Feasibility tests for treating shampoo and hair colorant wastewaters using anaerobic processes
Shaikh Z. Ahammad, A. Yakubu, J. Dolfing, C. Mota and D. W. Graham

ABSTRACT
Wastes from the personal care product (PCP) industry are often high in biodegradable carbon, which makes them amenable to aerobic biological treatment, although process costs are usually high due to aeration inefficiencies, high electricity demand and production of large amounts of sludge. As such, anaerobic treatment technologies are being considered to lower net energy costs by reducing air use and increasing methane production. To assess the amenability of PCP wastes to anaerobic treatment, methane yields and rates were quantified in different anaerobic reactors treating typical PCP wastes, including wastes from shampoo and hair colorant products. Overall, shampoo wastes were more amenable to methanogenesis with almost double the methane yields compared with colour wastes. To assess relevant microbial guilds, qPCR was performed on reactor biomass samples. Methanosetaetaeae abundances were always significantly higher than Methanosarcinaceae and Methanomicrobiales abundances ($P < 0.05$), and did not differ significantly between waste types. Although colour wastes were less amenable to anaerobic treatment than shampoo wastes, differences cannot be explained by relative microbial abundances and probably result from the presence of inhibiting compounds in hair colorants (e.g., oxidants) at higher levels. Results showed that anaerobic technologies have great potential for treating PCP wastes, but additional work is needed to establish the basis of elevated methane yields and inhibition, especially when colorant wastes are present.

Key words | anaerobic waste treatment, industrial wastes, methanogenesis, personal care products

INTRODUCTION
Personal care products (PCPs) are compounds used for the maintenance of hygiene, enhancement of beauty, and general well-being, and include both shampoo and hair colouring agents. Like all industrial products, wastes are generated in PCP manufacturing that have traditionally been treated using physico-chemical and/or aerobic biological treatment technologies (El-Gohary et al. 2010). PCP wastes tend to be very high in carbon, which makes aerobic treatment very effective. However, aerobic treatment is also costly due to the high energy required to provide air to the system as well as sludge management (Kasprzyk-Hordern et al. 2009). With the goal of reducing energy costs, anaerobic wastewater treatment systems are now being considered for PCP wastes, because anaerobic processes have lower operating costs, and due to possible energy recovery in the form of methane gas (Chynoweth et al. 2001). Furthermore, high strength wastewaters are often treated successfully by anaerobic biological systems, although this has not been commonly used in the PCP industry. Finally, anaerobic treatment technologies offer particular potential to developing countries (where many factories are now located) with limited resources because it converts carbon-rich wastes to potential energy, which is often in short supply (Brooks et al. 2008).

This study was undertaken to examine the potential for anaerobic treatment and associated microbial communities in the treatment of different PCP wastewaters. As background, a typical operating practice in the PCP industry is to manufacture different products at the same factory, but in separate dedicated batch reactors. The resulting rinse waters are captured in large central holding tanks. Therefore, resulting wastes are often diluted mixtures of residues that reflect the products made at each factory. In the present study, two major PCP products, shampoo and...
hair colorants, were chosen for examination because they are often produced at the same factories, but also usually have very different chemical constituents which could have varied effects on anaerobic processes. Shampoo wastes often have elevated surfactants content, whereas hair colour wastes include oxidants and ammonia, which can potentially inhibit anaerobic processes. Therefore, the focus of this study was to compare anaerobic methane yields and rates for two mixtures of shampoo and colour wastewaters to determine how the relative proportion of each constituent influences anaerobic biodegradability and methanogenesis. Such studies generate data that are valuable to the design of continuous reactors and subsequent scaling up endeavours.

**MATERIALS AND METHODS**

**Simulated waste composition**

Simulated wastes were prepared by mixing individual shampoos and hair colorants in ratios suggested by the PCP factory (personal communication; H. Bucholtz) as detailed in Tables 1 and 2. The recipe for making the synthetic effluents was obtained from the laboratory of a PCP industry; they determined the composition based on the amount of each waste generated from the different manufacturing units that contributed to the final effluent. These mixtures reflect the composition of the rinse water stream generated in the holding tanks; i.e. the waste water from the production of shampoos and colorants respectively. For the present experiments, waste constituents were mixed in two different proportions; 66.7% shampoo and 33.3% colour (by volume), which was defined as stream 1 (i.e., S1), and 33.3% shampoo and 66.7% colour, defined as stream 2 (S2). Characteristics of waste streams used in the experiment and a representative undiluted actual industrial effluent are listed in Table 3.

**Table 1 | Composition of shampoo effluent**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra douxe (vanilla)</td>
<td>0.177</td>
</tr>
<tr>
<td>Fructis (colour resist)</td>
<td>0.692</td>
</tr>
<tr>
<td>Ultra doux</td>
<td>0.527</td>
</tr>
<tr>
<td>Elseve (liss intense)</td>
<td>0.260</td>
</tr>
<tr>
<td>DOP (500 ml)</td>
<td>0.255</td>
</tr>
<tr>
<td>DOP petit (400 ml)</td>
<td>0.415</td>
</tr>
<tr>
<td>Mennen Sport</td>
<td>0.115</td>
</tr>
</tbody>
</table>

**Table 2 | Composition of hair colour effluent**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Base (g/L)</th>
<th>Colour (g/L)</th>
<th>Oxidant (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% colour</td>
<td>0.055</td>
<td>0.027</td>
<td>0.117</td>
</tr>
<tr>
<td>Belle colour</td>
<td>0.247</td>
<td>0.117</td>
<td>0.545</td>
</tr>
<tr>
<td>Nutrisse</td>
<td>0.302</td>
<td>0.140</td>
<td>0.660</td>
</tr>
<tr>
<td>Movida</td>
<td>0.080</td>
<td>0.035</td>
<td>0.172</td>
</tr>
</tbody>
</table>

**Table 3 | Characteristics of the waste streams**

<table>
<thead>
<tr>
<th>Parameter (mg/L)</th>
<th>S1</th>
<th>S2</th>
<th>Simulateda</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>1,360</td>
<td>1,609</td>
<td>6,500</td>
</tr>
<tr>
<td>TOC</td>
<td>218</td>
<td>235</td>
<td>1,071</td>
</tr>
<tr>
<td>TKN</td>
<td>7.2</td>
<td>10.3</td>
<td>36</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.9</td>
<td>4.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.3</td>
<td>0.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Sulphate</td>
<td>2.2</td>
<td>3.1</td>
<td>57.6</td>
</tr>
</tbody>
</table>

*Simulated example of undiluted effluent at a PCP factory based on recommendations from a L’Oreal factory.

**Experimental setup**

Screw cap air-tight bottles having a volume of 500 ml (Thermo Fisher, Netherlands) were used in all experiments. Typically, each bottle contained 200 ml effluent and 75 ml inoculum (see below for source), and the mixture was adjusted to pH 7, using 1 N HCl or NaOH, as required. The headspace air was removed by sparging with nitrogen gas and the bottles were maintained on an incubator-shaker at 30°C and mixed at 120 rpm.

Microbial inocula were obtained from a pilot anaerobic treatment plant operated by L’Oreal-International (Paris, France). As such, the biological seed used for the tests was acclimatized to PCP factory-related wastes. Nitrogen gas was sparged to remove air from the head space of two seed reactors. Further acclimation was then carried out at 50°C for 7 days in an incubator-shaker maintained at 120 rpm. The contents of two reactors then were combined; the sludge fraction was separated by gravity, the supernatant was discarded, and the resulting sludge was used to seed subsequent reactors in the experiment. The treatability tests were performed using six replicates per waste type (i.e., S1 and S2) over a 50-day period. Similar bottles and conditions were used as above, and headspace methane levels, chemical oxidation demand (COD), solids, pH and volatile fatty acid (VFA) levels were monitored over time.
Methane analysis and anaerobic biodegradability

The experiment was carried out in batch reactors (closed container) and the volume of the biogas produced was the volume of the headspace times the concentration of the biogas in the headspace. Head space methane concentration in each flask was measured using a gas chromatograph (Carlo Erba 5160 Mega GC) equipped with a Flame Ionisation Detector (FID) and HP-PLOT Q capillary column (30 m x 0.32 mm internal diameter) packed with 20 μm Q phase. The oven temperature was maintained at 35 °C and the injector and detector were kept at 300 °C. Hydrogen was used as carrier gas and fuel (1 ml/min, 65 kPa). 100 μl of head space gas sample was injected for each analysis. The actual methane potential of the waste streams was determined by using the biochemical methane potential (BMP) test described by Owens & Chynoweth (1993) and Angelidaki & Sanders (2004). Biochemical methane potential (BMP) was calculated from the amount of methane produced divided by the amount of initial COD of the waste stream introduced. BMP is expressed in ml CH₄/g COD at standard conditions of temperature and pressure (0 °C, 1 atm) (i.e., Biodegradability (%) = 100×BMP/(350 ml CH₄/g COD).

Chemical characterization of effluents and wastes

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to Standard Methods (APHA 1998) 2540E and 2540E, respectively. Chemical Oxygen Demand (COD) was measured using the photometric COD cell test kit (Spectroquant COD Cell Test Kit; Merck, Germany) according to the manufacturer’s instructions. All samples for COD were filtered through a 0.2 μm hydrophilic PES membrane filter (VWR, UK) prior to analysis. Total Carbon (TC) content of the samples was also analysed, using a Shimadzu TOC-5050A analyser where zero grade air was used as carrier gas and 25% phosphoric acid was used as inorganic catalyst solution.

Biochemical and molecular characterization of wastes and microbial communities

Different ancillary biochemical analyses were done to determine the volatile organic acids composition of waste influents and effluents over time. Specifically, total carbohydrates, proteins, lipids and VFAs were analyzed. Lowry’s method (Lowry et al. 1951) and the anthrone method (Dreywood 1946) were used to estimate proteins and carbohydrates, respectively, and their concentrations are expressed in equivalent bovine serum albumin (BSA) and in equivalent glucose. The lipid content was measured by solvent extraction using ethanol and chloroform (Cot et al. 2007).

Filtered (0.2 μm) samples were analysed to determine VFAs and anion concentrations. The analysis was done using an ion chromatograph (Dionex ICS-1000, USA) equipped with a conductivity detector. Ionpac column (ICE-ASI, 4 × 250 mm) and anion column (Ionpac AS14A, 4 × 250 mm analytical) were used for measuring VFAs and anions, respectively. Prior to the VFA analysis, samples were degassed by mixing equivalent of 0.5 M orthophosphoric acid, followed by sonication for 40 min.

DNA was extracted from reactor biosolids for qPCR microbial characterization using the Fast Soil DNA Kit (MP Biomed, USA) according to manufacturer’s instructions. Before qPCR analysis, DNA extracts were diluted (1:20) with molecular-grade water to minimize the presence of possible PCR inhibitors. Reactions were performed on a BioRad iCycler (Hercules, CA) qPCR system. Each 20 μl reaction mixture contains 3 μl of template DNA, 1 μl primers (10 pmoles/μL) and qPCR reagent (Primerdesign, UK). The primers used are listed in Table 4. The PCR programmes were followed as mentioned in the respective literature (Maeda et al. 2003; Yu et al. 2005).

RESULTS AND DISCUSSION

Anaerobic treatment processes are known to effectively treat high strength wastes (Letttinga 1995; McCarty 2001). In the case of the PCP wastes, waste CODs vary dramatically, ranging from as low as 2,000 to over 90,000 mg/L (Oliveira et al. 2009; El-Gohary et al. 2010), depending upon the specific factory and internal waste stream. However, some PCP industry wastes also contain toxic components, such as oxidants, which might inhibit the activity of the methanogens. Therefore, for the current proof-of-concept treatability study, wastes were diluted 5–4 times to reflect approximate waste data from a ‘typical’ factory that produces hair colour products and shampoo, to reduce potential toxicity issues and test their intrinsic capacity to generate methane. The tested wastewater characteristics are summarised in Table 3.
COD removal, CH₄ production and CH₄ yields

Different measures of treatability were assessed, although soluble COD (sCOD) removal levels and methane production rate are usually the best indicators of anaerobic microbial activity. Biochemical methane potential (BMP) is also useful for estimating anaerobic biodegradation capability and intrinsic methanogenesis because it describes the potential of the waste to produce methane per gram of available COD.

Methane production levels over time for the two wastes are shown in Figure 1 (based on six replicates). Results show that methane production was generally higher for S1 (mostly shampoo) than S2 (mostly colour), with actual mean methane production rates of 0.12 and 0.07 ml CH₄/day, respectively. COD of the waste streams in different reactors were measured at the beginning and at the end of the experiment. At the end of the experiment 64 and 45% COD removal were obtained from S1 and S2, respectively. COD removals obtained from different reactors were used to estimate the BMP of different waste streams. The BMP of S1 was 198.9 ± 41.5 ml CH₄/g COD (mean ± 95% confidence interval), whereas the BMP for S2 was only 101.2 ± 11.5 ml CH₄/g COD, which equate to 56.8 ± 11.8 and 28.9 ± 3.3% biodegradability, respectively.

S1 was significantly more amenable to anaerobic degradation than S2, which suggests that hair colour residues negatively influence methanogenesis. Although specific inhibitors were not quantified due to confidentiality issues, oxidants often found in hair colour products (see Table 2), such as peroxides, are legitimate potential inhibitors, especially given that methanogenesis requires highly reduced conditions. To partially support this speculation, other characteristics of the wastes were determined to assess whether significant differences existed on other chemical properties. Specifically, total lipid, carbohydrate and protein levels were quantified in both wastes, and the data are summarised in Table 5. Overall, lipid, carbohydrate and protein levels were higher in S2 than S1, which is consistent with slightly higher initial COD in S2. However, these data also imply that shampoo and colorant wastes are more similar than originally believed, and that the presence of methanogenic inhibitors in S2, such as oxidants, is a plausible explanation for differences in CH₄ production rates, BMP and waste biodegradability between the two wastes tested. The slight but distinct accumulation of VFA in S2 indicates that the observed reduction in CH₄ production was not due to substrate limitation, suggesting that

Table 5 | Biochemical constituents in waste stream

<table>
<thead>
<tr>
<th>Waste stream</th>
<th>Carbohydrate (mg/L glucose equivalent)</th>
<th>Protein (mg/L BSA equivalent)</th>
<th>Lipid (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>10.8 ± 0.3a</td>
<td>21.8 ± 0.5</td>
<td>752.4 ± 2.8</td>
</tr>
<tr>
<td>S2</td>
<td>14.2 ± 0.2</td>
<td>28.1 ± 0.7</td>
<td>769.1 ± 3.6</td>
</tr>
</tbody>
</table>

Note: *95% confidence interval.
the methanogens were indeed inhibited. An alternative explanation is that oxidants inhibited hydrolysis in S2 and that this led to a lower methanogenic activity. Another possible explanation would be that the differences in methane production were due to nutrient limitation mostly in colorant waste.

**VFA formation during degradation tests**

VFAs are intermediate compounds that are formed during anaerobic biological reactions and monitoring of VFA levels over time is often helpful for identifying potentially stressed conditions (e.g., reduced pH), which may lead to reduced methane production rates. Observed mean acetic and propionic acid levels (VFAs) measured over time are presented in Figure 2. Despite the increasing VFA levels measured as the experiment proceeded, VFAs were still relatively low compared with known inhibitory levels (Dogan et al. 2005), suggesting that appreciable VFA accumulation was not occurring in the reactors and it was not likely responsible for the differences in methane production rates.

**Microbial community analysis using qPCR**

To assess the microbial communities involved in the anaerobic degradation of colour and shampoo wastes, qPCR was used to quantify total eubacteria, the Methanosarcinaceae (MSC), Methanosaetaceae (MST) and Methanomicrobiales (MMB) guilds in the reactors. Eubacterial probes were used to estimate the total bacterial population, whereas MSC and MST estimate putative acetoclastic methanogens and MMB estimate hydrogenotrophic methanogens.

Although abundances of the three methanogen guilds differed relative to each other in the presence of both wastes (Figure 3), the abundances were not significantly different between wastes (P > 0.05). However, MST guild abundances were over two orders of magnitude higher than the other two guilds, implying MST is likely most important to methanogenesis on these simulated PCP wastes. MST reflects the acetoclastic methanogenic guild, which processes acetate to methane. Given that the absolute MST levels were similar for both wastes, it is unlikely that the relative MST abundance is a useful measure of biodegradation potential because other factors appear to be more important. Specifically, MST abundances are similar for S1 and S2 wastes, although methanogenic function is lower for S2, implying that organisms are present but less active in a waste with higher colorant content.

**CONCLUSIONS**

This study shows that shampoo waste is more amenable to anaerobic treatment processes than hair colour waste from PCP factories. Although an exact reason has not been identified, it is suspected that the presence of oxidants in colour wastes, possibly peroxides, is responsible for reduced anaerobic degradability. The toxic compounds present in colour waste might have inhibited the activity of hydrolysing microorganisms and/or the methanogens. Differences in methane production obtained from two wastes could be due to nutrient limitation mostly in colorant waste. The MST guild of acetoclastic methanogens was most and equally prevalent in the microbial communities treating both wastes, therefore we suggest reduced activity of the methanogens rather than lower abundances explains differences in methanogenesis rates. Overall, anaerobic
technologies have significant potential for treating PCP wastes, including the possibility of generating energy from methane produced. However, work is needed to enhance methane yields, especially for hair colour wastes, such that processes can be optimised for industrial applications.

ACKNOWLEDGEMENTS

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REFERENCES


El-Gohary, F., Tawfik, A. & Mahmoud, U. 2010 Comparative study between coagulation/precipitation (C/P) versus coagulation/dissolved air flotation (C/DAF) for pre-treatment of personal care products (PCPs) in wastewaters. *Desalination* 252, 106–112.


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