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Analysis of substrate degradation, metabolite formation and microbial community responses in sand bioreactors treating winery wastewater: A comparative study

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ABSTRACT

There is a global need for the implementation of more cost-effective green technologies for the treatment of effluent from wineries. However, systems reliant on microbial biodegradation may be adversely affected by the highly seasonal character of cellar waste. In this study, the biodegradation of two different formulations of winery effluent in sand bioreactors was compared. The degradation of organic substrates and formation of metabolites was monitored by physicochemical analyses of pore water and final effluent samples. Changes in the bacterial community structures were detected using molecular fingerprinting. In wastewater with an overall COD of 2027 mg/L, a formulation with a high concentration of acetate (800 mg COD/L) was more recalcitrant to degradation than a formulation with a high concentration of glucose (800 mg COD/L). Ethanol, glucose and phenolics were degraded preferentially in the deeper layers of the sand bioreactors (average Eh 25 mV) than in the superficial layers (average Eh 102 mV). The redox status also played a pivotal role on the bacterial community composition. The study yielded valuable insight that can be utilized in the design (configuration and operation) of full scale sand bioreactors.

1. Introduction

The wine industry is a significant contributor to the global environmental wastewater burden, generating between 1 and 4 L of effluent for each litre of wine produced (Bolzonella and Rosso, 2013). The bulk of winery wastewater emanates from cleaning equipment, vats and floors of wine cellars during seasonal activities associated with winemaking (Bories and Sire, 2010; Vlyssides et al., 2005). Winery effluent is typically characterized by a high chemical oxygen demand (COD) and low pH. However, the volume, COD range, and organic composition is directly related to cellar activities, including must production, fermentation processes, maturation/stabilization processes, and decanting, and is thus prone to seasonal variation (Bolzonella and Rosso, 2013; Vlyssides et al., 2005). Each grape varietal also has a unique organic fingerprint, so that, for example, wastewater generated during crushing of late harvest grape varietals has a comparatively high sugar and low phenolic content, while the converse is true for wastewater generated during the crushing of early harvest red grape varietals (Bolzonella and Rosso, 2013; Devesa-Rey et al., 2011; Malandra et al., 2003).

In many countries, effluent COD is used as a benchmark by regulatory authorities when determining wastewater discharge requirements for biodegradable industrial wastewater, and in most instances, no further characterization of the organic fraction is required (Andreoletta et al., 2009; Aybar et al., 2007; Mosse et al., 2011). However, in terms of biodegradability, there is considerable variation in the ratios of the different fractions of winery effluent, including readily biodegradable sugars, moderately biodegradable alcohols and slowly biodegradable/recalcitrant phenolics (Devesa-Rey et al., 2011; Malandra et al., 2003; Vlyssides et al., 2005).

There is a gap in the knowledge quest for a holistic understanding of biological organic winery wastewater treatment processes, particularly concerning the degradation and formation of different substrates and metabolites, respectively. There are scant

Abbreviations: SB, sand bioreactor; COD, chemical oxygen demand; GAE, gallic acid equivalents; SW, synthetic winery wastewater; VFA, volatile fatty acid.

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literature descriptions which detail the chemical composition of winery wastewater during or after treatment. Ganesh et al. (2010), distinguished between volatile fatty acid (VFA) and non-VFA COD in conventional activated sludge and membrane bioreactor systems treating winery effluent, and De la Varga et al. (2013), measured methane and carbon dioxide emissions in subsurface flow constructed wetlands. Other studies have limited the analyses to COD and/or total phenolic removal when assessing the performance of biological systems used for the treatment of winery wastewater.

Sand bioreactors (SBs) have been shown to be effective for the treatment of winery wastewater (Ramond et al., 2013; Welz et al., 2012). For comparative purposes, these systems may be seen as biological sand filters or (unplanted) constructed wetlands. The simplicity of SBs makes them particularly suited to small wineries that are not connected to municipal reticulation systems or do not have access to the expertise and/or funding required to operate sophisticated systems. In this study, SBs were amended with two different formulations of synthetic winery wastewater and results compared. The primary aim of the study was to determine how differences in wastewater composition would affect the metabolic processes of the functional microbial consortia. This was achieved by (i) chemically characterizing pore water samples taken at different spatial points in the systems, and (ii) determining the similarities in the microbial community structures in the same spatial niches before and after amendment with two different formulations of synthetic winery wastewater.

2. Materials and methods

2.1. Sand bioreactors (SBs)

In this study, three SBs, consisting of polyethylene structures (1.73 m in length, 1.06 m in width) containing river sand to a depth of 0.3 m, with a total volume of 0.502 m^3 and porosity of 292 ± 20 L/m^3 ($n = 6$), were used. Two of the SBs were designated as experimental replicates (SB1, SB2) and one was designated as a control (SBC) (Fig. 1). The hydraulic properties and local availability were the most important factors taken into consideration when choosing the sand substrate. The sand is readily available in the environs of the Cape Town, Stellenbosch and Paarl winemaking areas of South Africa and exhibits good hydraulic drainage properties with a hydraulic conductivity (k) of 2.8 × 10^{-4} m/s. The average saturated permeation rate of effluent flow from the three systems before feeding/amendment was 93 ± 29 L/m^3 sand day^{-1} ($n = 12$). All SBs were pre-inoculated with the same volume of sand taken from systems previously amended with winery wastewater and synthetic winery wastewater.

2.1.1. Set-up, medium and mode of operation

All systems were operated in batch mode with alternating periods of plugging, filling, unplugging (after 48 h), draining and resting. The systems were fed/amended twice weekly with basal nutrient solution or basal nutrient solution plus synthetic winery wastewater (Table 1). For approximately 2 h after unplugging, effluent outflow was rapid, after which it slowed to a drip. The influent was pumped via drip irrigation onto the inlet surface of the systems at a maximum pumping rate of 0.67 L/min. The outlets were located on the opposite (longitudinal) side to the inlet, and at the bottom of the polyethylene containers. The flow of wastewater thus took place both longitudinally and vertically towards the outlet.

2.1.2. Feeding and amendment procedures

In order to compare the bacterial communities, each SB was initially allowed to equilibrate for 14 weeks before amendment. During the equilibration period, all systems were fed with low

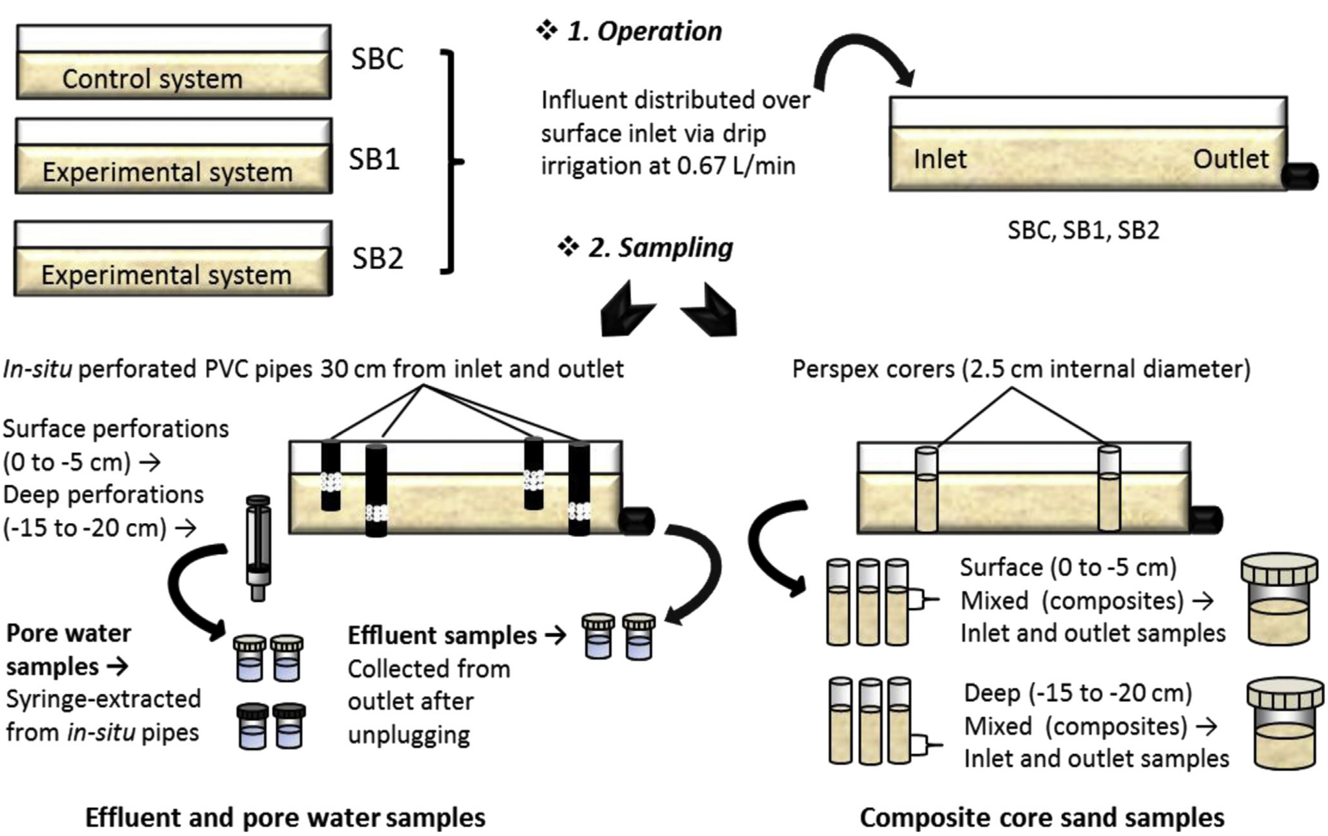

Fig. 1. Schematic diagram showing set-up, operation and sampling procedures used during the study.