (15–35%) was achieved in tests with initial concentrations ranging from 1500 to 1900 mg HAc/L and from 1000 to 1800 mg HPr/L. Concurrent with the removal of acids, pH increased between 0.3 and 0.45 units, to above the threshold pH for precipitation of CaCO\textsubscript{3}. Therefore, some 50–70% of dissolved Ca\textsuperscript{2+} was removed from solution. This study suggests that by using an equalization tank (prior to the anaerobic digester) to maintain the acetate and propionate concentrations to within their optimum range would help to alleviate clogging through the removal of dissolved Ca\textsuperscript{2+} and maximizing CH\textsubscript{4} production.

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1. Introduction

Leachate recirculation in bioreactor landfills is a landfill management strategy that has been employed to enhance biological degradation of organic refuse and waste settlement, while enhancing methane production, and reducing off-site leachate treatment. Recirculation involves collection of leachate at the base of the landfill and injecting it back into the waste cell, thus enhancing the biological activity of microorganisms by increasing the refuse moisture (Reinhart and Townsend, 1997).

Field studies of leachate collection and injection systems have observed that pipes transmitting leachate can experience significant amounts of clogging over time impairing the hydraulic performance, operation and service life of the bioreactor landfill (Brune et al., 1991; Turk et al., 1997; Fleming et al., 1999; Manning, 2000; Maliva et al., 2000; Yazdani et al., 2002; Bouchez et al., 2003; O’Brien, 2010). Based on the extensive body of research in leachate collection systems (Rowe et al., 2004), clogging is mainly produced by a combined effect of (1) suspended and attached (biofilm) microbial fermentation of volatile fatty acids (VFA), mainly acetate, propionate and butyrate, producing carbonic acid (H\textsubscript{2}CO\textsubscript{3}) and (2) CO\textsubscript{2} degassing from the leachate, both causing changes in pH. The clog material has been reported to be primarily composed of a very hard mineral encrustation (mainly Ca\textsuperscript{2+} carbonate minerals), the accumulation of inert suspended solids (ISS), soil particles, biological material and in situ biofilm development. In addition, the adhesive properties of extrapolymer saccharides (EPS) help retain the suspended particles at the biofilm surface, contributing significantly to the overall mass of the biofilm (Flemming et al., 2000) or clogging.

Given that the design and operation of current bioreactor landfills around the world have no control over leachate composition, uncontrolled biofilm growth is expected to occur. Therefore, limiting the influent acetate, propionate and butyrate concentrations through leachate methanogenesis prior to recirculation may limit biofilm growth and clog formation within the pipelines.

Although anaerobic digestion has been demonstrated to be the most efficient technology for the treatment of high strength organic landfill leachate (Ye et al., 2011; Kheradmand et al., 2010; Montusiewicz and Lebiöcka, 2011; Liu et al., 2011), most of the leachate digestion studies have used total COD and VFA content as the operational parameters to measure the effectiveness of the treatment performance (Timur and Öztüruk, 1999; Taconi et al., 2008; Nayono et al., 2010; Montusiewicz and Lebiöcka, 2011; Liu et al., 2011). In addition, landfill leachates from different landfills around the world and its changes in composition over time have been mainly documented using the same parameters (Kjeldsen et al., 2002; Rowe et al., 2004). There are only a few reports from some landfills in Europe and North America where individual VFA were sampled, as shown in Table 1.
Table 1
Leachate composition of acetate, propionate and butyrate from landfills in Europe and North America.

<table>
<thead>
<tr>
<th>Source</th>
<th>Acetate (mg HAc/L)</th>
<th>Propionate (mg HPr/L)</th>
<th>Butyrate (mg HBu/L)</th>
<th>Landfill</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoeks and Borst (1982)</td>
<td>1230–10,800</td>
<td>470–4390</td>
<td>1210–9810</td>
<td>na</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Cooke et al. (2005)</td>
<td>3533–4010</td>
<td>4125–4475</td>
<td>598–738</td>
<td>Keele Valley</td>
<td>Canada (ON)</td>
</tr>
<tr>
<td>Personal sampling</td>
<td>2000–7500</td>
<td>170–700</td>
<td>600–4100</td>
<td>Summit road</td>
<td>Canada (MB)</td>
</tr>
<tr>
<td>Owen and Manning (1997)</td>
<td>0–960</td>
<td>0–480</td>
<td>0–470</td>
<td>Maw green</td>
<td>England</td>
</tr>
<tr>
<td>Chian (1977)</td>
<td>1748</td>
<td>509</td>
<td>3075</td>
<td>na</td>
<td>US (IL)</td>
</tr>
</tbody>
</table>

Samples collected from the same landfill well during 1993 and 1994.

a: na: not available.
b: Standard deviation values for acetate, propionate and butyrate are 1699–1521, 1652–1497 and 458–558 of leachate collected during the performance of this study.
c: Samples collected from the same landfill well during 2008.
d: Impounded leachate from a semi-capped landfill cell.

2. Methods

The biomass used in this study was collected from the anaerobic sequencing batch reactor (ASBR) used by Lozecznik et al. (2010), where the biomass was acclimated in the ASBR with landfill leachate at a hydraulic retention time (HRT) of 24 h and solid retention time (SRT) of 40 days. The total suspended solids (TSS) concentration in the biomass was approximately 100 g/L at 24% of VSS. The synthetic leachate was prepared following the formula used by VanGulck and Rowe (2004) that emulated the leachate characteristics of the Keele Valley Landfill in Ontario, Canada, collected between June and August 1993. This solution consisted mainly of acetate, propionate and butyrate (0.12 M, 0.067 M and 0.01 M, respectively), various salts and a trace metal solution.

Past studies of clogging in drainage material (VanGulck et al., 2003; Cooke et al., 2005) had tested synthetic and real leachate from Keele Valley Landfill at an approximate molar ratio of 1:1 for acetate and propionate. From Table 1, several molar ratios are reported from different landfills around the world, so testing different acetate to propionate concentration values and molar proportions will represent closely to what occurs in field conditions.

This laboratory study was performed using Balch tubes (Belco Glass Co.) with a working volume of 27 mL, containing 5 mL of media (synthetic leachate) and 5 mL of inoculum (biomass). The final VFA concentration of the medium tested (leachate and biomass) was expected to change as equal volume of synthetic leachate and biomass were digested, and are shown in Table 2. Butyrate was maintained constant at 25 mmol/L. A total of 12 Balch tubes were prepared for each of the leachate combinations, sampling three Balch tubes (triplicate) each at times 0, 24, 48 and 72 h after the anaerobic digestion cycle. Due to the amount of tubes assessed, this study was conducted over two consecutive weeks. During the first week, tests 1, 2 and 3 were completed (higher acetate than propionate concentration values) and during the second week tests 4, 5 and 6 were finished. Test 3 and 4 contained roughly the same acetate and propionate concentration values to compare the reproducibility of the results, as shown in Table 2.

Each tube was tested for pH, acetate, propionate and butyrate concentrations at 0, 24, 48 and 72 h. Biogas content (CO2 and CH4) was tested at 24, 48 and 72 h and was measured from the gas phase. An initial leachate pH of seven was chosen to ensure that

Table 2
Average (µ) and standard deviation (σ) of measured acetate, propionate and butyrate concentrations, pH and approximate acetate to propionate molar ratio of the test media at T = 0 h from tests 1 to 6 after inoculation.

<table>
<thead>
<tr>
<th>Test</th>
<th>Acetate ([mg HAc/L])</th>
<th>Propionate ([mg HPr/L])</th>
<th>Molar Ratio ([HAc/HPr])</th>
<th>Butyrate ([mg HBu/L])</th>
<th>pH (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>µ µ σ</td>
<td>µ µ σ</td>
<td>µ µ σ</td>
<td>µ µ σ</td>
<td>µ µ σ</td>
</tr>
<tr>
<td>1</td>
<td>2389 92</td>
<td>586 13</td>
<td>4.08 0.01</td>
<td>927 21</td>
<td>6.97 0.01</td>
</tr>
<tr>
<td>2</td>
<td>1831 41</td>
<td>1025 25</td>
<td>1.79 0.01</td>
<td>1001 23</td>
<td>7.00 0.01</td>
</tr>
<tr>
<td>3</td>
<td>1527 15</td>
<td>1787 16</td>
<td>0.85 0.01</td>
<td>1086 21</td>
<td>6.97 0.01</td>
</tr>
<tr>
<td>4</td>
<td>1387 359</td>
<td>1423 380</td>
<td>0.97 0.01</td>
<td>864 191</td>
<td>6.99 0.01</td>
</tr>
<tr>
<td>5</td>
<td>1124 32</td>
<td>2168 60</td>
<td>0.52 0.01</td>
<td>1108 47</td>
<td>6.99 0.01</td>
</tr>
<tr>
<td>6</td>
<td>505 40</td>
<td>3313 318</td>
<td>0.15 0.01</td>
<td>738 51</td>
<td>6.97 0.01</td>
</tr>
</tbody>
</table>
no precipitation of calcium occurred and to represent real leachate values observed at landfills. The tubes were air-sealed with butyl-rubber stoppers, crimped with aluminum seals and were gassed and degassed (1:4 min) four times with 100% nitrogen (N2) to maintain anaerobic conditions (Daniels et al., 1986) at 1.5 atm overpressure.

The Balch tubes were placed on an orbital shaker (Scientific Co. Inc.) at a medium speed inside of a temperature controlled chamber at mesophilic temperature (35 °C). The pH and temperature were measured using an ORION 5STAR MULTI WPHH equipped with the appropriate electrical probes and calibrated for changes in temperature. Ca2+ was measured using a HACH universal digital titrator kit with the proper reagents to measure hardness for Ca2+.

VFA concentrations in the samples were analyzed by a Varian CP 3800 gas chromatograph (GC) equipped with a flame ionization detector, CP-8400 autosampler and WCOT fused silica 25 m × 0.32 mm internal diameter (ID) coating FFAP-CB capillary column. The optimized GC operating conditions were: 270 °C in the injector and 300 °C in the detector. Temperature in the oven was initially set at 70 °C and then ramped up to 140 °C at the rate of 10 °C/min, from 140 °C to 200 °C at the rate of 25 °C/min, and then from 200 °C to 240 °C at the rate of 30 °C/min. The column was maintained at 240 °C for 3.97 min to let the residual contaminated flush out. The total running time was 15 min. The gas flow rates were: helium in the column at 6.5 mL/min, hydrogen at 30 mL/min, and air at 300 mL/min. Crotonic acid was added as an internal standard to enhance the analytical reproducibility and quantitative accuracy were obtained for all VFAs. Following Standard Methods (APHA, 21st Edition, 2005), samples were acidified to pH 2 using 85% phosphoric acid and then filtered by 0.22 μm syringe filter prior to injection.

CH4 and CO2 from the Balch tubes were analyzed by a Varian CP 3800 gas chromatographer (GC) equipped with a thermal conductivity detector. CO2 was separated from other components using CP-Porabond Q fused silica column (25 m × 0.53 mm ID) and CH4 was resolved using fused silica 25 m × 0.53 mm ID coating molecular sieve 5A column together with the second CP-Porabond Q fused silica column. The optimized GC operating conditions were 250 °C in the injector and 180 °C in the detector. Temperature in the oven was initially set at 40 °C for 1 min and then ramped up to 100 °C at the rate of 20 °C/min for a total running time of 15 min. The flow rate of carrier gas helium in the column was a constant 3 mL/min. Samples were taken directly from the Balch tubes and injected into the GC, the volume of sample loop was 250 μL. Acetate, propionate and butyrate concentrations were measured in duplicates from each Balch tube. The pH, CO2 and CH4 were measured once from each Balch tube and Ca2+ values were measured from two of the three Balch tubes collected at different times. CO2 and CH4 production was corrected for the amounts dissolved in the liquid phase using Henry's law, and for CO2, the Henderson–Hasselbach equation as well.

3. Results and discussion

3.1. Acetate, propionate and butyrate concentrations over time

Fig. 1 shows the different average concentrations of acetate, propionate and butyrate over time for the different digestion tests. The highest average removal values of acetate after 72 h of digestion were attained in tests 2, 3 and 4, averaging 1466, 1482 and 1698 mg HAc/L (24–29 mmol/L), respectively, indicating nearly complete removal of available acetate (between 80% and 100%). These tests also had the highest removal of butyrate (44% and 55%); in the case of propionate, test 5 shows the highest removal value at 565 mg HPr/L (7.6 mmol/L), and at the same time, the second highest percentage removal of acetate (approximately 88%). Tests 2 and 5 attained similar amounts of acetate removal after 72 h.

Test 1 with the highest concentration of acetate (2400 mg HAc/L – see Table 2), showed 50% of acetate removal after 72 h of digestion (>1000 mg L−1 removed), but no propionate removal was measured. Test 6 with the highest concentration of propionate (3313 mg HPr/L) showed very little removal of butyrate and virtually no propionate removal during the 72 h of digestion. Despite removing 30% of the acetate over the 72 h of digestion, this only represented 10% of the acetate removal observed in tests 2, 3 and 4. Overall, test 6 shows the worst performance for VFA removal. For tests 2, 3, 4 and 5, acetate and propionate removal values were observed after 72 h of digestion. These results suggest that equalization of high initial concentrations of acetate and propionate is required in order to maximize the anaerobic treatment of leachate, favouring the activity of syntrophic bacteria.

Since test 5 showed approximately 20% removal of propionate during the 72 h of digestion, syntrophic communities were also present within the inoculum.

3.2. pH and dissolved Ca2+ concentrations

Given the removal of acetate in all tests, and butyrate and propionate in some of the tests, pH values were expected to increase, as shown in Fig. 2. The largest increase in pH was attained for tests 3 and 4, followed by test 2. This is consistent with the acetate and butyrate removal values observed in Fig. 1. Lozecznik et al. (2010) measured in the headspace as shown in Figs. 2 and 3. The largest increase in pH (Figs. 2 and 3). The removal of Ca2+ will have direct implications on the amount of CaCO3 as inert suspend solids (ISS) within the biomass, may have a negative effect on the activity of the biomass (Yu et al., 2001; Fernandez-Nava et al., 2008) and thereby hinder the fermentation of VFA in the long term.

3.3. CH4 and CO2

As VFA’s were removed, biogas was formed within the headspace of the Balch tubes. Fig. 3 shows the average CO2 and CH4 (mg/L) concentrations measured in the headspace of the Balch tubes after 24, 48 and 72 h of digestion for tests 1–6.

During tests 2, 3 and 4, a rapid increase of the CH4/CO2 ratio was measured in the headspace as shown in Fig. 4. Tests 1 and 5 show a slow CH4/CO2 ratio increase during the first 48 h, while test 6 does not show a CH4/CO2 ratio increase during this study. These differences of CH4/CO2 ratio in the headspace over time are explained as the effect of VFA removal on the pH values, and the increase of CO2 converted into carbonate, eventually causing the removal of dissolved Ca2+ as CaCO3. As pH in the medium and CO2 in the headspace of the Balch tubes increased (see Figs. 2 and 3), a shift in the carbonate equilibrium of the medium was expected, increasing the carbonate availability within the tubes. This was verified by calculating the average dissolved CO2 concentrations, HCO3− and CO32− in the medium using Henry’s law and the Henderson–Hasselbach equation, and is shown in Table 3. These calculations of CO2 concentration in the medium used the measured pH of the medium and CO2 concentration in the headspace at 24, 48 and 72 h of digestion for tests 1–6.
To verify the quality of our gas measurements relative to the substrate concentration, a mass balance was performed for the 72 h digestion period. In order to account for the dissolution of gases (mostly \( \text{CO}_2 \)) into the medium and the carbon loss, \( \text{CO}_2 \), \( \text{HCO}_3^- \), and \( \text{CO}_3^{2-} \) were calculated as explained above and shown in Table 3. Due to the pH values measured within the Balch tubes, it was expected to find most of the C converted into \( \text{HCO}_3^- \), as shown in Table 3. The conversion of dissolved \( \text{CO}_2 \) to \( \text{HCO}_3^- \) was calculated using a pKa of 6.3 and a pressure of 1.5 atm. In addition, the carbon loss due to dissolved \( \text{Ca}^{2+} \) removed as \( \text{CaCO}_3 \) was also determined. Table 4 shows the \( \text{CH}_4/\text{CO}_2 \) ratio calculated from the removal of acetate, propionate and butyrate values removed after 72 h of digestion. Column (b) of Table 4 indicates the \( \text{CH}_4/\text{CO}_2 \) ratio calculated from the values measured within the headspace and calculated from the dissolved phase. Finally, column (c) shows the ratio of carbon formed versus carbon consumed, calculated as \( \text{CH}_4 \) and \( \text{CO}_2 \) presence in the gas and dissolved phases against the carbon removed by the consumption of acetate, propionate and butyrate after 72 h of digestion. From Table 4, it can be observed that tests 1–5 present similar theoretical and measured \( \text{CH}_4/\text{CO}_2 \) ratios, showing a balance of gases formed in both phases, and carbon balance from products (gases) and substrate (VFA). The inhibition of methanogenesis observed in test 6 (high propionate) gave rise to a different \( \text{CH}_4/\text{CO}_2 \) measured than theoretical ratio value expected. The reason of higher carbon formed than consumed may be attributed to the lower substrate consumed and gases produced (\( \text{CH}_4 \) and \( \text{CO}_2 \)) and errors associated with the measurements.

![Fig. 1. Average of (1) acetate, (2) propionate and (3) butyrate in tests (a) 1, 2 and 3 and (b) 4, 5 and 6 with time.](image)

![Fig. 2. Variation of pH in tests 1–6 with time.](image)
From Fig. 3 and Table 3, the following observations can be made.

- The average CH₄ increased (ranging from 70 to 403 mg/L) for all tests, except test 6 between 48 and 72 h of digestion. The highest amount of average CH₄ (134 mg/L/day) was achieved during test 2 after 72 h of digestion, followed by test 1, 3 and 4 (117, 128 and 131 mg/L/day).
- Methanogenesis stopped after 48 h in test 6.
- The CH₄/CO₂ ratio increased in the gas phase over time for all tests. It was highest for test 4 achieving an average value of 1.45 after 72 h of digestion.
- Total dissolved CO₂ (CO₂, HCO₃⁻, CO₃²⁻) increased over time for all tests, except test 6 between 48 and 72 h.

3.4. Mechanisms of CaCO₃ precipitation

To better represent the mechanisms observed within the digestion tests (especially tests 2, 3 and 4) performed in this study, a conceptual framework that identifies key variables affecting the precipitation of CaCO₃(s) during methanogenesis of leachate under sealed conditions is presented in Fig. 5. Fig. 5. depicts a flowchart describing the relationship between VFA removal and the mechanisms of CaCO₃ precipitation observed in tests 2, 3 and 4. When optimum influent acetate and propionate concentrations are metered into the digester, VFA removal is maximized thereby increasing the pH of the medium and producing
CO₂ and CH₄ as biogas most quickly. The increase of CO₂(g) in the headspace increases the partial pressure and concentration of the gas, increasing the concentration of CO₂(aq). This increase in CO₂(aq) destabilizes the carbonate equilibrium of the medium, with more CO₂ converted to bicarbonate. This increase in carbonate is further accentuated by the pH increase, as the pH goes further away from the pKₐ. This increase in bicarbonate and the availability of significant amount of Ca²⁺ within the leachate leads to CaCO₃(aq) which precipitates as CaCO₃(s).

4. Conclusions

This study has shown that methanogenesis of leachate is influenced by the ratio of acetate and propionate. It also shows that methanogenesis of VFA increases the digester pH and the concentration of carbonate in solution, influencing the precipitation of CaCO₃.

It is suggested that if leachate undergoes methanogenesis prior to re-injection into a bioreactor landfill, it may protect the pipes and other engineered landfill systems against CaCO₃ precipitation and its detrimental effects, while allowing for methane recovery from the digester gas phase. However, blending leachates from different cells to maintain the balance between acetate and propionate concentrations is highly recommended.

Table 4

<table>
<thead>
<tr>
<th>Tests</th>
<th>(a) CH₄/CO₂</th>
<th>(b) CH₄/CO₂</th>
<th>(c) Cformed/Cconsumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>1.17</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>1.36</td>
<td>0.8</td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>1.45</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<td>1.01</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>0.39</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Note: Pressure at 1.5 atm.

Fig. 5. A flowchart describing the relationship between VFA methanogenesis and the mechanisms of CaCO₃ precipitation (inorganic clogging) in the Balch tubes as a model for inorganic clogging in landfill pipes.

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References


