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Full Length Research Paper

Anaerobic digestion of fungally pre-treated wine distillery wastewater

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The combination of fungal pre-treatment with *Trametes pubescens* and anaerobic digestion were tested for the removal of chemical oxygen demand (COD) and phenolic compounds from wine distillery wastewater. The COD removal efficiency after fungal pre-treatment reached 53.3%. During digestion, pH buffering was achieved using $CaCO_3$ and K_2HPO_4. This provided a stable environment inside digester for efficient and time-independent COD removal. The total COD removal efficiency reached 99.5%, and the system proved able to eliminate shock COD loads, as indicated by the concentrations of sludge and volatile fatty acids. Complex changes of phenolic compounds are suspected in anaerobic digestion system, and are investigated further.

Key words: Chemical oxygen demand (COD), fungi, polyphenol, stillate and vinasse.

INTRODUCTION

Wine distilleries produce limited volumes of high-strength wastewater during the wine season (Nogales et al., 2005). The composition of wine distillery wastewaters (WDWs) is highly variable, depending on the raw material distilled and the production parameters (Benitez et al., 1999). Raw materials processed can include wine, lees, and pressed grapes. Wine distillery wastewaters contain high concentrations of nutrients, such as different classes of organic compounds, nitrates and phosphates (Bustamante et al., 2005). As a result, wastewater discharge, irrigation or reuse cannot be undertaken without prior treatment (DWAF, 1996). Most distilleries practise biological treatment of their wastewaters using either anaerobic or aerobic conditions, both of which lead to reduction in biological oxygen demand (BOD) and chemical oxygen demand (COD) (Pandey et al., 2003). Yeoh (1997) reported WDW COD concentrations of >100 g/l.

The antibacterial activity of WDW has resulted in investigation of several treatment methods, for example, chemical oxidation, anaerobic digestion, use of activated sludge systems, dilution of WDW before treatment and treatment with fungi (Lacina et al., 2003). Aerobic processes have been known for a long time to be effective in removing toxic and antibacterial organic compounds in wastewaters (Lacina et al., 2003). However, they are often connected with high operating costs, and also generate large quantities of waste sludge which must be disposed of (Benitez et al., 1999). Fungi have been used effectively as a pre-treatment for anaerobic digestion of other materials with high phenolic content, such as molasses and olive mill wastewater (Lacina et al., 2003). The phenolic compounds in such materials exert antimicrobial activity inside biological wastewater treatment systems, inhibiting the effectiveness of the treatment (Lacina et al., 2003). In such cases, fungal pre-treatment under aerobic conditions makes it possible to obtain 51 - 100% phenol removal; good decolourisation (31 - 100%); <85.4% BOD reductions, and production of enzymes capable of degrading xenobiotics e.g., laccase (EC 1.10.3.2; Lacina et al., 2003).

The search for sustainable treatment systems capable of minimizing energy consumption has encouraged the use of anaerobic biological systems, even in cases where the main goal is to eliminate the biodegradable and dissolved fraction of carbonaceous substrates (Rajeshwari et al., 2000). This is due to the possibility of using the biogas produced for meeting the energy demands of the process and/or recovering part of the

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operating costs (Vlissidis and Zouboulis, 1993). These anaerobic treatment systems have been used mainly for high strength organic wastewaters such as 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 process and thus hinders complete removal of COD (Vlissidis and Zouboulis, 1993). An improvement in digestion efficiency can be brought about by either modifying the digester design or incorporating appropriate advanced operating techniques or a pretreatment step (Rajeshwari et al., 2000). In this study, a high rate anaerobic digester was investigated for removal of phenolic compounds from a wastewater obtained from Olafbergh Distilleries Inc. (Worcester, South Africa) that was fungally pre-treated in the laboratory.

The anaerobic digester was inoculated with 2.5 l of methanogenic sludge, 0.5 l of fungally pre-treated WDW and 7.0 l nutrient broth (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 hydraulic retention time (HRT) was 48 h, while the sludge retention time (SRT) was 100 d. The digester contents were allowed to settle for one hour every 48 h to enable supernatant removal from the digester and replacment with an equal volume of fresh feed. Digester performance was monitored by determination of the following feed and supernatant parameters: pH, soluble COD (COD_s), total concentration of phenolic compounds and volatile fatty acids (VFAs), turbidity and colour.

RESULTS AND DISCUSSION

The influence of aerobic pre-treatment on the parameters of WDW can be seen from the data in Table 1. The total concentration of phenolic compounds in the untreated WDW was 522.9 mg/l, while the COD_s value was 15000 mg/l. The total concentration of phenolics dropped to 144.0 mg/l after the fungal pre-treatment, and the COD_s concentration after pre-treatment was 7000 mg/l. Nollet and Verstraete (2004) reported that during anaerobic digestion reductive acetogens and methanogens compete for H_2 as a substrate. The domination of methanogens, i.e. higher productivity of methane than acetate indicates stability of the anaerobic digester (Vlissidis and Zouboulis, 1993). It was reported that methanogens had the competitive advantage over acetogens for H_2 as a substrate in the pH range from 7.0 to 7.5, while acetogens dominated at pH levels around 6.5 (Nollet and Verstraete, 2004). As a result, pH of the fungally pre-treated WDW (FTWDW) had to be increased by additions of $CaCO_3$ and K_2HPO_4 to ensure system stability by stimulating methanogenic activity, and at the same time encourage higher removal of organic components from FT WDW. Colour and turbidity of WDW increased after fungal pre-treatment.

The pH values and the VFA concentration profile with time are shown in Figure 1. During the 100 day study period, pH values fluctuated between 6.98 and 8.63, with peak values above 8 reached on days 16, 18, 38, 58, 84, 92, 96, and 98. The pH was very stable and mostly in the methanogenic range, especially between days 18 and 36. Additions of $CaCO_3$ (Merck Chemicals) were investigated for pH buffering by adding at 2 g/l from day 0 to day 4. Due to the lack of sufficient buffering, a combination of $CaCO_3$ and K_2HPO_4 was introduced into the system from day 6 of digester operation. The concentration of $CaCO_3$ was kept at 2 g/l from day 6 until day 14, and decreased to 0.5 g/l after this point until the end of the experiment on day 100. The concentration of K_2HPO_4 in the feed was kept at 1 g/l from day 6 until day 8, and changed to 0.5 g/l from day 10 until the end of the experiment on day 100. At day 0 the VFA concentration was 614 mg/l, it increased to 729 mg/l on day 2. A gradual decrease in VFA concentrations was recorded until day 10 of digester operation, with the minimum value reaching 72.9 mg/l on day 10. This coincides with a decrease in COD_s values (Figure 2). This indicated degradation of higher molecular weight compounds into new VFA molecules and their subsequent removal. As from day 6 VFA concentration was always below 300 mg/l.

Based on previously obtained data, micronutrient amendments were supplied as 50 mg/l of $Fe(NO_3)_3$ added from days 18 to 24; $Co(NO_3)_3$ from days 26 to 32, and $Ni(NO_3)_3$ from days 34 to 38 (Merck Chemicals). During the period of addition of micronutrients the concentration of VFAs was below 100 mg/l. There was VFA accumulation between days 56 and 62, with a maximum of 911 mg/l being recorded on day 62, at pH value of 7.04. As the pH on that day was within the methanogenic range, this high concentration was probably not indicative of the digester failure. Volatile fatty acid accumulation during this period was due to increased organic rate from 5% (v/v) to 30% (v/v). When comparing data in Figures 1 and 2, it can be seen that the COD_s value on day 62 was

Table 1. Characteristics of untreated (UT) and fungally pre-treated (FT) WDW.

Parameter	UT WDW	FT WDW*
pH	3.83	6.7
Colour (A_{500})	1.29	4.76
Turbidity (FAU)	0.74	1.29
Phenols (mg/l)	522.9	144.0
COD_s (g/l)	15	7
Total N (mg/l)	4.2	3.3
Total P (mg/l)	40.0	10.2
NH_4 (mg/l)	0.24	0.24
NO_3 (mg/l)	124.8	98.8
PO_4^{3-}(mg/l)	163.6	18.8

*Fungal pre-treatment of WDW was conducted using *Trametes pubescens* (Melamane et al., 2006) followed by digestion in a 10 l high rate anaerobic digester.

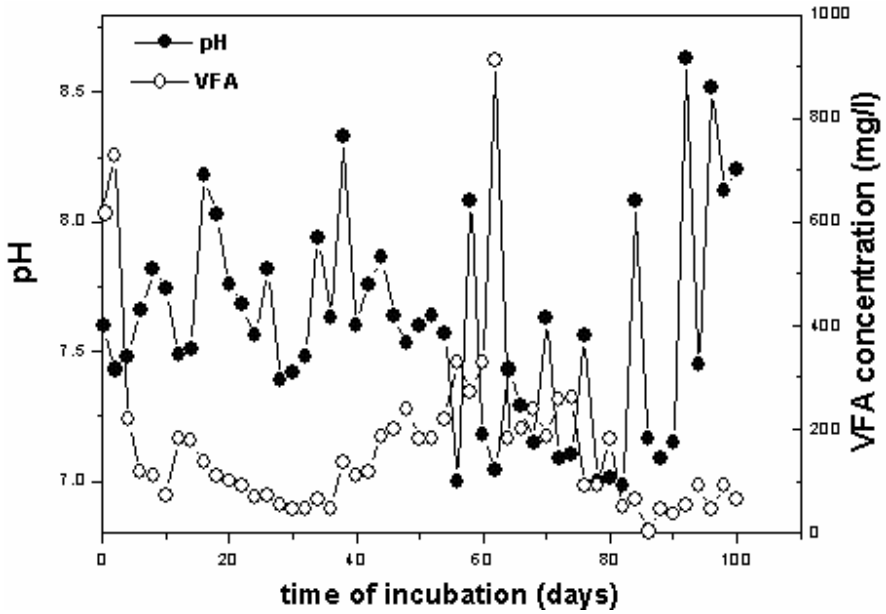

Figure 1. Digester pH and VFA concentration as a function of time during anaerobic digestion. The digester feed was a mixture of nutrient broth and fungally pre-treated WDW, and the WDW concentration of the feed concentration was increased incrementally from 5 % (v/v) between day 0 and day 36, to 10% (v/v) from day 38 to day 44 to 15 % (v/v) from day 46 to day 50; to 20 % and finally to 30% (v/v) from day 52 to day 100. The pH values were measured using a Cyberscan 2500 pH meter (Eutech Instruments). The concentration of VFAs was determined by titration (SCA, 1979).

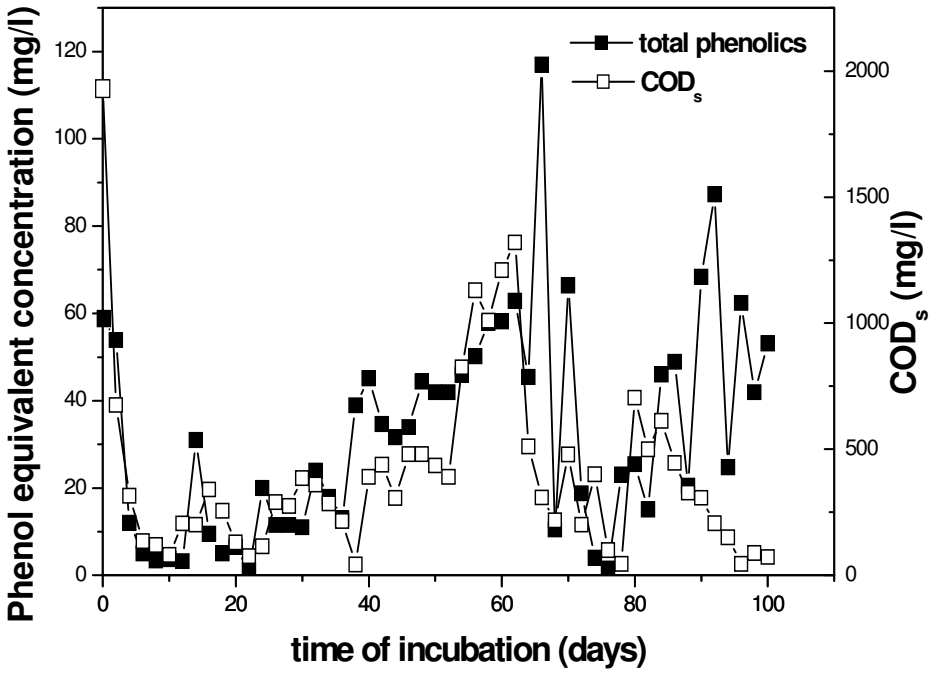

Figure 2. The total concentration of phenolics and COD_s as a function of time during anaerobic digestion. The total concentration of phenolic compounds was measured using Folin-Ciocalteu's method with phenol as the standard, expressed in mg phenol equivalent/l (Khan, 2005). Concentrations of COD_s, phosphates, nitrates and ammonia were measured according to *Standard Methods* (APHA et al., 1998). Colour and turbidity were measured using a Specroquant Nova 60 (Merck Chemicals Pty Ltd, Johannesburg).

1320 mg/l, and thus coincided with the maximum in VFA concentrations. This indicates that more refractory organic matter was loaded into the system during feed refills of this period and the digester needed time to degrade the respective organic components. As can be seen from Figure 1, VFA concentration dropped to 182.2 mg/l on day 64, suggesting robustness of the digester. Soluble COD values and the total concentration of polyphenols are summarised in Figure 2. Soluble COD dropped from the initial value of 1935 mg/l to 80 mg/l on day 10. The values increased to 340 mg/l on day 16, and subsequently fluctuated between 42 and 1320 mg/l until the end of the experiment, with peak values of 385 mg/l on day 30, 825 mg/l on day 54, 1320 mg/l on day 62 and 704 mg/l on day 80. The final COD_s value was 72 mg/l. The fungal pre-treatment led to 53.3% removal of the initial COD_s, while the total COD_s removal was 99.5%. These values are comparable those observed by others (Lacina et al., 2003). From day 0 until day 8, the total concentration of phenolics decreased from the initial value of 58.9 mg/l to 3.4 mg/l. For the remainder of the experiment, the concentrations fluctuated between 3.7 and 117.0 mg/l. The observed trend might be explained by the changes in the molecular weight of the individual phenolic compounds (Melamane et al., 2006). A detailed study of this trend is currently underway. The MLSS values fluctuated during the experiment, however, they never decreased below the initial value of 13.64 g/l. The final MLSS concentration was equal to 21.80 g/l (data not shown). The ability of the system to handle shocks of high organic loadings, the high removal efficiency of CODs, and the MLSS data indicate suitability of fungal pre-treatment followed by high rate anaerobic digestion for the treatment of WDW.

Conclusion

Fungal pre-treatment of WDW led to a significant reduction in COD_s and polyphenols in the studied WDW. The COD_s removal efficiency after fungal pre-treatment reached 53.3 %. The pH of the fungally pre-treated wastewater reached 6.7, reducing the pH buffering requirements for anaerobic digestion. The latter was conducted under pH buffering using a mixture of $CaCO_3$ and K_2HPO_4, which provided stable environment inside the bioreactor system for efficient COD_s. The total COD_s removal efficiency reached 99.5%, and the system proved able to eliminate shock loads of high input COD_s concentrations.

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