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Phenolic removal processes in biological sand filters, sand columns and microcosms

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HIGHLIGHTS

- At low influent concentrations, phenolics in winery wastewater and model synthetic wastewater were completely removed by a combination of biotic and abiotic influences in biological sand filters.
- High influent concentrations of model phenolics resulted in accumulation of metabolites in biological sand filters. Increased hydraulic conductivity strongly suggested a concomitant loss of biomass/biofilm due to accumulation of toxic concentrations of catechol.
- Acclimation of microbial populations to vanillin and gallic acid resulted in enhanced biodegradation of these phenolics when compared to a non-acclimated population.

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ABSTRACT

This study evaluated the removal processes involved in the removal of the phenolic component of winery wastewater in biological sand filters, sand columns and sand microcosms. It was found that at low influent phenolic concentrations, complete organic removal was accomplished, but at high concentrations, there was incomplete substrate removal and an accumulation of potentially toxic metabolites, including catechol. The sand provided a suitable substrate for the treatment of phenolic-laden waste, and both biotic (48%) and abiotic (52%) removal mechanisms effected the removal of model phenolics. Prior acclimation of microbial communities increased the biodegradation rate of phenolic acids significantly.

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1. Introduction

The winemaking process generates copious amounts of cellar effluent: it has been estimated that South Africa produces one billion liters and Australia 5–9 billion liters of winery wastewater per annum (Mosse et al., 2011; Sheridan et al., 2011). Winery effluent requires treatment before discharge, but remediation is complicated by the fact that the composition and volume fluctuates on a seasonal basis, depending on cellar activities (Arienzo et al., 2009; Malandra et al., 2003; Mosse et al., 2011). Typical chemical oxygen demand (COD) values of 800 to 12 800 mg/L are found during the vinification period, resulting from the presence of high concentrations of organic molecules with variable degradation rates (Malandra et al., 2003). Simple sugars, organic acids and alcohols commonly found in winery wastewater are readily biodegradable, while the phenolic component is characteristically slowly biodegradable (Serrano et al., 2010).

Plant phenolics may be toxic to microbes. It has been demonstrated that tannins, which are abundant in red wines, can inhibit microbial activity by precipitation of key metabolic proteins (Arienzo et al., 2009). The levels of phenolic compounds in winery wastewater, particularly in the effluent emanating from the production of red wine, are likely to inhibit microbial activity in soils, affecting soil and plant health (Mosse et al., 2011). It has been shown that the phenolic component of winery wastewater can adversely affect the growth of a variety of aquatic and non-aquatic plants, including cash crops (Arienzo et al., 2009).

Small to medium-sized wineries in rural areas are often not connected to municipal reticulation systems for the treatment of winery effluent and cannot afford to operate sophisticated

Abbreviations: BSF, biological sand filter; COD, chemical oxygen demand; COD_t, theoretical COD; COD_m, measured COD; CW, constructed wetland; GAE, gallic acid equivalents; $biotic_i$, indicating biotic removal; $abiotic_i$, indicating abiotic removal.

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biological treatment systems (Christen et al., 2010). In these wineries, constructed wetlands (CWs) and biological sand filters (BSFs), such as the FILTER system are ideal systems for the treatment and re-use of wastewater as they have low energy and maintenance requirements, are tolerant of seasonal input fluxes and do not require lengthy start-up and shut down periods (Christen et al., 2010).

In the environment, the fate of phenolics is influenced by both biotic and abiotic factors; it is important to understand these processes in order to apply appropriate design principles to BSFs and CWs used to treat phenolic-laden waste. Unlike biological wastewater treatment systems that incorporate sludge wasting, these systems can become saturated with recalcitrant organic chemicals with time. Hence, sufficient biodegradation and mineralization of a critical proportion of phenolics must occur to prevent accumulation and leaching of potentially harmful chemicals from BSFs and CWs treating cellar effluent.

There are a number of previous reports describing the overall removal of total phenolics from agri-industrial wastewaters including winery wastewater, olive mill wastewater and coffee processing wastewater in BSFs and CWs. This study was designed not only to quantify the overall removal of common winery phenolics from cellar effluent in BSFs, but also to gain insight into the biotic and abiotic removal processes taking place during the remediation of the phenolic component of winery effluent. BSFs were used is an alternative to CWs because of the potentially phytotoxic nature of winery effluent.

2. Methods

2.1. Influent and effluent analyses

2.1.1. Chemical oxygen demand

Chemical oxygen demand (COD) was quantified using a Hanna® instruments (Smithfield, USA) C214 multiparameter bench photometer, C9800 digester and low range (1–150 mg/L: HI93754A-25) and medium range (0–1 500 mg/L: HI93754B-25) COD kits, following the manufacturer's instructions.

2.1.2. Phenolics, sugars, acids and alcohols

Total phenolics were determined using the Folin–Ciocalteau (FC) micro method for total phenolics in wine, based on the method reported by Slinkard and Singleton (1977), using Folin–Ciocalteau reagent (Merck®, Whitehouse Station, USA, Cat No: 1.09001.0500). Gallic acid monohydrate (Sigma–Aldrich®, St. Louis, USA Cat No: 27645) standards were prepared in-house and results were expressed as mg/L in gallic acid equivalents (GAE) determined from a standard graph.

Individual phenolics, sugars, acids and alcohols in the effluent were identified and quantified using reverse phase HPLC: samples were separated using a Merck® Hitachi Lachrom instrument equipped with an L-7400 UV detector. For the detection of phenolics, a Waters® (Milford, USA) Spherisorb® S50DSI analytical cartridge was used with deionised water, methanol and glacial acetic acid (Merck® uniVAR, Cat No: SAAR1021020LC) (80:20:2.5) as the mobile phase. The wavelength, flow rate and time were set at 280 nm, 0.5 mL/min and 60 min, respectively. Acids and alcohols were analyzed by HPLC using a Phenomenex® Rezex RHM-monosaccharide H+(8% cross-linkage) column according to the method described by La'Zaro et al. (1989), with a L-7400 ultraviolet detector (210 nm) and an Agilent® refractive index detector being used for the detection of acids and alcohols, respectively. Where possible, organic molecules were identified by spiking experiments and quantified using relevant standard graphs prepared from HPLC chromatograms.

The theoretical COD (COD_t) values of gallic acid, vanillin (Sigma–Aldrich®, Cat No: V1104), vanillic acid (Sigma–Aldrich®, Cat No: V-2250); catechol (Sigma–Aldrich®, Cat No: C9510); and acetic acid were calculated using Eq. (1). The relationship between COD and COD_t was established by COD measurement of triplicate phenolic solutions to give a figure, termed "measured COD" (COD_m), which was used in subsequent mass balance calculations:

$$COD_t = 8(4x + y - 2z)/(12x + y + 16z)\text{mgCOD/mgC}_x\text{H}_y\text{O}_z \quad (1)$$

Gallic acid: COD_t 1.12 mgCOD/mg and COD_m 1.02 ± 0.01 mgCOD/mg;

Vanillin: COD_t 1.79 mgCOD/mg and COD_m 1.81 ± 0.005 mgCOD/mg;

Vanillic acid: COD_t 1.52 mgCOD/mg and COD_m 1.51 ± 0.014 mgCOD/mg;

Catechol: COD_t 1.89 mgCOD/mg and COD_m1.92 ± 0.007 mgCOD/mg;

Acetic acid: COD_t 1.07 mgCOD/mg and COD_m 1.07 ± 0.003 mgCOD/mg.

2.2. Experimental set-up, design and procedure

The relationship between the three experimental phases (BSFs, columns and microcosms) is shown graphically in Fig. 1.

2.2.1. Biological sand filters

Four identical, unplanted, experimental BSFs, each consisting of river sand to a volume of ~0.5 m^3, void space of 0.08 m^3 and a depth of 0.3 m were inoculated in a ratio of 1:4 with sediment from a local wetland treating winery wastewater. The final BSF sediment consisted of 1% clay, 7% silt, 4% fine sand, 12% medium sand and 76% coarse sand. The elemental composition of the sediment per kilogram sand was as follows: 6 mg P/kg; 1.9 g C/kg; 0.07 cmol(+) Na/kg; 0.05 cmol(+) K/kg; 1.64 cmol(+) Ca/kg; 0.21 cmol(+) Mg/kg; 0.61 mg Cu/kg; 1.0 mg Zn/kg; 1.9 mg Mn/kg; 0.10 mg B/kg; 63.03 mg Fe/kg; 7.42 mg S/kg. The pH of the sediment was 7.7.

All BSFs were maintained in an outdoor, undercover environment in order to avoid exposure to precipitation events. The systems were operated in a hybrid mode of vertical and horizontal subsurface flow i.e. effluent was sprayed uniformly at a rate of 0.68 L/min over the inlet zone and allowed to gravitate longitudinally and vertically towards the outlet. Bi-weekly inundation, followed by gradient-directed drainage ensured that the mode of operation was biased towards classical vertical subsurface flow.

Two replicates (A and C) served as control BSFs and two replicates (B and D), served as test BSFs. All four BSFs received a bi-weekly basal influent solution consisting of 0.3 g yeast extract (Biolab®, Midrand, RSA Cat No: HG000BX6.500) and 0.3 g D (+) glucose (Merck® chemically pure Cat No: SAAR2676020EM) dissolved in 12.5 L tap water, for the duration of the equilibration and experimental periods, the former continuing for a minimum of 16 weeks (Ramond et al., 2012). During the experimental period, BSF B was amended with winery wastewater diluted in a ratio of 1:5 (2.5 L in 12.5 L) for a period 17 weeks, while BSF D was amended with increasing concentrations of gallic acid and vanillin for 9 weeks (Table 1).

The hydraulic conductivity (HC) was determined by measuring the volume of effluent collected between 1–2 h after the start of amendment and results were expressed as L/h $m^3 sand^{-1}$. It had previously been established that outflow was consistent during this period (data not shown).

2.2.2. Sand columns

Without physically disrupting the sediment structure, six Perspex samplers (250 mm in length and 35 mm in diameter) were used to extract core samples (sediment mass 579 ± 11 g) from an

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