Treatment of wine distillery wastewater by high rate anaerobic digestion

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Abstract Wine distillery wastewaters (WDW) are acidic and have a high content of potential organic pollutants. This causes high chemical oxygen demand (COD) values. Polyphenols constitute a significant portion of this COD, and limit the efficiency of biological treatment of WDWs. WDW starting parameters were as follows: pH 3.83, 4,185 mg/l soluble COD (CODs) and 674.6 mg/l of phenols. During operation, amendments of CaCO3 and K2HPO4, individually or in combination, were required for buffering the digester. Volatile fatty acid concentrations were < 300 mg/l throughout the study, indicating degradation of organic acids present. Mean CODs removal efficiency for the 130 day study was 87%, while the mean polyphenol, removal efficiency was 63%. Addition of 50 mg/l Fe3+ between days 86 and 92 increased the removal efficiencies of CODs to 97% and of polyphenols to 65%. Addition of Co3+ improved removal efficiencies to 97% for CODs and 92% for polyphenols. Optimization of anaerobic treatment was achieved at 30% WDW feed strength. Removal efficiencies of 92% and 84% were recorded at increased feed strength from days 108 to 130. High removal efficiencies of CODs and polyphenols after day 82 were attributed to the addition of macronutrients and micronutrients that caused pH stability and thus stimulated microbial activity.

Keywords Anaerobic digestion; chemical oxygen demand; polyphenols; volatile fatty acids; wine distillery wastewater

Introduction

Wine distilleries produce large volumes of wastewaters known as vinasse or wine distillery wastewaters (WDWs; Keyser et al., 2003). These wastewaters are acidic and have high concentrations of potential organic pollutants. The composition of WDW varies depending on the type of raw material distilled (Borja et al., 1993; Keyser et al., 2003). The concentration of oxidizable organic substances, expressed as chemical oxygen demand (COD), ranges from 20 to 150 g/l (Perez et al., 2004). In addition to oxygen demand pollution, WDWs contain polyphenols. These include gallic acid, p-coumaric acid and gentisic acid (Borja et al., 1993). Polyphenols cause WDWs to impart strong antibacterial effects, and the toxicity and inhibitory properties of these aromatic compounds have been previously shown to affect anaerobic digesters used for biological treatment of WDWs (Goodwin et al., 2001).

In the last few years the search for sustainable treatment systems capable of minimizing energy consumption has encouraged the use of anaerobic biological wastewater treatment systems, even in cases where the main goal is to eliminate the biodegradable and dissolved fraction of carbonaceous substrates (Rajeshwari et al., 2000; van Lier et al., 2001). These anaerobic treatment systems have been used mainly for high strength organic wastewaters such as brewery and distillery wastewaters (Sales et al., 1987). Although anaerobic digestion of this type of wastewater is feasible and appealing from an energy point of view, the presence of polyphenols slows down the treatment process and thus hinders the complete removal of COD. An improvement in digestion efficiency can be brought about by either modifying the digester design or incorporating appropriate advanced
operating techniques (Rajeshwari et al., 2000). The objective of this study was to investigate a high rate anaerobic digester for the removal of COD and polyphenols from WDW.

**Materials and methods**

A 10 l anaerobic digester was set up in a controlled temperature environment of 30°C with mixing to suspend the sludge solids. It was operated at a 48 h hydraulic retention time (HRT) using a batch feeding system; every 48 h the digester would be allowed a one hour settling period (Figure 1), after which the supernatant was withdrawn and replaced with an equal volume of fresh feed. The mean cell residence time (MCRT) was 130 days.

The digester was inoculated with 2.5 l of methanogenic sludge, 0.5 l of WDW and 7.0 l nutrient broth, a general purpose medium used for the cultivation of a wide range of bacteria which are not fastidious in their food requirements (containing 1 g/l meat extract, 2 g/l yeast extract, 5 g/l peptone and 8 g/l AnaLar grade sodium chloride; Merck Chemicals (Pty) Ltd, Johannesburg). The digester feed was a mixture of nutrient broth and WDW, and the concentration of WDW was increased incrementally from 5% (v/v) between days 0 and 52, to 10% (v/v) between days 108 and 112; to 15% (v/v) between days 114 and 120; to 20% between days 122 to 124 and finally to 30% (v/v) between days 126 and 130. CaCO₃ (2,000 mg/l) was added to buffer the pH value of the system from days 62 to 73. From day 73 until day 84, this was replaced with a mixture of CaCO₃ and K₂HPO₄ (1 g/l each, UniLAB grade, Merck). Finally, only K₂HPO₄ (1 g/l) was used to buffer the system between days 84 until 130. Micronutrient amendments were supplied as 50 mg/l of Fe(NO₃)₃ (UniLAB, Merck) added from days 86 to 92; Co(NO₃)₃ (UniLAB, Merck) from days 94 to 100, and Ni(NO₃)₃ (UniLAB, Merck) from days 102 to 106.

Digester performance was monitored by determination of feed and supernatant parameters. The digester agitation was switched off every 48 h and the solids allowed to settle for one hour. The supernatant was then removed from the digester and replaced with equal volume of fresh feed. Samples of the supernatant withdrawn were retained at 4°C for a maximum of 48 hours before analysis.

The feed and supernatant parameters analyzed were pH, soluble COD (CODₗ) and the concentrations of phosphates, nitrates, ammonia, polyphenols and volatile fatty acids (VFAs). The pH values were measured using a Cyberscan 2500 pH meter (Eutech Instruments, Johannesburg, South Africa). Colorimetric reagent test kits (Merck Chemicals (Pty) Ltd, Johannesburg), based on the principles of Standard Methods (1998), were used to measure CODₗ (Spectroquant reagent test 14538/9 analogous to method number 5220-D), phosphates (14543 analogous to 4500-P-E), nitrates (14773, analogous to 4500-NO₃-E) and ammonia (14752, analogous to 4500-NH₃-F). Concentrations of
total VFAs were determined according to a standard titration method (SCA, 1979). Polyphenols were measured using the Folin–Ciocalteu’s spectrophotometric method by Box (1983). Analysis for polyphenols was conducted by aliquoting 100 μl of the sample and mixing it with 1.6 ml of distilled water and 250 μl of Folin–Ciocalteu’s reagent. The mixture was vortexed briefly and 1.5 ml (100 g/l) aqueous solution of Na₂CO₃ (Merck Chemicals (Pty) Ltd, Johannesburg) was added. Samples were made up to a volume of 10 ml with distilled water, vortexed briefly and incubated in the dark at room temperature for 2 hours. After incubation, absorbance at 765 nm was measured using multi-wavelength multi wellplate reader (PowerWave, Bio-Tek Instrument Inc). Calibration curves were measured using phenol (AnaLAR, Merck) as a standard, and polyphenol concentrations were expressed as mg of phenol per litre (mg/l). Mixed liquor suspended solids (MLSS) in the digester were determined by withdrawal of a sample during digester mixing and analysis according to Standard Methods (1998).

Results and discussion

Data for raw and treated WDW are summarized in Table 1. Before anaerobic digestion, the WDW-containing feed had an acidic pH of 3.83 and contained 4,185 mg/l CODs and 674.6 mg/l of phenols. After digestion the pH value increased to 7.05. The supernatant also showed improvement in colour, A₅₀₀ decreased from 1.29 to 0.59 and turbidity from 0.74 to 0.33 FAU. Decreases in concentrations of phenols, and CODs were indicative of the efficiency of the high rate anaerobic digester during treatment of WDW.

The results in Table 1 are indicative of the robustness of the digester during operation. The pH and VFA profiles measured are shown in Figure 2. The pH of the digester supernatant increased from the initial value of 7.3 to 9.2 between days 0 and 8. From day 8 until day 16, a gradual decrease in pH was recorded, with the minimum value of 6.0 recorded on day 16. Subsequently, pH increased again to reach 9.2 on day 20, and then a gradual decrease was recorded to the value of 6.2 on day 42. Then a sharp spike in pH to 12.3 was recorded on day 52, suggesting a complete breakdown of the anaerobic system. The digester had to be revived and pH buffering had to be introduced, which accounts for the approximately constant value of 7.05 recorded between days 54 and 130. The digester was revived by first removing half of the contents and replacing them with fresh methanogenic sludge, feed at 5% (v/v) of WDW strength in nutrient broth and allowing recovery until day 62. The buffering was accomplished using addition of CaCO₃ or the CaCO₃/K₂HPO₄ mixture (see Materials and methods).

The VFA concentration decreased from 1,639.3 mg/l on day 0 to 45.5 mg/l on day 14, indicating breakdown of readily available CODs in the digester. From day 16 until day 52, the VFA concentration increased to 900 mg/l. Peak values of 475 mg/l and 640 mg/l were recorded on days 32 and 38, respectively. We hypothesize that increase of VFA concentration could be thereafter attributed to digestion of complex polyphenols thus releasing organic acids, and this hypothesis is currently being tested. After revival of the system and implementation of pH buffering, VFA concentration reached a maximum equal to 550 mg/l on day 76 and 340 mg/l on day 116. The residual concentration of VFA

<table>
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<th>Parameter</th>
<th>Influent (± standard deviation)</th>
<th>Effluent (± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.83 ± 0.006</td>
<td>7.05 ± 0.010</td>
</tr>
<tr>
<td>Colour (A₅₀₀)</td>
<td>1.29 ± 0.020</td>
<td>0.59 ± 0.026</td>
</tr>
<tr>
<td>Turbidity (FAU)</td>
<td>0.74 ± 0.030</td>
<td>0.33 ± 0.030</td>
</tr>
<tr>
<td>Phenol (mg/l)</td>
<td>674.60 ± 23.34</td>
<td>9.25 ± 0.640</td>
</tr>
<tr>
<td>COD₅₀₀ (mg/l)</td>
<td>4,185 ± 28</td>
<td>55 ± 9</td>
</tr>
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was 200 mg/l on day 130. Recovery of pH from acidic range coupled with decrease in VFA concentration from days 16 to 50 further confirmed use of WDW as COD. After digester revival (between days 54 and 62), CaCO₃ was added to buffer the digester, followed by a combination of CaCO₃ and K₂HPO₄, and K₂HPO₄ alone. Buffer capacity in the digester was improved when K₂HPO₄ was added alone, compared to the use of a combination of CaCO₃ and K₂HPO₄ or CaCO₃ alone. VFA concentrations fluctuated less after the induction of pH buffering (Figure 2). Addition of K₂HPO₄ alone also led to pH stability and robustness of the digester and VFAs were digested even at increased organic loading rates.

Removal efficiencies of CODₐ and polyphenols as a function of the time of incubation are shown in Figure 3. For CODₐ, the values fluctuated between days 0 and 20, with the
average value equal to 66% and the minimum equal to 40% recorded on day 20. Fluctuations continued until day 52, with two distinct minima at 52% at day 38 and 40% recorded at day 52. The introduction of the K₂HPO₄ buffer helped to stabilize the removal efficiency for CODs and polyphenols, even at increased organic loading rates. The overall CODs and polyphenol removal efficiencies for the 130 day study were 87% and 63%, respectively. Addition of 50 mg/l Fe³⁺ as Fe(NO₃)₃ between days 86 and 92 increased the removal efficiencies of CODs to 97% and of polyphenols to 65%. Addition of 50 mg/l Co³⁺ as Co(NO₃)₃ decreased CODs removal efficiency from 97 to 92% between days 94 and 100; while polyphenol removal efficiency increased from 65 to 93%. Addition of 50 mg/l Ni³⁺ as Ni(NO₃)₃ decreased CODs and polyphenol removal efficiencies to 74% and 70%, respectively. Similar results have been reported before (Sharma and Singh, 2001), and might indicate the possible physiological significance of ferric ions to sludge under the conditions prevailing in the digester. The CODs removal efficiency for the study period from days 108 to 130 improved from 74 to 92% and from 70 to 84% for polyphenols, despite increased organic loading rates (see Materials and Methods). The improvement of CODs and polyphenol removal efficiencies further confirmed the robustness of the digester.

These results further confirmed methanogenic activity during treatment of WDW. Removal efficiencies of CODs obtained in this study were higher than those obtained by Wolmarans and de Villiers (2002) who used an upflow activated sludge bed or blanket (UASB) reactor to treat distillery wastewater. Addition of macro and micronutrients caused pH stability and thus stimulated microbial activity. In the digester organic loading rates had to be gradually increased from 5 to 30% WDW feed strength as shock loading rates employed at the beginning led to dramatic decrease in pH and thus a decrease in methanogenic activity.

Nitrate (N–NO₃⁻) and ammonium (N–NH₄⁺) concentrations measured in the supernatant during WDW anaerobic digestion are shown in Figure 4. Nitrate concentration reached peak values of 37.3 mg/l on day 2, 40.8 mg/l on day 8, 67.0 mg/l on day 20, 62.1 mg/l on day 52, and 56.8 mg/l on day 74. When the organic loading rate was increased the volumetric fraction of WDW reached 30% of WDW in the feed, nitrate
concentration dropped below 10 mg/l. Data in Figure 4 indicates that when nitrate concentration peaked ammonium concentration was at its lowest and vice versa, i.e., when nitrates were being used up, ammonium was being formed or released into the medium. Mutual relationship of the nitrate and ammonium concentrations in an anaerobic digester originates from the possible occurrence of the following processes: nitrification/denitrification, dissimilatory nitrate reduction (anaerobic respiration with nitrate as the terminal electron acceptor), the reduction of \( \text{N} - \text{NO}_3^- \) to \( \text{NH}_4^+ \) coupled with uptake into activated sludge cells and its utilization in protein synthesis and other physiological processes, followed by the release of \( \text{NH}_4^+ \) after cell lysis (Ruiz et al., 2006).

Although a detailed study of the significance of individual processes was not conducted for this experimental system, the observed temporal trends in nitrate and ammonium concentrations allowed the following to be postulated: nitrates were reduced into ammonium cations by denitrifying bacteria, or bacteria utilizing nitrate as the terminal acceptor of electrons during anaerobic respiration. Ammonium cations produced were successively assimilated by the other microbial cells present in the activated sludge, and used to meet the needs of protein synthesis and other physiological processes. As the particular cells died off, the proteins were degraded, and the ammonium was released into the supernatant of the experimental system. Here it could be transformed into nitrate, or the nitrates were supplied by the replenished feed after supernatant removal for analyzes. A mass balance of nitrogen over the experimental system was not conducted, so other processes mentioned could have occurred simultaneously, leading to the loss of nitrogen from the experimental system via the evolution of \( \text{N}_2 \) or \( \text{NO}_x \) gases.

Micronutrients were added as their respective nitrates (see Materials and Methods); the addition of \( \text{Fe(NO}_3)_3 \) between days 86 and 92 did not lead to any significant changes in the concentration of ammonium or nitrates, which ranged from 2.5 to 3.0 mg/l for ammonium and 1.1 to 3.4 mg/l for nitrates. On the other hand, additions of \( \text{Co(NO}_3)_3 \) and \( \text{Ni(NO}_3)_3 \) led to a significant decrease in ammonium concentrations to values below the limits of detection for the analytical method used, and an increase in the concentration of nitrates, with peak values of 11.4 mg/l on day 94 and 9.9 mg/l on day 104. This might point to the possible toxicity of \( \text{Ni}^{3+} \) to the microorganisms responsible for the denitrification or dissimilatory nitrate reduction to ammonium. A lot has been speculated and contradictory data have been published in the potential and proven toxicity of the ammonia/ammonium acid-base equilibrium towards anaerobic wastewater treatment systems (Calli et al., 2005). After introduction of pH buffering and with the addition of iron as a micronutrient, the observed ammonium concentrations were not toxic to the sludge present in the experimental digester. This might be an indication of the suitability of the studied experimental system for the reduction of nitrogen loading of the studied WDW.

Figure 5 shows the supernatant phosphate (\( \text{P} - \text{PO}_4^{3-} \)) and the MLSS concentrations measured during anaerobic treatment of WDW. From days 0 to 26 the concentration fluctuated between 113 and 96 mg/l. There was a major decrease in phosphate concentration from days 28 to 50 which led to the digester failure on day 52, when the pH increased rapidly to 12.34, solubilizing the contents of the digester.

When phosphate concentration was measured during this period, a drastic decrease in phosphate concentration was observed up to day 72 even when \( \text{CaCO}_3 \) (2,000 mg/l) was added as a buffer. Addition of a combination of \( \text{K}_2\text{HPO}_4 \) and \( \text{CaCO}_3 \) (1,000 mg/l each) from days 74 to 84 stabilized phosphate concentration at approximately 60 mg/l. As from days 86 to 130 only \( \text{K}_2\text{HPO}_4 \) (1 g/l) was used to buffer resulting in increased phosphate concentration even at increased organic loading rates. The digester MLSS concentration fluctuated during the course of the anaerobic digestion with peak values of 9 g/l on day 10, 13 g/l on day 20, 15.4 g/l from days 40 until day 42, and continued to increase after
introduction of pH buffering to a final value of 13.44 g/l. This seemed to be sufficient biomass to degrade polyphenols and COD₃ in the WDW.

Conclusions
Average COD removal for the 130 day study was 87%. Average polyphenol removal efficiency for the 130 day study was 63%. Optimization of anaerobic treatment for WDW was achieved at 30% wastewater strength. Additions of CaCO₃ (2,000 mg/l), CaCO₃ and K₂HPO₄ (in combination with a concentration of 1,000 mg/l each), and K₂HPO₄ (1,000 mg/l) were essential for buffering the bioreactor and addition of 50 mg/l Fe³⁺ increased COD₃ removal efficiency further to 95%.

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