

Characterisation of winery wastewater from continuous flow settling basins and waste stabilisation ponds over the course of 1 year: implications for biological wastewater treatment and land application

P. J. Welz, G. Holtman, R. Haldenwang and M. le Roes-Hill

ABSTRACT

Wineries generate 0.2 to 4 L of wastewater per litre of wine produced. Many cellars make use of irrigation as a means of disposal, either directly or after storage. In order to consider the potential downstream impacts of storage/no storage, this study critically compared the seasonal organic and inorganic composition of fresh winery effluent with effluent that had been stored in waste stabilisation ponds. Ethanol and short chain volatile fatty acids were the main contributors to chemical oxygen demand (COD), with average concentrations of 2,086 and 882 mgCOD/L, respectively. Total phenolics were typically present in concentrations <100 mg/L. The concentration of sodium from cleaning agents was higher in the non-crush season, while the converse was true for organics. The effluent was nitrogen-deficient for biological treatment, with COD:N ratios of 0.09 to 1.2. There was an accumulation of propionic and butyric acid during storage. The composition of the pond effluent was more stable in character, and it is possible that bacterial and algal nitrogen fixation in such systems may enhance biological wastewater treatment by natural nitrogen supplementation. It is therefore recommended that if land requirements can be met, winery effluent should be stored in ponds prior to treatment.

Key words | characterisation, organics, seasonal variation, waste stabilisation pond, winery wastewater

INTRODUCTION

Studies have found that for each litre of wine that is produced, between 0.2 and 4 L of wastewater is generated (Vlyssides *et al.* 2005; Bolzonella & Rosso 2013). Most of this effluent originates from a variety of seasonal cleaning activities associated with winemaking, and typically has a high chemical oxygen demand (COD) concentration and low pH (Vlyssides *et al.* 2005; Bories & Sire 2010). The volume and chemical composition (inorganic and organic) of the wastewater are highly variable and depend on the grape varietal, the influent wash-water chemistry, the cellar activities from which it is generated, and the cleaning/sanitising products employed (Vlyssides *et al.* 2005;

Bolzonella & Rosso 2013). From an inorganic perspective, sodium-based cleaning and sanitising products are still used in most wineries (Bories & Sire 2010; Mosse *et al.* 2013). Caustic soda (NaOH), in particular, is very effective and affordable, a fact which is retarding the move to more environmentally friendly alternatives. From an organic perspective, cellar activities (e.g. grape crushing, fermentation, maturation/stabilisation, decanting and bottling) largely determine the effluent character (Bories & Sire 2010). For instance, the concentration of ethanol is high when vats are emptied and washed out, but low when crushing equipment is cleaned. The converse applies for sugars.

The grape varietal used to make the wine may also have a major impact on the organic character, especially during the crush season. For example, when comparing the crushing of late harvest white grapes with early harvest red varieties, fresh wastewater is characterised by high

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sugar/low total phenolic, or low sugar/high total phenolic concentrations, respectively (Malandra *et al.* 2003; Devesa-Rey *et al.* 2011; Bolzonella & Rosso 2013).

Most winery wastewater is discharged into municipal reticulation systems or irrigated onto agricultural or other nearby land. Chemical and/or biological treatment to enhance the 'quality' of the water may precede disposal (Aybar *et al.* 2007; Andreoletta *et al.* 2009; Mosse *et al.* 2011a). Ideally wineries should make it their responsibility to understand any adverse environmental impacts that may arise from discharging liquid and solid waste products, and institute measures to mitigate these.

Whether stemming from the need to comply with legislative requirements and/or an environmentally aware ethos of individual winemakers, most cellars test their effluent for selected parameters. The frequency of sampling and range of parameters differs from winery to winery and country to country, and results are not widely published and interpreted, or accessible. To assist wineries and wastewater treatment engineers/consultants to design and/or implement effective treatment systems, and government agencies to benchmark legislative discharge requirements, there is a need for more comprehensive studies on the characterisation and critical evaluation of different components of winery effluent. This study focused on the characterisation and comparison of winery effluent from: (i) waste stabilisation ponds (WSPs) at two large wineries that crush >10,000 tons of grapes/annum, (ii) 'fresh' wastewater from a small winery generating approximately 800 m³ wastewater per annum, and (iii) 'fresh' wastewater from a medium-sized winery generating approximately 5,000 m³ of wastewater per annum. Samples were taken monthly for a period of 1 year, with weekly samples being taken over the high crush season.

MATERIALS AND METHODS

Sample sites and collection

Four wineries in the Western Cape Province of South Africa were identified. In all instances, the effluent was disposed of via irrigation on adjacent grasslands/pastureland dedicated to this purpose. This is historically the primary means of effluent disposal at small to medium-sized wineries in South Africa. In order to compare the character of freshly produced effluent with stored effluent, samples were taken from two concrete settling basins ((SBs) Wineries A and B), where effluent was rapidly pre-settled and pH adjusted,

and from two WSPs into which effluent was pumped and stored prior to irrigation (Wineries C and D). All the study sites were located within a 50 km radius of one another. The wineries all crush grapes, and produce and bottle wines on the premises, which is typical of many wineries in South Africa. The ponds only serve as repositories for wastewater storage prior to irrigation, not as treatment systems, and are the only two such systems in the area. Likewise, the SBs only serve as rudimentary systems for settling larger particles that may otherwise block the irrigation infrastructure. The dimensions (length × width × depth) are as follows: Winery A (11 m × 5 m × 1 m), Winery B (8 m × 5 m × 1 m), Winery C (100 m × 40 m × 3 m), Winery D (50 m × 16 m × 3.5 m). The water column height, especially in the WSP at Winery C, varies throughout the year, depending on inflow and abstraction for irrigation, which is not routinely monitored. The retention times in all systems are highly variable, and it is assumed that they are lowest during the crushing and bottling seasons. Samples were taken each month for a period of 1 year, and each week during the 'high' crush season. Three samples were taken from each site at each sampling instance. For the ponds, three sites spread around the perimeter were identified and the same sites were used each month. For the SBs, samples were taken close to the inlet, at the middle of the basins, and close to the outlet. All samples were taken on the same day each month.

For total dissolved solids and total suspended solids, grab samples were taken in 1 L glass screw-capped Schott® bottles. For the remaining analyses, grab samples were taken in sterile 50 mL Falcon tubes. All samples were transported immediately to the laboratory for analysis.

Characterisation of effluent

pH

The pH was determined on the day of sample collection using a pH700 meter and probe (Eutech Instruments, Singapore), according to the manufacturer's instructions.

COD, total nitrogen, total phosphorus, nitrate, nitrite and ammonia

The COD, total nitrogen (TN), total phosphorus (TP), nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonia (NH₃) were determined on unfiltered wastewater samples using a Merck Spectroquant® Pharo instrument (Merck, Darmstadt, Germany), together with Merck Spectroquant® cell tests or reagents

according to the manufacturer's instructions: COD (cell test: cat no. 1.14540 and 1.14541), TN (cell test: cat no. 1.14537), TP (cell test: cat no. 1.14543), NO_3^- (reagent kit: cat no. 1.09713), NO_2^- (cell test: 1.14547) and NH_3 (reagent kit: 1.00683). The COD was determined on the day of sampling. Due to time constraints, other parameters were determined the following day. All samples were kept at 4 °C until analysis.

Total phenolics

The concentration of total phenolics was determined on unfiltered wastewater samples using the Folin-Ciocalteu micro method for total phenolics in wine, based on the method reported by Slinkard & Singleton (1977), using Folin-Ciocalteu reagent (Merck).

Sodium and potassium

Concentrations of sodium and potassium were determined on unfiltered wastewater samples using a Varian[®] MPX ICP-OES spectrophotometer (Agilent Technologies, Santa Clara, USA) at Bemlab (Pty) Ltd (Strand, South Africa).

Volatile fatty acids (VFAs)

Two different methods were used to determine the concentrations of VFAs in the samples. Firstly, a spectrophotometric method was used to determine the overall VFA concentration in unfiltered samples, and secondly, high-performance liquid chromatography (HPLC) was used to quantify the concentrations of acetic, propionic and butyric acids in filtered samples. The unfiltered samples included possible contributions from organic solids, while the filtered samples did not. The total VFA concentration was determined using a Merck Spectroquant[®] Pharo spectrophotometer and the Hach (Loveland, USA) esterification method 8196, according to the manufacturer's instructions. Concentrations were determined using a graph prepared using standard solutions of acetic acid, and concentrations were given in mg acetate/L.

Sugars, ethanol, glycerol, VFAs and organic acids

Glucose, fructose, maltose, ethanol, lactic acid, tartaric acid, malic acid, succinic acid, acetic acid, propionic acid, butyric acid and glycerol were identified and quantified using reverse phase HPLC with filtered wastewater samples: samples were separated using a Phenomenex (Torrance, USA) Rezex RHM-monosaccharide H^+ (8% cross-linkage) column and an

Agilent Technologies 1100 series (Santa Clara, USA) equipped with a diode array ultraviolet detector set at 210 nm, and an Agilent Technologies 1200 series refractive index detector. The mobile phase consisted of 1 mM H_2SO_4 solution at pH 2.5 and the sample acquisition time and flow rate were set at 60 min and 0.550 mL/min, respectively.

The identities of the organic molecules were confirmed by spiking experiments. Standard graphs were prepared by plotting the absorbance against the concentrations of relevant standard solutions prepared from analytical grade chemicals obtained from Sigma-Aldrich (St Louis, USA). Concentrations were determined from the standard graphs using instrument software (EZChrom, Agilent Technologies). To determine the relative contributions of individual organics to the overall COD, all concentrations were converted into COD terms using the method described by Welz *et al.* (2011).

Statistical analyses

All statistical analyses were performed using Microsoft Excel 2010. Significance of temporal differences was determined using two-factor analysis of variance without replication. Correlations were determined using a two-tailed t-test (two samples of unequal variance, 95% confidence).

RESULTS AND DISCUSSION

Sampling bias

Due to low hydraulic retention times (HRTs), samples taken from the SBs during high flow periods were seen as snapshots of the total wastewater generated each month, and it was recognised that this commonly employed sampling strategy does not account for temporal variations. Conversely, it was assumed that the samples taken from the WSPs were a better reflection of the monthly average.

COD

COD concentrations in SBs and WSPs

In a recent review, Iannou *et al.* (2015) reported the average influent concentration of 40 systems being used to treat winery wastewater to be 11,886 mgCOD/L (range: 320 to 49,105 mgCOD/L). In this study, the lowest and highest COD concentrations were determined in samples taken from the SBs at the small winery (Winery A) and the

medium-sized winery (Winery B), respectively (Winery A: average 905 mgCOD/L, range 28 to 7,265 mgCOD/L; Winery B: average 10,906 mgCOD/L; range 675 to 76,900 mgCOD/L) (Figure 1(a) and 1(b)). The average COD concentration in the effluent at Winery B was similar to the literature average reported by *Iaonnou et al. (2015)*, while the concentration at Winery A was considered low. There was one high outlier in the results from Winery B (January 2015). During this month, in order to de-sludge the basin before the crush season, no fresh wastewater was added at the inlet. Evaporation of the liquid fraction resulted in the concentration of solids leading to anomalously high results for most parameters at Winery B during January, particularly in the sample taken at the inlet. However, even with removal of the outlier, there was an average ten-fold difference in COD concentration between the two SBs (Wineries A and B), confirming the common perception that cellar practices can significantly improve the quality of wastewater produced by individual wineries.

The concentration of organics and inorganics in winery effluent is a direct reflection of cellar practices (*Sheridan et al. 2011*). A number of best practices to reduce the volume and/or 'strength' of wastewater are applicable to the wine industry. These range from simple procedures

such as mopping up spills before washing down with water, the installation of self-closing nozzles on hoses, installation of effective devices for trapping solids, and creating staff awareness about the need to conserve water, to more expensive options such as the installation of cleaning-in-place equipment. In some instances attempts to reduce water consumption lead to an increase in the concentration of the inorganic and/or organic fraction of the wastewater, unless this is addressed simultaneously.

In contrast to the results from the SBs, samples taken from the WSPs exhibited similar COD concentration averages (Winery C: 5,143 mgCOD/L, range 470 to 8,840 mg/L; Winery D: 5,534 mgCOD/L; range 700 to 13,730 mgCOD/L) (Figure 1(c) and 1(d)), which were lower than the literature average reported by *Iaonnou et al. (2015)*.

Monthly variation in COD concentrations

In the case of the SBs, there were random monthly variations in COD concentrations (Figure 1). In contrast, similar monthly trends were exhibited by the samples from the WSPs. The highest concentrations in the WSP samples occurred during the crush season in 2015, and were notably

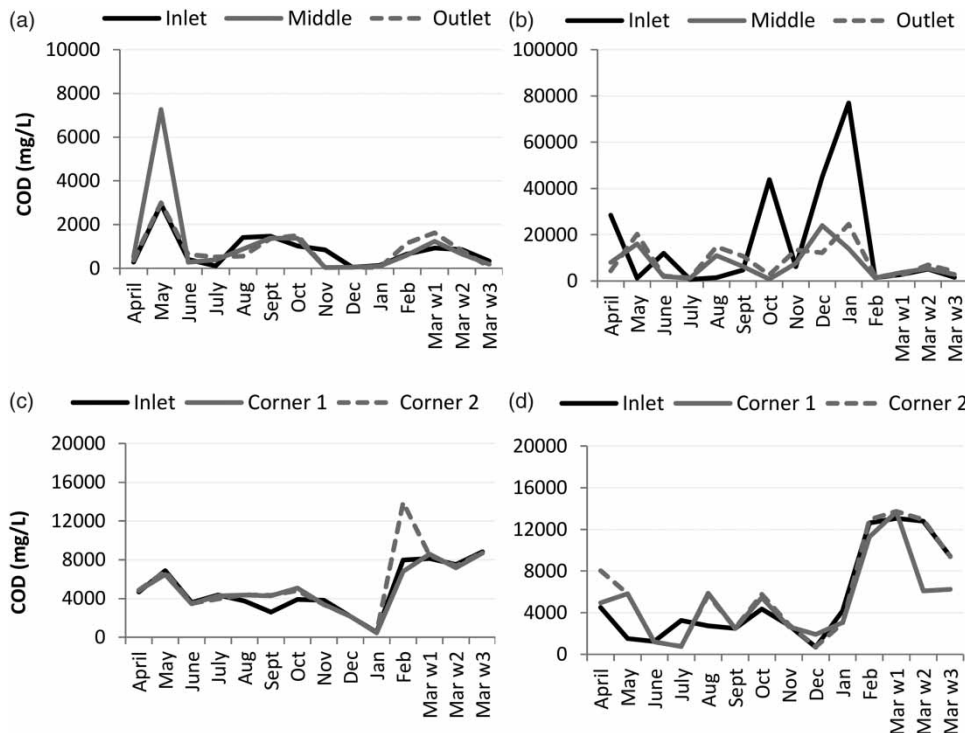


Figure 1 | COD concentrations measured in samples taken from the SBs at a small winery (a) and a medium-sized winery (b), and from WSPs at two large wineries (c) and (d) from April 2015 to March 2016.

higher at Winery D. In January 2015, there was an algal bloom in the pond at Winery C, which coincided with the lowest COD concentrations. It is postulated that the algae may have contributed to organic biodegradation at Winery C during this period. The algal growth abated during February 2015, and no visible growth was seen in either of the ponds at any other stage during the study period. This contrasts with ponds treating municipal effluent, where algae are typically abundant, and suggests that unless winery wastewater is dilute, it may be toxic to algae endemic to the area. It is unlikely that nutrient (N) limitation prevented algal growth in the winery ponds, as many algal species are capable of atmospheric nitrogen fixation.

The monthly variation in the COD concentration in samples taken from the SB at Winery A, and both WSPs (Wineries C and D) was highly significant ($p < 0.01$), but less significant in samples taken from the SB at Winery B ($0.01 < p < 0.05$), and it is postulated that this may have been related to the 'snapshot' sampling bias alluded earlier.

Compliance of COD concentrations with legislative requirements for irrigation

It has been shown that the typical biological oxygen demand (BOD_5)/COD ratio of winery wastewater is in the order of 0.5 to 0.8 (Quayle *et al.* 2009; Bolzonella & Rosso 2013), and is highest during the crush season (Oliveira & Duarte 2014). In South Africa, discharge standards are based on COD limits (and other parameters) applicable to the daily volume of wastewater that is applied via irrigation. In Australia, guidelines are based on BOD, take into account the area of land that is irrigated, and are applicable over a longer period (limit of 1,500 kgBOD/ha/month; Iannou *et al.* 2015). The Australian version is more complex for the wineries to calculate, but is more scientifically valid because the irrigation area is taken into account. This is important, because it has been shown that over-irrigation of land with winery wastewater results in significant soil degradation and microbial toxicity (Mosse *et al.* 2011b).

In terms of COD, the wastewater from 96% of samples from Winery A complied with South African legislative requirements for discharge via irrigation ($< 5,000$ mg COD/L for volumes < 500 m³/day or < 400 mg COD/L for volumes > 500 m³/day). Only 43%, 43% and 32% of samples from Wineries B, C and D complied with the upper COD discharge limit of 5,000 mg/L, and none with the lower limit of < 400 .

It is clear that winery wastewater should undergo more intensive treatment before being discharged to the

environment by irrigation or other means. Many different systems have been applied (with varying degrees of success) for the treatment of winery effluent, and have recently been reviewed by Lofrano & Meric (2016) and Inannou *et al.* (2015).

The organic fraction of winery wastewater

From an organic perspective, knowledge of COD concentration alone in winery effluent does not provide sufficient information for engineers wishing to design effective biological wastewater treatment systems, or for studying the functioning of such systems (Sheridan *et al.* 2011; Welz *et al.* 2012, 2014). This is because some of the organic components are highly biodegradable (e.g. sugars and alcohols), while others are recalcitrant and/or toxic to microbial communities and/or plants (e.g. many (poly)phenolics) (Arienzo *et al.* 2009a; Mosse *et al.* 2011b; Welz *et al.* 2012).

Non-phenolic organics

A variety of sugars, organic acids and alcohols contribute to the COD make-up of winery wastewater. In 'snapshot' samples taken from four wineries during the crush season, Malandra *et al.* (2003) identified 26 non-phenolic organics, and found that the fermentable sugars, glucose (0 to 1,800 mg/L), and fructose (0 to 1,530 mg/L) constituted almost half of the COD in some samples, but were absent in 33% and 11% ($n = 9$), respectively. The authors also reported that acetic acid (27 to 663 mg/L) and ethanol (concentrations not given), contributed significantly to the overall COD. In a seasonal winery wastewater characterisation study, Sheridan *et al.* (2011) found ethanol and acetic acid to be the primary contributors to COD in two wineries, and did not detect glucose in any of the samples taken throughout the year. Apart from sugars, ethanol and acetic acid, other organics that have been reported to be present in relatively high concentrations are tartaric acid (0 to 530 mg/L), malic acid (0 to 70 mg/L), lactic acid (0 to 350 mg/L), succinic acid (40 to 80 mg/L), and glycerol (140 to 320 mg/L) (Mosse *et al.* 2011a; Bolzonella & Rosso 2013; Malandra *et al.* 2003). In light of previous findings, the range of non-phenolic organics quantified during this study was rationalised to short chain VFAs (acetic acid, propionic acid, butyric acid), ethanol, glycerol, tartaric acid, malic acid, lactic acid, succinic acid, glucose, fructose and maltose.

VFAs. For this study, the short chain VFAs commonly found in winery wastewater, namely acetic acid, propionic

acid and butyric acid, were considered. Previous studies have reported that acetic acid, which is a ubiquitous molecule involved in the central and subsidiary bacterial metabolic pathways, constitutes the major fraction of these VFAs (Malandra *et al.* 2003; Sheridan *et al.* 2011; Welz *et al.* 2014). Acetic acid can be absorbed and serve as a substrate for dissimilatory acetate utilisation, or assimilated and excreted by microorganisms (Wolfe 2005). It is the major substrate for the formation of propionic and butyric acid, and can be formed from the degradation of almost any organic molecule, including ethanol and (poly)phenolics (Welz *et al.* 2011, 2012). Although the short chain VFAs are generally considered as readily biodegradable molecules, this is not always the case; accumulation appears to be influenced significantly by the redox status of the environment (Conrad & Klose 2011; Welz *et al.* 2014).

VFAs can be formed from residuals in the winery, thereby constituting a fraction of the 'fresh' wastewater, or they can be formed from other organic molecules during the storage of wastewater. The high organic loads and HRTs in sedimentation basins and ponds in the absence of oxygen may favour anaerobic digestion, with concomitant VFA production. If anaerobic digestion is being considered as a method of COD reduction, it is important to note that acidification linked to high VFA concentrations can have a negative effect on methanogenic communities (Akuzawa *et al.* 2011).

In this study, VFAs were found in all of the samples from Wineries B, C and D, and in 75% of samples from the SB of (small) Winery A (Figure 2(a)). No hypotheses on the origin of VFAs in the samples from Wineries C and D are offered, because of the relatively long HRT in the ponds. Indeed, the results from Winery B, where there was a relatively short HRT, strongly suggest that significant formation occurred in the SB, because: (i) apart from samples taken in October 2014, December 2014 and January 2015, the concentration of VFAs was lowest in samples taken from the inlet, and highest in samples taken from the outlet of the SB (Figure 2(b)), suggesting sequential formation during wastewater movement through the SB; (ii) the highest concentrations of VFAs were found during the low flow/high HRT period in January 2015 (7,951–11,254 mg acetate/L), when VFAs were also the most substantial contributors to overall COD (Figure 2(a) and 2(c)); and (iii) low concentrations were found during the high flow/low HRT crush season.

However, there were three sampling instances when VFA concentrations were highest at the inlet of the SB basin at Winery B (demarcated by dotted circles in Figure 2(b)), indicating that VFAs were also formed in the cellar, and were

present in the incoming wastewater. On a monthly basis, the percentage of VFAs comprising the overall COD in the SBs was highly erratic, reflecting variable seasonal inputs from the cellar, when compared to the relative percentages in the WSPs, which were more stable (Figure 2(c) and 2(d)).

The concentrations of soluble short chain VFAs, acetic, propionic and butyric acids were measured and totalled. These molecules are considered to be readily biodegradable. In the SBs, there were instances where none of these molecules were detected, and also instances where only acetic acid was detected (Figure 3(a) and 3(b)). In contrast, all three VFAs were found in all of the samples from the WSPs, and propionic and butyric acid were typically found in higher concentrations than in the 'fresh' wastewater from the SBs (Figure 3(c) and 3(d)). The formation of propionic acid and the particularly malodorous butyric acid on storage of winery wastewater upon standing is problematic.

Ethanol. In this study, ethanol typically comprised a high fraction of the overall COD in the wastewater from both SBs and WSPs. If the origin of ethanol in winery wastewater could be definitely determined, it could be used as a proxy for product loss, and the need to identify best practices to reduce this. The highest concentrations determined were 2,377 mg/L, 21,000 mg/L, 1,539 mg/L and 4,896 mg/L from Wineries A through D, respectively. In samples from the SB at Winery A, the highest contributions to overall COD were seen out of crush season, constituting >50% of the COD in October and January (Figure 4(a)). In 'fresh' samples from the SB at Winery B, ethanol contributed to >50% of the overall COD in 50% of the samples, and >80% in 25% of the samples (Figure 4(a)). In these samples there was also a significant negative correlation between the contribution of VFAs and ethanol to the overall COD ($r = -0.76$; $p = <0.001$). In other words, when the VFA concentration was high, the ethanol concentration was relatively low, and vice versa.

Between 0 and 67% of the overall COD was comprised of ethanol in samples taken from the WSPs. Similar and clear seasonal changes were demonstrated at both wineries, with the lowest concentrations being seen in the non-crush period between September 2014 and January 2015 (Figure 4(b)). The contribution of ethanol to overall COD in the WSPs was typically lower than that from the 'fresh' wastewater at Winery B, and no negative correlation was seen between the % contribution of VFAs and ethanol.

These results highlight the fact that the complex organic formation/degradation kinetics has a notable impact on the composition of wastewater that has been exposed to the

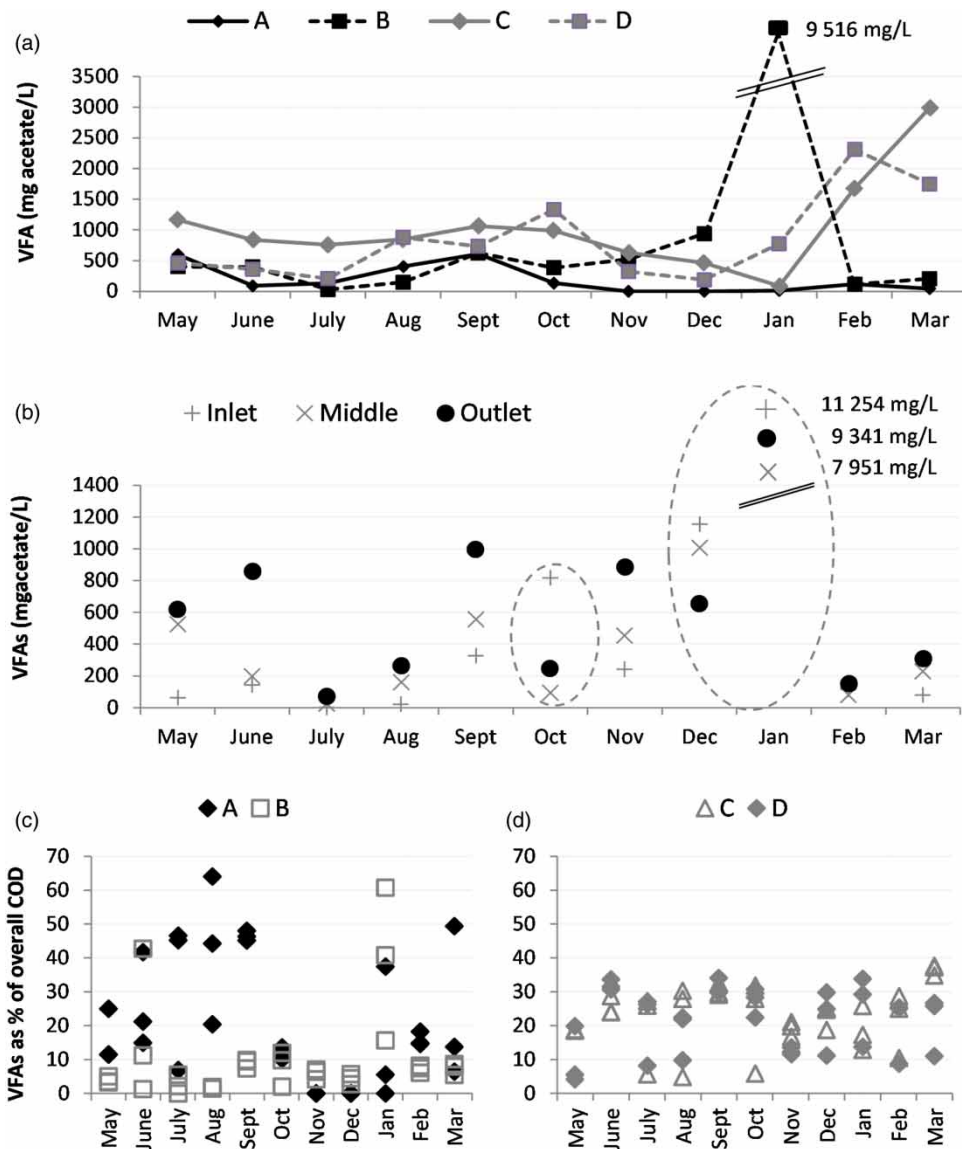


Figure 2 | Average monthly VFA concentrations (a), VFA concentrations at the inlet, middle and outlet of the SB at Winery B (b), % contribution of VFAs to overall COD in both SBs (c), and both WSPs (d).

environment for protracted time periods when compared to wastewater that has been recently generated. From a wastewater treatment perspective, ethanol, even in concentrations $>20,000$ mg/L, is readily biodegradable in biological wastewater treatment systems once the microbial community structure and function has acclimated to the prevailing chemical environment (Welz et al. 2011; Ma et al. 2013). Therefore, the presence of ethanol in the concentrations found in this study should not be deemed problematic.

Glycerol, organic acids and sugars. Holistically, ethanol and VFAs were major contributors to overall COD in the winery wastewater analysed during this study, while organic acids, glycerol and sugars were minor contributors (Table 1).

Of the minor organics, glycerol and tartaric acid were the most prevalent. The highest concentrations of glycerol ($>7,000$ mg/L) were found in the samples from the SBs, with the highest concentrations in both WSPs being notably lower ($<2,500$ mg/L), probably because of the highly biodegradable nature of this molecule. Malic acid, lactic acid and succinic acid were less prevalent, and found in lower concentrations in the samples from all sites. Relatively high concentrations of sugars (glucose, fructose and maltose) were typically only found around the crush period (results not shown). With the exception of Winery B, the sugars were prevalent in $<40\%$ of the samples. Although high concentrations of glucose were found during the crush period in the WSPs, this sugar

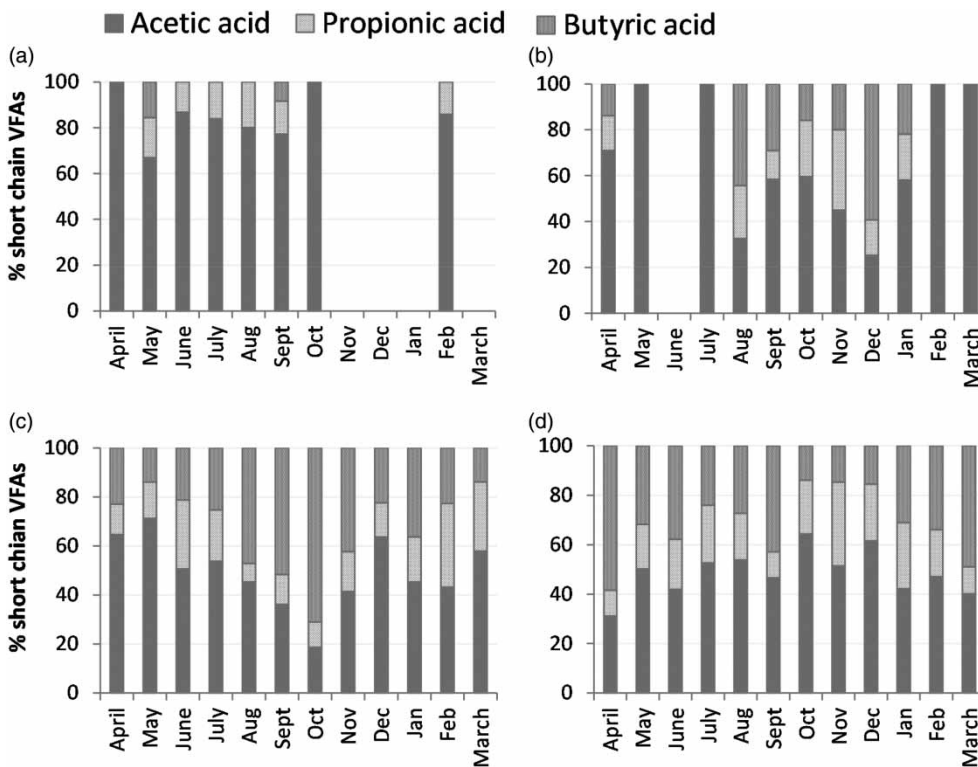


Figure 3 | Relative contributions of the short chain fatty acids from the SBS at Winery A (a) and Winery B (b), and from the WSPs at Winery C (c) and Winery D (d).

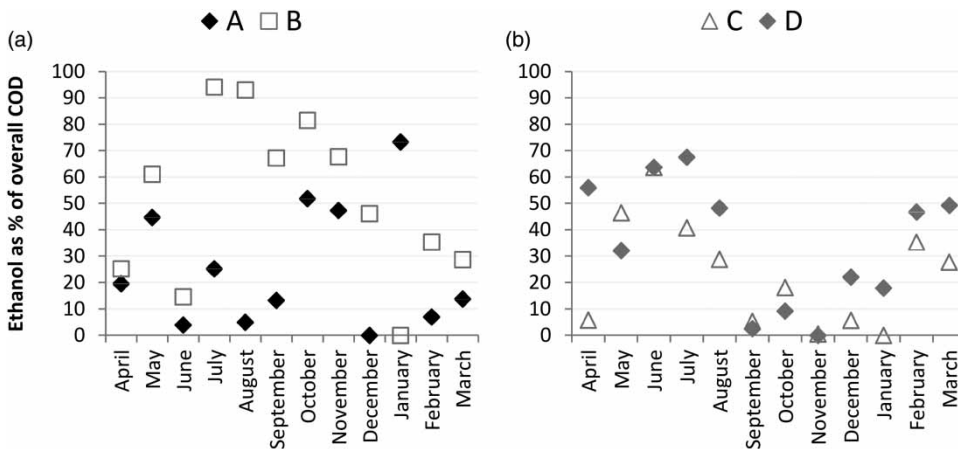


Figure 4 | Average % contribution of ethanol to the overall COD in both SBS (a) and both WSPs (b).

was only detected in samples taken from the inlet, reflecting the highly degradable nature of this molecule.

Total phenolics

It is generally accepted that the (poly)phenolic component of winery wastewater is the least degradable fraction. Once discharged to the environment, the different molecules can

polymerise/depolymerise, adsorb/desorb onto soil particles, undergo biotic and/or abiotic degradation, or remain recalcitrant to degradation (Coliarieti *et al.* 2002; Mutabaruka *et al.* 2007). Of concern to the wine industry is the fact that high concentrations of (poly)phenolics have been associated with phytotoxicity and microbial toxicity (Tharayil *et al.* 2006; Qu & Wang 2008, Welz *et al.* 2012). There are many factors that influence these toxic effects, most

Table 1 | Prevalence, concentration range and seasonality of minor non-phenolic organics in winery wastewater samples

		Winery A	Winery B	Winery C	Winery D
Glycerol	Prevalence (%)	50	94	50	67
	Conc. range (mg/L)	0 to 7,123	0 to 7,948	0 to 1,643	0 to 2,355
	Seasonality	No	No	No	No
Tartaric acid	Prevalence (%)	70	93	78	75
	Conc. range (mg/L)	0 to 392	0 to 1,794	0 to 165	0 to 352
	Seasonality	No	No	No	No
Malic acid	Prevalence (%)	22	64	14	22
	Conc. range (mg/L)	0 to 238	0 to 583	0 to 138	0 to 125
	Seasonality	No	No	No	No
Lactic acid	Prevalence (%)	8	11	17	22
	Conc. range (mg/L)	0 to 33	0 to 770	0 to 10	0 to 269
	Seasonality	No	Pre-crush	No	No
Succinic acid	Prevalence (%)	20	64	25	31
	Conc. range (mg/L)	0 to 230	0 to 1,121	0 to 938	0 to 333
	Seasonality	No	No	No	No
Glucose	Prevalence (%)	19	22	14	22
	Conc. range (mg/L)	0 to 1,596	0 to 653	0 to 1,450	0 to 626
	Seasonality	Crush	Crush	Crush*	Crush*
Fructose	Prevalence (%)	36	83	19	31
	Conc. range (mg/L)	0 to 412	0 to 238	0 to 12	0 to 506
	Seasonality	Crush	Crush	No	Crush
Maltose	Prevalence (%)	31	56	36	33
	Conc. range (mg/L)	0 to 889	0 to 718	0 to 45	0 to 14
	Seasonality	No	No	Crush	Crush

*Inlet samples.

notably the concentration and the chemical species of the particular molecule/s, and the physicochemistry of the soil horizon (Inderjit & Mallik 1997; Coliarjeti et al. 2002; Tharayil et al. 2006; Chen et al. 2009).

In this study, the percentage contribution of total phenolics to the COD was small, ranging from 0.2 to 4.2%, with the highest percentages being found during the bottling season. There were significant correlations ($r = 0.98, 0.82, 0.80, 0.50$; $p < 0.050$ from Wineries A through D, respectively) between COD and total phenolics in the samples. In keeping with elevated temporal COD values, 10 samples from Winery B, and 1 sample from Winery D, exhibited concentrations >100 mgGAE/L (110 to 3,531 mgGAE/L, and 110 mgGAE/L, respectively (GAE = gallic acid equivalents)). The total phenolic concentration in the remainder of the samples from Wineries A, C and D were < 100 mgGAE/L throughout the study period.

Given the complexity of the subject, and the lack of available literature, it was beyond the scope of this study to determine the detailed phenolic profile and relate this to the potential environmental threat of the winery wastewater. Only one such study is reported in the literature,

where three hardy wetland macrophyte species (*Phragmites australis*, *Schoenoplectus validus*, *Juncus ingens*) were unable to survive in diluted winery wastewater with a total phenolic concentration as low as 2.5 mg/L (Arienzo et al. 2009a). However, it was not definitively established which component (or combinations) in the wastewater were responsible for the phytotoxicity. There is therefore a clear need for more in-depth research in this area. This would require formulating complex synthetic phenolic cocktails to mimic those found in 'typical' winery wastewaters. These cocktails would then need to be applied with and without other organic and inorganic fractions in toxicity assays.

The inorganic fraction of winery wastewater

Origin of inorganics in winery wastewater

Inorganics in winery wastewater originate from cleaning and sanitising agents, and from grape skins, juice and wine (Mosse et al. 2011a; Conradie et al. 2014; Versari et al. 2014). The range of inorganic cleaning products used in

wineries include alkalis (sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium metasilicate (Na₂SiO₃), trisodium phosphate (Na₃PO₄), sodium carbonate (Na₂CO₃), and acids (phosphoric acid (H₃PO₄)). Caustic soda (NaOH) is also used as a sanitising agent. Other sanitising agents include quaternary ammonium compounds, peracetic acid compounds, hydrogen peroxide (H₂O₂), ozone, sulphur (S) compounds and heat. Depending on cellar practices, the cleaning and sanitising agents are expected to contribute mainly Na and/or K to the inorganic fraction of winery effluent, but some phosphorus/phosphate (P/PO₄³⁻), and NH₃/NH₄⁺ is also likely to originate from this source.

The concentration of inorganics in grapes varies according to the soil geochemistry, agricultural practices, and the plant uptake (which depends on the grape varietal and rootstock) (Versari *et al.* 2014; Bimpilas *et al.* 2015). In addition to Na, K, N and P, Bustamante *et al.* (2005) found high concentrations of Ca (286 mg/L), Mg (33 mg/L) and Fe (12 mg/L), as well as lower concentrations of Mn (310 µg/L), Cu (790 µg/L), Zn (580 µg/L), Co (170 µg/L), Cr (150 µg/L), Pb (1,090 µg/L), Cd (60 µg/L) and Ni (120 µg/L) in 21 samples of winery/distillery wastewater.

While the inorganic fraction emanating from the grapes, and to a large extent the influent wash-water, is unavoidable, the fraction from the cleaning and sanitising agents can be decreased by instituting best practice principles for wastewater management in wineries. This study therefore focussed on quantifying the inorganics that may have originated from cleaning and sanitising agents (Na and K), and those that can be present in cleaning agents and are also important nutrients in biological wastewater treatment processes (N and P).

Sodium and potassium concentrations in winery wastewater samples

NaOH is widely used as a cleaning and sanitising agent because it is inexpensive and effective. However, high concentrations of sodium in irrigation water can have a severe impact on the soil structure. In contrast, the addition of K in moderate concentrations is seen as beneficial, making KOH an environmentally-preferable alternative for cellar use. Unfortunately, it has not been widely adopted because it is significantly more expensive and is perceived to be less effective (Arienzo *et al.* 2009b).

To assist in preventing soil sodicity, the sodium adsorption ratio (SAR) was developed as a simplistic measure of the suitability of saline wastewater for irrigation (Equation (1)), where cations are expressed in mmol/L). The World Health

Organization SAR guideline for this parameter for irrigation is 3 to 12 (Oliveira & Duarte 2014).

$$\frac{[\text{Na}]}{\sqrt{([\text{Ca}] + [\text{Mg}])}} \quad (1)$$

Oliveira & Duarte (2014) measured the SAR in treated winery wastewater from three wineries over a period of 3 years, and found the average to be 13 ± 2 (winery with lowest SAR) to 23 ± 6 (winery with highest SAR), all higher than the WHO recommendation.

In this study, the average winery effluent K concentrations were significantly higher ($p < 0.01$) than the Na counterparts at each winery (Table 2).

There were significant temporal trends in the concentrations of Na and K in the samples from all four wineries ($p < 0.01$) (Figure 5). In the case of Na, this contrasts with results obtained by Sheridan *et al.* (2011), who found that the Na concentration from one winery was relatively stable around the 50 mg/L mark ($n = 10$ samples over 1 year). In the WSP samples, elevated Na concentrations were found in all samples taken between August and December, and in the samples from Winery A, from August to November (Figure 5(a)). It was clear from the results that the highest concentrations of cleaning agents are found in winery wastewater during the non-crush season, which is expected as high concentrations of caustic soda are typically used for cleaning tanks/fermenters (Figure 5(b)).

Unlike Na, which emanates mainly from cleaning agents, significant quantities of K in winery wastewater can also be derived from grapes and wine. In all samples, the K concentrations remained < 150 mg after the crush season in spring and into early winter (April to July). In samples from the SBs, there was a sustained spike in K from August to October at Winery A, which correlated

Table 2 | Average sodium and potassium concentrations measured in the effluent from four wineries over a period of 1 year

	Na (mg/L)		K (mg/L)	
	Average $n = 36$ for each winery	Range	Average $n = 36$ for each winery	Range $n = 36$
Winery A	23 ± 19	5–67	59 ± 83	5–329
Winery B	73 ± 54	18–574	130 ± 96	11–383
Winery C	73 ± 35	24–116	265 ± 108	28–389
Winery D	55 ± 50	11–142	210 ± 106	43–334

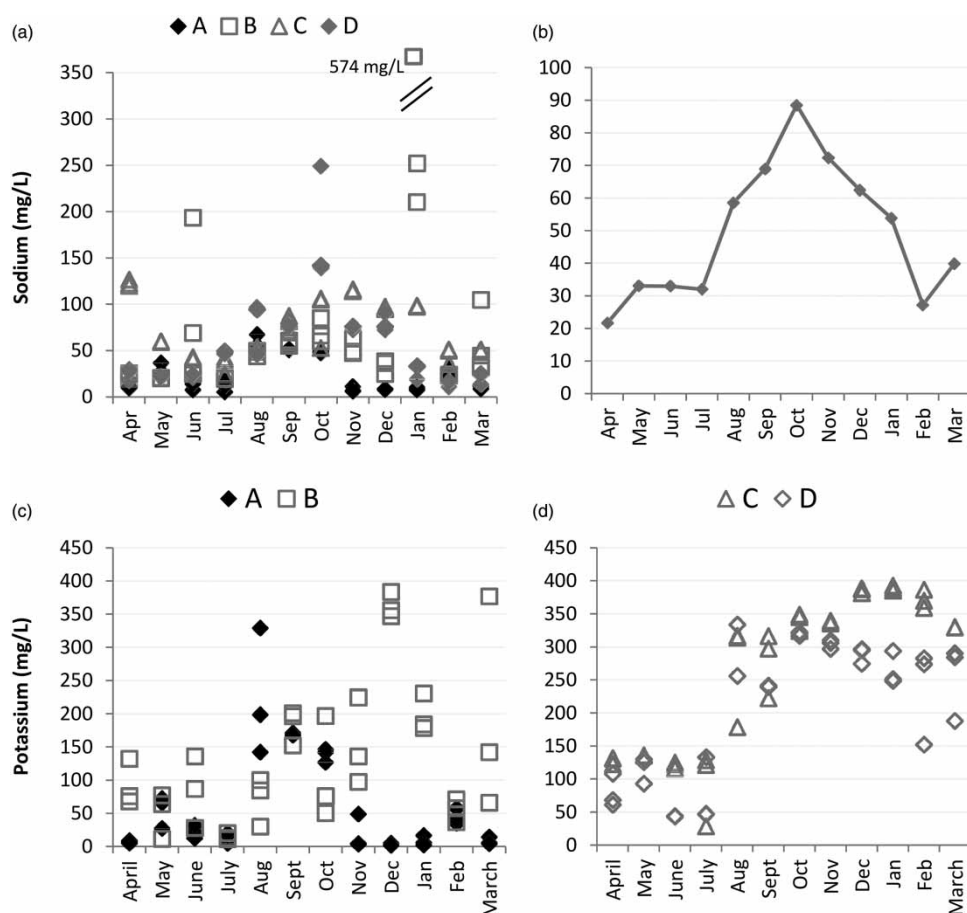


Figure 5 | Individual monthly wastewater sodium concentrations in all samples (a); average combined wastewater sodium concentrations from all sampling sites, with outliers removed [outliers: Winery B (January, June outlet); Winery C: April; Winery D: October site 3] (b); monthly wastewater potassium concentrations from all sampling sites in the SBs (c); and monthly wastewater potassium concentrations from all sampling sites in the WSPs (d).

with increased Na, but otherwise the monthly distribution was random (Figure 5(c)). In the WSPs (Figure 5(d)), which are a more accurate reflection of monthly trends, it is evident that the K levels in winery wastewater were affected by input from grape, grape juice and wine, as well as cleaning products, because as with Na, there was an increase in August. However, in contrast to the Na results, this was sustained during the crush period (February to March) when the COD values were highest.

Nitrogen and phosphorus in winery wastewater

If released to the environment, the presence of N and P in wastewater can lead to eutrophication and the death of aquatic organisms, including amphibians and fish (William *et al.* 2011). In this study, high concentrations of N (up to 176 mg/L), comprised mainly of different ratios of $\text{NH}_3/\text{NH}_4^+$ and NO_3^- , were found in the SB at Winery B (Figure 6(a)). The majority of the samples from the other

wineries fell below 10 mg/L (Figure 6(b)), with the notable exception of four samples from the WSP at Winery C taken in January and February (range: 10 to 40 mg/L). This coincided with an algal bloom in the pond. It is hypothesised that algal atmospheric N-fixation was responsible for the spike. Analysis of the TN results from the WSPs is confounded by the fact that microbial nitrogen fixation, nitrification and denitrification can take place in such systems, so that N can be both added and/or removed during storage.

Unlike N, P can neither be added to nor removed from a system by fixation or volatilisation via mineralisation. Concentrations >1 mg/L can lead to eutrophication of water bodies, but it is also an essential nutrient for plant growth (Correl 1998; William *et al.* 2011). It can precipitate into sediments in inland waters, become complexed with components of soil (e.g. iron), or be removed with microbial biomass in settled sludge of suspended growth wastewater treatment systems (Correl 1998; Wetzel 2001). The importance of P removal from winery wastewater is therefore dependent

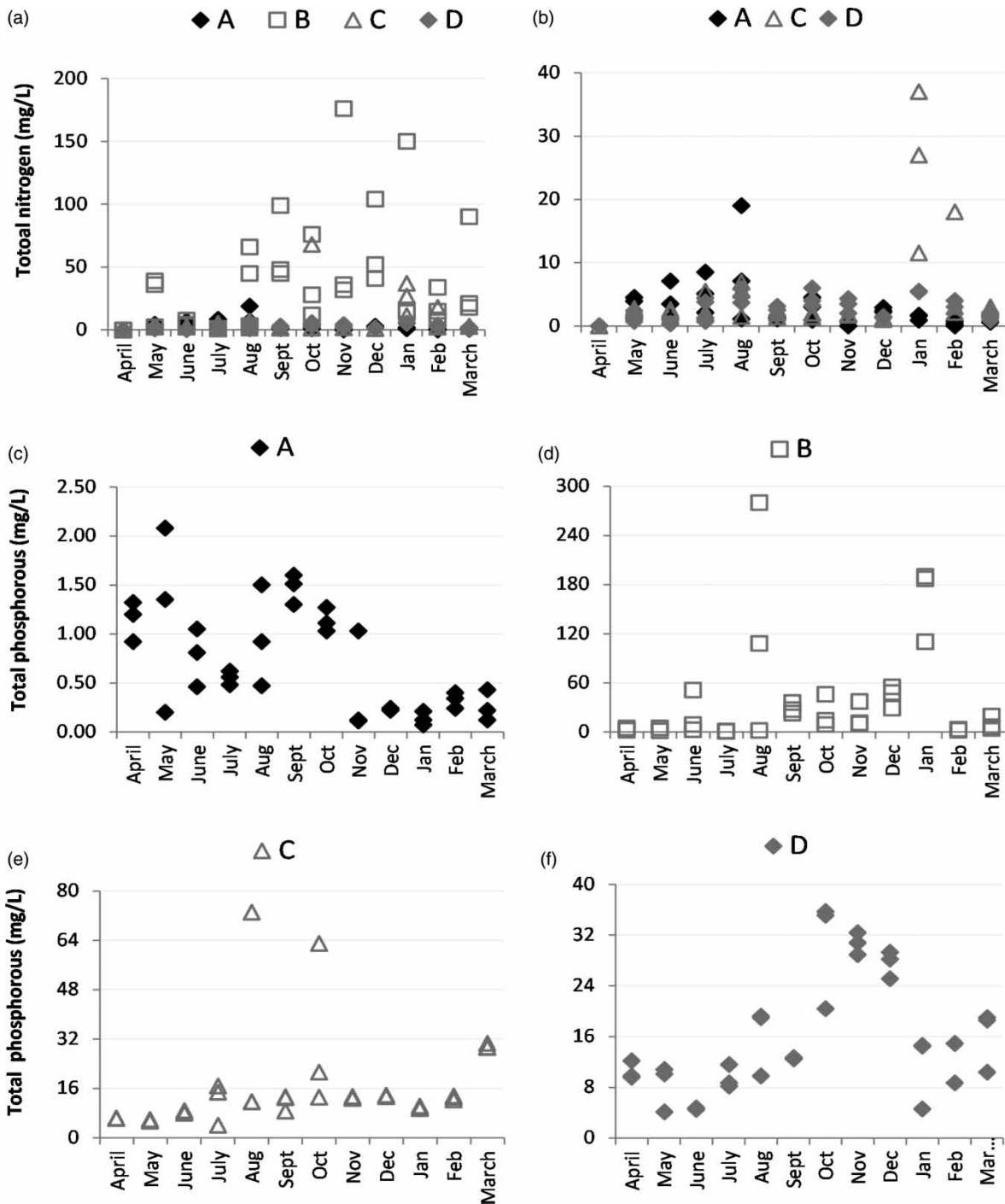


Figure 6 | Monthly wastewater TN concentrations in samples from all wineries (a), and all wineries except the SB at Winery B (b); and monthly total phosphorus concentrations in all samples from the SBs at Winery A and B (c) and (d), and WSPs at Winery C and D (e) and (f).

on a number of factors, most important being the destination of the final effluent. If the wastewater is being used for irrigation, the P concentrations and the soil properties will determine whether the seepage or run-off poses an environmental threat to inland water bodies. In this study, high

concentrations of P were intermittently found in the WSPs and the SB at Winery B, in the order of Winery B > Winery C > Winery D > Winery A (Figure 6(c)–6(f)). The concentration of this element is thus an important parameter to consider when monitoring winery wastewater for discharge.

Apart from the environmental significance of N and P, they are the most important functional nutrients required for biological systems treating organic-rich wastewaters, such as winery wastewater. In terms of the COD:N:P ratio, the ideal metabolic requirement is approximately 100:5:1, but differs according to the treatment system and the composition of the COD (O'Flaherty & Gray 2013). Winery wastewater may be nutrient-limited in terms of N and P. To remedy this, additional nutrients are sometimes added, or high C:N wastewater is treated simultaneously with domestic wastewater (Ganesh et al. 2009). However, addition of N and P may lead to excess nutrients in the treated effluent (Rodriguez-Caballero et al. 2012). In this study, the N, and to a lesser extent, P values were less than those suggested for biological wastewater treatment systems (Table 3). There is a possibility that N deficiency could be overcome by microbial nitrogen fixation in systems with relatively high HRTs, as has been shown with kraft mill wastewater in WSPs (Clark et al. 1997).

The pH of winery wastewater

Previous studies have reported the pH ranges of winery wastewater generated during the crush and non-crush as 4 to 6, and 6 to 8, respectively (Bolzonella & Rosso 2013). The lower values during the crush season have been linked to the presence of lower concentrations of caustic (alkaline) cleaning products. In samples taken from the SB at Winery A, the pH values fell in a relatively narrow range (between 5.0 and 7.2), but in the SB at Winery B, there were large monthly variations, ranging from 3.6 in August to 10.5 in June. There was no obvious distinction in the pH between the crush and non-crush seasons in the samples from the SBs. As expected, the pH changes in the samples taken from the WSPs were less erratic, and with

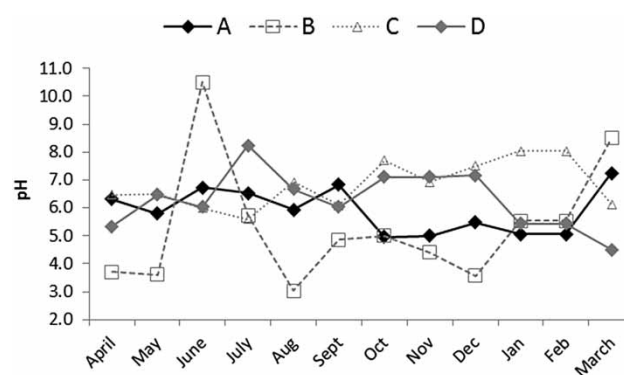


Figure 7 | Average ($n = 3$) monthly pH values in winery wastewater samples taken from SBs at Winery A and B, and the WSPs at Winery C and D.

the exception of the samples from Winery D in March, all monthly averages fell to between pH 5 and 8 (Figure 7).

CONCLUSIONS

In terms of wastewater remediation, the compositional variability of winery effluent presents a challenge during the design and operation of many physicochemical and biological treatment systems. In this study, it was shown that storage of effluent in ponds smoothed the variable nature of winery wastewater. Ponds can therefore potentially serve as both storage and equalisation basins to minimise fluctuations in pH, and volumetric and mass loading prior to further downstream remediation processes. Up-front equalisation could therefore be considered as best practice for winery wastewater treatment. In addition, previous studies have shown that when high C:N wastewater is treated in ponds, the environment becomes conducive for bacterial and/or algal nitrogen fixation. The nitrogen-deficient nature of winery effluent could potentially be overcome by nutrient 'self-balancing' in ponds. This would circumvent the common practice of adding nutrients to improve the performance of conventional biological treatment systems. Not only does nitrogen addition add to the cost and complexity of treatment, but residual nitrogen can result in eutrophication if the treated wastewater is discharged to the aquatic environment. Therefore, provided land requirements can be met, it is feasible to use ponds to pre-treat winery wastewater in order to improve the performance of downstream biological treatment systems.

The organic character of winery wastewater changes considerably during storage in ponds. In particular, there is a significant accumulation of VFAs. Research into (i) the reasons for this propensity, including the role of nitrogen

Table 3 | The ratio of essential nutrients measured in winery wastewater samples

	COD:N (100:X)		COD:P (100:X)	
	Average X $n = 36$ for each winery	Range	Average X $n = 36$ for each winery	Range
Winery A	1.20 ± 1.61	<0.01–6.3	0.41 ± 0.94	0.02–3.96
Winery B	0.65 ± 0.81	0.07–2.84	1.29 ± 2.79	0.01–12.47
Winery C	0.57 ± 1.61	0.04–7.18	0.59 ± 0.83	0.09–2.83
Winery C*	0.06 ± 0.05	0.04–0.16	N/A	N/A
Winery D	0.09 ± 0.06	0.01–0.21	0.70 ± 1.00	0.06–4.63

*Outliers from algal bloom period removed from Winery C. Ideal value of X for N = 5. Ideal value of X for P = 1 (O'Flaherty & Gray 2013).

limitation, (ii) the implications of high VFA concentrations on wastewater treatment processes, and (iii) the effect of VFA load on the environment, is recommended.

Finally, the results of this study reiterate previous research findings that: (i) although there are commonalities, the composition of winery wastewater is highly variable on a temporal and site basis, (ii) the highest concentrations of organics, and lowest concentrations of inorganics (from cleaning products) are found during the crush season, with the converse being true for the non-crush period, and (iii) without treatment, winery wastewater is generally not suitable for irrigation purposes in terms of current legislative requirements, and may lead to environmental degradation.

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