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Biodegradation of organics and accumulation of metabolites in experimental biological sand filters used for the treatment of synthetic winery wastewater: A mesocosm study

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ABSTRACT

Winery effluent is characterized by the presence of high concentrations of organic molecules. Biological sand filters are ideal systems for the bioremediation of this waste at small wineries. In this study, synthetic winery wastewater, consisting of phenolics and high concentrations of ethanol and acetate was treated in experimental biological sand filters operated in batch mode. The organic substrates and metabolites in effluent and pore water samples, taken from four niches (superficial and deep; inlet and outlet), were identified and quantified. The highest COD concentrations were measured at the deep inlet and the lowest at the deep outlet, reflecting the establishment of degradation gradients from inlet to outlet due to plug flow. Ethanol was the preferred substrate in all niches but contrary to expectations, the biodegradation of ethanol and phenolics took place preferentially under lower redox conditions, in the deep niches. There was an accumulation of acetate and propionate, with propionate being found in notably higher ratios in the deep niches. The acidic influent (pH 3.5) was neutralized.

1. Introduction

According to the International Organisation of Vine and Wine (www.oiv.int), the global production of wine in 2013 was 265×10^9 L. It is estimated that between 1 and 4 L of wastewater is generated for every litre of wine produced [4], making the industry a significant contributor to the global environmental wastewater burden. Winery effluent is of variable nature, both in terms of quantity and quality, and treatment presents a unique set of challenges in wine producing areas across the globe [1,18,20]. The effluent lends itself to biological remediation methodologies as it is characterized by a high chemical oxygen demand (COD) associated with a largely biodegradable organic fraction [4,20]. Once remediated, the wastewater is generally suitable for irrigation or other re-use purposes, provided *in situ* or *ex situ* measures are taken to reduce the inorganic content (K^+ and/or Na^+) [20]. Irrigation of salt-accumulating cash crops provides an ideal example of how this can be achieved using the principle of beneficiation [2,3].

The organic make-up of winery wastewater is variable and seasonal. For example, ethanol and simple sugars are typically present in wastewater generated from the washing of crushing equipment and post-fermentation equipment, respectively [4,5,18]. In addition, the sugars, organic acids, and alcohols are considered readily biodegradable, while the phenolic component is typically more recalcitrant [8,18,30]. For successful bioremediation of the organic fraction, the prevailing environmental conditions should support the growth and appropriate metabolic activities of the functional microbial consortia [13,29]. Conventional reactor-type biological treatment systems generally fit these criteria. However, they are expensive to install, are energy intensive and require skilled operation, making them particularly unsuitable for smaller cellars [7].

Constructed wetlands are sustainable wastewater treatment alternatives, and there are reports from wine producing countries around the world on the use of these systems for the reduction of COD in winery wastewater [11,16,21]. While it has been demonstrated that slow filtration of winery wastewater through soil and sand can effectively treat winery wastewater, the effluent has also been shown to be phytotoxic to the plants used in constructed wetlands [1,7]. Biological sand filters (BSFs) do not contain plants, are potentially affordable to install and operate, are tolerant of seasonal input fluxes and experimental systems have demonstrated excellent potential for treatment and re-use of wastewater at small to medium sized cellars. In BSFs, the sand substratum provides

Abbreviations: BSF, biological sand filter; COD, chemical oxygen demand; GAE, gallic acid equivalents.

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Table 1
Physicochemical properties of the sand substrate used in the study.

Mechanical fraction (%) ($n = 3$)				
Clay	Silt	Fine sand	Medium sand	Coarse sand
3.9 ± 1.1	1.7 ± 0.6	24.9 ± 3.9	41.8 ± 1.0	27.7 ± 2.1

Available metal(loids) (mg/kg) ($n = 3$)							
P	K	Cu	Zn	Mn	B	Fe	As
4.6 ± 1.5	7.3 ± 3.1	0.6 ± 0	0.4 ± 0.2	0.5 ± 0.2	0.1 ± 0	12.7 ± 5.1	0.3 ± 0.2

[*] Exchangeable cations and cation exchange capacity (CEC) [cmol(+)/kg] ($n = 3$)				
CEC	Na	K	Ca	Mg
1.65 ± 0.13	0.10 ± 0.01	0.02 ± 0.01	12.23 ± 0.13	0.17 ± 0.02

Elemental analysis (%) ($n = 3$)								
SiO_2	CaO	AlO_3	Fe_2O_3	K_2O	MgO	Na_2O	P_2O_5	TiO_2
84.59 ± 0.50	7.66 ± 0.03	0.31 ± 0.02	0.07 ± 0	0.15 ± 0	0.15 ± 0	0.21 ± 0	0.03 ± 0.03	0.04 ± 0.01

[*] <0.1 mg/kg Pb, Hg, Cd, Sb.

a large surface area for biofilm attachment and may contain co-factors required for microbial metabolism [6,12,37]. Although it has been argued that the longevity of soil/sand based systems such as constructed wetlands (and by inference, BSFs), is decreased by clogging with solids, it has also been shown that effective pre-treatment to reduce/remove suspended solids can prevent clogging [9,11,14,30]. Many smaller wineries already store effluent in ponds or settling tanks to remove suspended solids prior to disposal via irrigation.

The fundamentals of start-up procedures, overall COD removal and the effect of winery wastewater on the bacterial community structure in experimental BSFs have previously been elucidated and published; these studies have shown that significant remediation of whole winery wastewater, as well as selected common readily biodegradable and slowly biodegradable fractions of winery wastewater (~15,000 mg L^{-1} ethanol, ~3500 mg L^{-1} phenolics) can be achieved in these systems [25–27,35,36]. However, the biodegradability of winery wastewater can be significantly influenced by the chemical make-up and pre-treatment [18,31]. For example, if winery effluent is stored, acetic acid bacteria may excrete acetate if they lack the appropriate catabolic enzymes, resulting in the accumulation of acetate [15,35].

To date, the *in situ* use of BSFs at wine cellars, which is the ultimate goal of this cumulative work, has not been described. To ensure the successful operational design of full-scale BSF systems, an understanding of the rates of biodegradation, biotransformation and mineralization of the various fractions of winery effluent is required.

To achieve these aims, it is important to determine spatio-temporal and physicochemical associations with long term biodegradative performance and the degradation kinetics of different fractions of winery effluent. Building on the knowledge from previous work, this study serves to introduce and discuss these complex interactions. To this end, results of amendment of BSF replicates with different concentrations of complex synthetic winery wastewater with a similar composition to that found in settled pond effluent, are described.

2. Materials and methods

2.1. Biological sand filters (BSFs)

Three BSFs, consisting of locally available polyethylene (PE) tanks (173 cm in length, 106 cm in width) containing river sand to a depth of 30 cm, were used in this study. The experimental systems were kept in an undercover environment to mimic field temperatures, but to avoid complicating experimental interpretation associated with dilution of the wastewater by precipitation. The PE tanks are reasonably priced and easily transportable and it is intended to pilot the use of these tanks (containing sand) in parallel and series in an integrated wastewater treatment system *in situ* at a wine cellar later in 2014. Findings from the experimental systems can thus be extrapolated and results compared to the experimental systems. One BSF was designated as a control (BSF A) and two as experimental replicates (BSF B, BSF C). Reproducibility experiments were previously conducted using up to 4 replicates; in these experiments, similar inter-replicate trends in the microbial community structure and function were found, justifying the use of two replicates, especially for long-term, large mesocosm experiments with considerable space, personnel and cost requirements [23,37].

The sand is readily available from a large, local quarry site in Phillipi, Cape Town. The physicochemical parameters of this sand are given in Table 1.

2.1.1. Set-up, medium and mode of operation

The hydraulic conductivity of the sand used in the BSFs is 0.28 mm/s, which allows rapid drainage of the systems (unpublished results). The systems were operated in batch mode with the outlets being plugged prior to feeding/amendment. This prevented the bulk flow of wastewater from one area of the systems to another once plugged and filled with wastewater. Consequently, the batch mode of operation was suited to the calculation of degradation kinetics, determined from analysis of pore water samples taken at different time periods after amendment (Section 2.3.5).

The volume of wastewater/nutrient solution used for feeding/amendment (40 L) was the volume required to completely saturate the systems. Basal nutrient solution and/or synthetic wastewater were drip-fed onto the surface of the BSF at the inlet using a peristaltic pump and a perforated PE irrigation pipe at the rate of 0.67 L/min (Section 2.1.2). The drainage ports were located at the bottom of the tanks on the opposite (longitudinal) ends to the inlets. The wastewater thus permeated longitudinally and vertically towards the outlet during filling, and again during emptying (after unplugging).

In order to establish (i) the effect of hydraulic retention time on system performance, (ii) long-term performance, and (iii) the degradation kinetics, four different, concurrent strategies were used: the systems were plugged (i) for a period of 24 h, three times weekly, for the first four weeks, (ii) for a period of 48 h, twice weekly from week 5 to 19, (iii) for a protracted period (226 h) at week 20/21,