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ORIGINAL ARTICLE

# Anaerobic digestion of primary winery wastewater sludge and evaluation of the character of the digestate as a potential fertilizer

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## Abstract
Sludge is generated from settling of winery wastewater from seasonal cellar activities. Most primary winery wastewater sludge is generated during the crush season and is often disposed to landfill. This practice is an economic and environmental burden and ignores the potential for valorization. In this study, a series of biochemical methane potential tests were conducted using seasonal batches of sludge with and without the addition of selected micronutrients (Co, Cu, Ni) at different inoculum to substrate ratios and temperatures (ambient vs 37 °C). The highest specific $CH_4$ yields were obtained at an inoculum to substrate ratio of 4 with the addition of micronutrients from sludge collected during ($206 \pm 2.7$ mL$CH_4$/gVS$_{added}$) and after ($177 \pm 1.4$ mL$CH_4$/gVS$_{added}$) the crush season at 37 °C and ambient temperatures (15.3 to 27.1 °C), respectively. In some instances, digestion at 37 °C appeared to promote inhibited steady-state conditions leading to lower $CH_4$ yields than at ambient temperatures. This suggested that the sludge could be easily digested without heating, particularly during the warmer months of grape harvesting and crushing. The composition of the digestate indicated that it may be suitable as an agricultural fertilizer, with high concentrations of N (21.5 to 27.7 g/kg dry weight) and C (229 to 277 g/kg dry weight), as well as the presence of all essential micronutrients.

**Keywords** Anaerobic digestion · Fertilizer · Irrigation · Reuse · Valorization · Waste

## 1 Introduction

Solid and liquid wastes are generated by the wine industry through winemaking processes. Solid wastes from winemaking include lees, fining agents, filtration aids, and agricultural waste known as grape marc or pomace (skins, seeds, and stems). Winery wastewater (WWW) is generated when cellar equipment and floors are washed and/or sterilized and contains solid grape residuals, chemicals, and filter aids in variable quantities [1]. Over the last three decades, a number of industrial biorefineries have been set up to extract value-added products from grape pomace and lees including bioactive compounds such as phenolics, dietary fiber, cellulose, grapeseed oil, and tartaric acid [2–8]. Valorization approaches that have been explored on a more theoretical level include fermentation of pomace for bioethanol production, energy production from gasification or pyrolysis of pomace, and extraction of condensed tannins for use as wood adhesives [9–13]. Grape pomace has also been composted, vermi-composted, or co-composted with substrates such as green waste, municipal waste activated sludge (WAS), olive mill waste, and spent winery filter material as a lower value valorization option [14–18]. Co-composting may enhance the quality of the compost [14, 16]. Application of such compost to agricultural land may increase plant yield, as in the case of fertilizer obtained from co-composting of pomace, WWW, olive mill sludge, olive mill wastewater, and green waste, which was shown to increase radish yield [17].

Primary winery wastewater sludge (PWWS) and secondary winery wastewater sludge (SWWS) are indirect solid wastes that are generated by the wine industry. Unlike direct forms of solid winery wastes, WWS is not suitable for higher value valorization. Indeed, some wineries contract commercial companies to remove WWS and

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dispose it to landfill, which is (i) an economic burden on the industry, (ii) a burden on landfill sites, and (iii) ignores the potential for valorization of the organic-rich waste.

In water-stressed countries, including South Africa, Australia, and some areas of the USA, cellar effluent is often used for beneficial irrigation after primary settling and pH adjustment if required. This results in the generation of copious amounts of PWWS. Conversely, in many wineries, especially those in Europe, WWW is either discharged into municipal sewers for concurrent treatment with domestic wastewater or it undergoes secondary treatment in situ generating SWWS that differs in character from PWWS [19, 20]. Although SWWS and PWWS differ in nature, both are malodorous and potentially toxic to the environment, mainly due to high concentrations of (poly) phenolics and volatile fatty acids (VFA). It has been shown that stabilized SWWS from constructed wetlands can be used directly as a soil conditioner [19] and that SWWS from a continuous activated sludge (CAS) process is a suitable substrate for anaerobic digestion (AD) [21].

From economies of scale, composting and AD are generally preferred for decentralized and centralized biodegradation of organic matter, respectively [22]. However, composting can be labor-intensive because compost piles need regular turning and stalks may need to be ground [14]. In addition, a large spatial footprint is required as the process is slow. Anaerobic digestion occurs at a faster rate, requires a smaller spatial footprint, and emits less greenhouse gases than composting, provided the biogas from AD is utilized [22]. Promising results have been obtained from AD of grape pomace, lees, and grape marc [21, 23–26]. Arguably, in contrast to WWS, these are already being used for extraction of more valuable products than biogas. Nonetheless, these studies indicate that valorization of WWS via AD may be a viable remediation approach. Previous studies of AD of primary and secondary WWS were ineffective ($<30$ m$LCH_4$/gVS) in relation to other winery wastes like grape marc [27]. To the best of our knowledge, no other studies have been conducted on the AD of PWWS, including the addition of micronutrients and the use of digestate as a fertilizer.

The aims of this study were therefore to determine the biochemical methane potential (BMP) of PWWS from a winery during and after the crush season at different inoculum to substrate (ISR) ratios, with and without the addition of micronutrients at different temperatures (ambient and 37 °C). Furthermore, the digestates were characterized and compared with commercial organic fertilizers in terms of their macro- and micronutrient compositions.

## 2 Materials and methods

### 2.1 Biochemical methane potential experiments

The inoculum was prepared by randomly feeding the digestate obtained from previous AD experiments [28] with PWWS for > 6 months before the start of the first experiments. The composition of the biogas produced by the inoculum was monitored on a weekly basis. The inoculum was deemed ready for use once the $CH_4$% of the biogas was > 60%.

Approximately 100 L of PWWS was collected from during and after the crush season. The PWWS was transported to the laboratory immediately and thoroughly homogenized using a blender. The PWSS was fully characterized together with the inoculum. As the microbial communities are naturally dynamic in nature, the inoculum for each of the four experiments was characterized separately.

Two experiments were conducted with both the crush and the post-crush PWSS, giving a total of four sets of experiments: (i) crush experiment 1, (ii) crush experiment 2, (iii) post-crush experiment 1, and (iv) post-crush experiment 2. Experiment 1 for both PWWS substrates was conducted at ambient temperature (20.7 °C ± 2.0 °C) using a combination of two variables inoculum to substrate ratio (ISR) (2:1, 3:1, 4:1) and nutrient addition (nutrients/no nutrients) (Table 1). The character of the PWSS taken during the crush

**Table 1** Parameters used for experiments 1 and 2

| | Crush season PWWS | | | Post-crush season PWWS | | |
|---|---|---|---|---|---|---|
| | Experiment 1 | Experiment 2 | | Experiment 1 | Experiment 2 | |
| Temperature | Ambient | Ambient | 37 °C | Ambient | Ambient | 37 °C |
| ISR 2 | ✓ | ✗ | ✗ | ✓ | ✗ | ✗ |
| ISR 2 + N | ✓ | ✗ | ✗ | ✓ | ✗ | ✗ |
| ISR 3 | ✓ | ✗ | ✗ | ✓ | ✗ | ✗ |
| ISR 3 + N | ✓ | ✗ | ✗ | ✓ | ✗ | ✗ |
| ISR 4 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| ISR 4 + N | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

*PWWS*, primary winery wastewater sludge; *ISR*, inoculum to substrate ratio; *N*, nutrients (5 mg/L $Cu^{2+}$, 20 mg/L $Ni^{2+}$, 20 mg/L $Co^{2+}$)

season informed the choice of (micro)nutrients (5 mg/L copper ($Cu^{2+}$), 20 mg/L nickel ($Ni^{2+}$), 20 mg/L cobalt ($Co^{2+}$)). These metabolic co-factors were deficient in both batches of PWWS [29, 30]. Three controls were included in each experiment: inoculum only, substrate only, and inoculum + acetate, all with and without nutrients. All BMP experiments, including controls, were conducted in duplicate.

The experimental set-ups for the variable combination resulting in the most efficient performance in terms of specific biogas and $CH_4$ generation in experiment 1 were repeated at ambient (19.4 °C ± 1.77 °C) and mesophilic (37 °C) temperatures simultaneously to determine the effect of these temperatures on AD efficiency (experiment 2). The sets of experiments were first performed during the crush season using the crush season sludge, followed by the post-crush season sludge.

For reference, the results for characterization of all of the reactor contents at the beginning and end of the experiments are provided in the Supplementary material (Supplementary Tables 1 and 2).

### 2.2 Biogas measurements

Biogas was collected directly from the reactors into 2-L Supelco (Bellefonte, USA) foil gas sampling bags. The volume of biogas was determined three times per week on designated days by extracting the gas from each bag into graduated gas tight syringes. Volumes were measured at 20.7 °C ± 2.0 °C and pressure of 1008.7 ± 2.7 hpa. The composition of the biogas was determined using a Geotech (Warwickshire, UK) Biogas 5000 instrument. The performance of AD was assessed using the specific total biogas and $CH_4$ yields based on volatile solids added (mL/gVS).

### 2.3 Physicochemical analyses

Total solid (TS) and total volatile solid (TVS) concentrations were determined by standard methods (weight loss on ignition at 105 °C and 550 °C), respectively. A Merck (Darmstadt, Germany) Spectroquant Prove® 600 spectrophotometer together with Merck cell tests or kits was used to determine chemical oxygen demand (COD) (cat no: 14555), volatile fatty acids (VFA) measured as total volatile organic acids (VOAt) (cat no: 01763), total organic carbon (TOC) (cat no: 14879), total nitrogen (TN) (cat no: 14537), total alkalinity (TAlk) (cat no: 101758), total phosphorous (TP) (cat no: 14729), ammonia as $NH_4^+$ (cat no: 00683), sulfate $SO_4^{2-}$ (cat no: 14791), sulfide ($S^{2-}$) (cat no: 14779), and chloride ($Cl^-$) (cat no: 14789) concentrations, according to manufacturers' instructions. Quantification of carbon, hydrogen, nitrogen, and sulfur (CHNS) concentrations (%wt.wt) was determined at the Central Analytical Facility of Stellenbosh University using an Elementar (Hamburg, Germany) Vario EL cube Elemental analyzer according to the manufacturers' instructions. Major and minor elements were quantified at the same facility using a Thermo ICap 6200 ICP-AES instrument for trace analyses, while ultra-trace analyses were performed on an Agilent (Santa Clara, USA) 7900 ICP-MS instrument, according to manufacturers' instructions.

## 3 Results and discussion

### 3.1 Characterization of wastewater, primary winery wastewater sludge, and inoculum and theoretical suitability of sludge for anaerobic digestion

#### 3.1.1 Organic fractions, ammonia, and sulfides

Due to the seasonal nature of winery operations, there is a considerable intra- and inter-site variation in the characteristics of WWW and by inference in PWSS [1]. Samples of WWW taken from the same winery over the crush season exhibited a high COD:N ratio of 226 (Table 2). High COD:N ratios are typical of WWW, and a source of N is often added to secondary wastewater treatment systems to assist bioremediation [1]. Notably lower COD:N ratios were measured in the PWWS (12.9 and 24.1 for the crush and post-crush season PWWS, respectively). It was therefore noted that N partitioned preferentially into the PWWS and indicated that the character of the PWWS cannot be extrapolated directly from that of the WWW.

In this study, the C content from the (CHNS) elemental analysis was notably higher in the post-crush than the crush season PWWS, but the converse was true for the TOC. The post-crush PWWS exhibited significantly higher (ANOVA, $p > 0.05$) concentrations of TS, TVS, and COD than the crush PWWS (Table 2). The higher TOC in the crush season PWWS was due to the presence of fresh pomace residues from washing equipment and floors during the crushing. The post-crush PWWS still contained some pomace residues and solubilized degradation products because the settling delta at the winery had not been completely de-sludged after the crush period. Overall, the organic character of the PWSS adequately reflected seasonal operational variabilities.

Theoretically, the C:N ratio of the crush season PWWS (11.6) and post-crush season PWWS (7.1) were both below optimal for AD of carbonaceous substrates, which has been widely reported as 20–30 [31]. The AD process for carbonaceous substrates with lower C:N ratios may be susceptible to instability due to the potential accumulation of $NH_4^+$ and VFAs. High concentrations of VFAs can inhibit methanogenesis leading to bioreactor instability or failure [32]. In this study, The VFAs contributed 21% and 32% to the crush

**Table 2** Characteristics of primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments as well as parameters measured in the wastewater over the same period (average and standard deviation from the mean, $n=3$)

| Parameter ($n=3$) | Crush PWWS | Crush inoculum 1 | Crush inoculum 2 | Post-crush PWWS | Post-crush inoculum 1 | Post-crush inoculum 2 | Winery effluent |
|---|---|---|---|---|---|---|---|
| Elemental CHNS analysis (g/kg dry weight) | | | | | | | |
| C | 29.4 ± 2.45 | 32.5 ± 2.2 | 25.2 ± 2.9 | 40.1 ± 1.3 | 26.5 ± 0.3 | 20.4 ± 3.9 | NA |
| H | 0.55 ± 0.05 | 4.86 ± 0.55 | 4.85 ± 0.42 | 6.61 ± 0.43 | 3.62 ± 0.52 | 2.28 ± 0.64 | NA |
| N | 2.54 ± 0.04 | 2.86 ± 0.16 | 1.89 ± 0.25 | 5.67 ± 0.14 | 2.25 ± 0.20 | 1.78 ± 0.39 | NA |
| S | 0.38 ± 0.04 | 0.89 ± 0.05 | 0.57 ± 0.15 | 0.34 ± 0.03 | 0.38 ± 0.07 | 0.38 ± 0.08 | NA |
| C:N | 11.6 | 11.6 | 13.3 | 7.1 | 11.8 | 11.5 | NA |
| Characterization (g/L wet weight homogenized sludge, g/L wastewater) | | | | | | | |
| COD | 149 ± 5 | 35.5 ± 1.0 | 206 ± 14 | 346 ± 26 | 95.2 ± 8.8 | 71.7 ± 5.6 | 6.79 ± 1.20 |
| TP | 0.70 ± 0.01 | 0.17 ± 0.01 | 0.82 ± 0.20 | 1.65 ± 0.13 | 0.49 ± 0.09 | 0.44 ± 0.06 | 0.20 ± 0.07 |
| TN | 11.5 ± 0.5 | 2.3 ± 0.4 | 2.5 ± 1.07 | 14.3 ± 0.5 | 2.4 ± 0.8 | 4.5 ± 0.1 | 0.03 ± 0.02 |
| VFA | 28.5 ± 0.7 | 8.98 ± 0.35 | 44.6 ± 10.4 | 104 ± 5 | 26.1 ± 1.1 | 23.3 ± 2.36 | 1.37 ± 0.15 |
| $HS^-$ | 0.08 ± 0.01 | 0.06 ± 0.01 | 0.08 ± 0.01 | 0.75 ± 0.02 | 0.11 ± 0.01 | 0.13 ± 0.02 | ND |
| $NH_4^+$-N | 1.89 ± 0.04 | 1.3 ± 0.17 | 5.39 ± 0.06 | 1.18 ± 0.35 | 1.02 ± 0.21 | 1.16 ± 0.13 | ND |
| TS | 173 ± 10 | 257 ± 7 | 62.3 ± 4.1 | 301 ± 14 | 145 ± 2 | 117 ± 1 | ND |
| VS | 91.9 ± 11 | 97.8 ± 8.7 | 32.8 ± 22 | 247 ± 9 | 67.9 ± 1.5 | 85.3 ± 1.4 | ND |
| COD:VS | 1.62 | 0.36 | 6.28 | 1.40 | 1.40 | 0.84 | ND |
| VS:TS | 0.53 | 0.38 | 0.53 | 0.82 | 0.49 | 0.72 | ND |

*PWWS*, primary winery wastewater sludge; *Inoculum 1*, inoculum for ambient experiments (experiment 1); *NA*, not applicable; *ND*, not determined; *Inoculum 2*, inoculum used for ambient vs mesosphilic experiments (experiment 2)

and post-crush season PWWS CODs, respectively, based on the theoretical COD yield of acetic acid [33]. Similarly, the stoichiometric contribution of VFAs to the TOC for the crush and post-crush PWWS was 40% and 62%, respectively. In this case, the total VFA concentrations of the PWWS and inocula (Table 2) were above previously reported inhibitory thresholds of 5.80–6.90 g/L [34].

Ammonia in WWW emanates from cellar cleaning products and/or from hydrolysis of proteins from lees and grapes. The pH, temperature, C:N ratio, and elemental concentrations influence the degree of toxicity of $NH_4^+$ on the sensitive methanogenic archaea during AD [35]. Hence, a range of inhibitory concentrations (IC) has been reported in literature, from 2 to 14 g/L as total $NH_3$-N and 0.053 to 1.45 g/L as $NH_3$ [29]. In this study, the $NH_4^+$ concentrations in the PWWS fell within these previously reported ranges for inhibition of AD (Table 2).

The $S^{2-}$ concentration measured in the post-crush PWWS was 89% higher than the concentration measured in the crush season PWWS (0.75 g/L and 0.08 g/L, respectively). For hydrogenotrophic and aceticlastic methanogens, $IC_{50}$ ranges reported in literature for $H_2S$ are 0.043–0.125 g/L and 0.014–0.060 g/L, respectively [36]. In this study, the measured equivalent $H_2S$ concentrations for the PWWS substrates were within the inhibitory range, particularly for aceticlastic methanogens. However, $HS^-$ is likely to ppt. as metal sulfides during AD. This can alleviate toxicity by reducing the $HS^-$ concentration, and in some instances, reducing the bioavailability of concentrations of toxic cations by co-ppt. Conversely, AD can be retarded if the bioavailabilities of essential metabolic co-factors are reduced to below functional microbial requirements.

Although the measured VFA, $NH_4^+$, and $HS^-$ concentrations were above inhibitory values reported in literature, this does not necessarily translate into poor performance as each substrate and inoculum is unique. Indeed, it has been shown that substrates that appear unsuitable for AD may yield good AD results after long-term acclimation of the functional microbial communities to the particular physicochemical milieu provided by the substrate [37].

### 3.1.2 Elemental composition of primary winery wastewater sludge and inoculum

The elemental composition of grapes varies according to the soil geochemistry, the agricultural practices, and the grape varietal and rootstock. Potassium ($K^+$), the element found in the highest concentrations in the crush season PWWS, is found in high concentrations in grapes and can also be present in cellar cleaning products. Sodium is also typically present in high concentrations in WWW because caustic soda (NaOH) is the main cleaning and sterilizing agent used in most cellars [1]. However, $Na^+$ tends to remain in the soluble fraction of WWW [38], while much of the K remains in the PWWS because it is a constituent of the pomace. This is reflected by the relatively low concentrations of

Na compared with K in the PWWS, particularly in the crush season PWWS (Table 3).

In the case of metals, a large range of concentrations expected to inhibit AD has been reported in literature. This is due to variabilities in substrates, the degree of functional microbial acclimation to the intended substrates, substance synergism, and antagonism and operational differences [39]. Acclimated microbial communities may adapt to prevent metal toxicity or deficiency by altering their rate limiting flux and/or protecting key enzymes from reactive metals [40]. Soluble microbial products (SMPs) and extracellular polymeric substances (EPS) also play significant roles in chelating metals, thereby reducing their bioavailability to microbes in anaerobic digesters [40]. Metals are not only inhibitory—many are also essential metabolic co-factors, and it is common practice to supplement AD reactors with a co-factor cocktail to enhance AD. In this study, the metabolic co-factors Ni, Zn, Co, and Cu were either within or below the optimal range for AD [29], hence the addition of Ni, Co, and Zn as micronutrients in the experiments.

## 3.2 Biochemical methane potential tests

The quality and quantity of $CH_4$ generated from AD are fundamentally dependent on the oxidation state of the organic C. Higher $CH_4$ yields are associated with more reduced substrates [41]. Sludge characteristics are often used to infer $CH_4$ generation potential [42]. Buswell's equation has been used for decades to estimate the theoretical BMP of organic substrates assuming 100% conversion into carbon dioxide ($CO_2$) and $CH_4$ [43]. However, the theoretical yield is usually overestimated as the methanogenic archaea are highly susceptible to inhibition [29, 35]. The yields were therefore determined experimentally in this study.

### 3.2.1 Anaerobic digestion of primary winery wastewater sludge from the crush season

The first set of BMP experiments was conducted using PWWS taken during the crush season. Although it is well known that stable mesophilic (30–38 °C) and thermophilic (40–60 °C) temperatures typically increase the rate of AD, digestion still occurs under ambient conditions in some temperate and tropical climates [44]. In order to ascertain whether AD of PWWS could feasibly be conducted without having to install and operate sophisticated heating equipment, the BMP experiments were first conducted in the laboratory under ambient conditions in a non-temperature-controlled environment. Most WWW (and hence PWWS) is generated during the crush period, which falls within the

**Table 3** Elemental cation concentrations in primary winery wastewater sludge and inoculum used in the biochemical methane potential experiments

| | Crush PWWS | Crush inoculum 1 | Crush inoculum 2 | Post-crush PWWS | Post-crush inoculum 1 | Post-crush inoculum 2 |
|---|---|---|---|---|---|---|
| Major elemental parameters (g/kg dry weight) | | | | | | |
| Fe | 3.83 ± 0.13 | 3.59 ± 0.11 | 4.23 ± 0.95 | 2.96 ± 0.12 | 4.85 ± 0.85 | 5.02 ± 0.36 |
| Al | 11.1 ± 0.14 | 7.18 ± 0.24 | 10.2 ± 2.23 | 10.7 ± 0.22 | 13.0 ± 2.03 | 10.0 ± 1.16 |
| Ca | 7.37 ± 0.45 | 22.1 ± 0.19 | 10.4 ± 2.07 | 0.91 ± 0.12 | 10.3 ± 1.15 | 14.4 ± 3.03 |
| K | 51.8 ± 4.70 | 43.2 ± 1.39 | 29.5 ± 4.17 | 3.73 ± 0.12 | 61.5 ± 24.7 | 39.8 ± 15.7 |
| Mg | 1.60 ± 0.23 | 3.74 ± 0.19 | 2.32 ± 0.44 | 1.21 ± 0.41 | 2.19 ± 0.21 | 1.99 ± 0.13 |
| Na | 1.03 ± 0.16 | 30.9 ± 0.99 | 3.40 ± 0.09 | 0.18 ± 0.05 | 4.72 ± 1.61 | 3.75 ± 1.69 |
| P | 2.85 ± 0.12 | 5.24 ± 0.15 | 4.14 ± 0.93 | 5.40 ± 0.11 | 2.91 ± 0.05 | 3.80 ± 0.31 |
| Si | 0.91 ± 0.01 | 1.39 ± 0.12 | 0.65 ± 0.11 | 2.52 ± 0.53 | 1.57 ± 0.27 | 1.78 ± 0.35 |
| Minor elemental parameters (mg/kg dry weight) | | | | | | |
| B | 68.1 ± 48.3 | 54.6 ± 5.60 | 40.5 ± 7.58 | BDL | 45.3 ± 2.00 | 46.4 ± 2.50 |
| Mn | 39.7 ± 1.60 | 333 ± 3.80 | 93.0 ± 17,0 | 23.4 ± 2.20 | 74.5 ± 13.5 | 80.3 ± 11.3 |
| Co | 1.30 ± 0.50 | 16.9 ± 0.83 | 26.3 ± 5.84 | BDL | 20.4 ± 8.90 | BDL |
| Ni | 36.9 ± 4.9 | 11.6 ± 0.70 | 52.9 ± 12.1 | 12.9 ± 3.10 | 49.5 ± 5.40 | 36.0 ± 6.30 |
| Cu | 147 ± 12.7 | 168 ± 8.10 | 157 ± 34.5 | 120 ± 3.00 | 208 ± 30.3 | 197 ± 79.4 |
| Zn | 300 ± 15.0 | 1443 ± 71 | 449 ± 100 | 354 ± 2.4 | 511 ± 82.6 | 385 ± 35.2 |
| Sr | 21.6 ± 1.70 | 90.4 ± 0.70 | 51.14 ± 9.67 | 67.1 ± 11.9 | 39.1 ± 3.00 | 67.1 ± 11.9 |
| Mo | 3.01 ± 1.30 | 5.0 ± 0.2 | 1.70 ± 0.47 | ND | ND | ND |
| Pb | 34.2 ± 6.40 | 19.1 ± 6.70 | 24.3 ± 7.30 | 34.7 ± 8.60 | 29.7 ± 6.30 | 35.1 ± 10.5 |
| Ba | 133 ± 8.60 | 42.8 ± 4.90 | 72.9 ± 19.2 | 69.8 ± 2.50 | 114 ± 29.6 | 114 ± 13.1 |

*BDL*, below detection limit; *PWWS*, primary winery wastewater sludge; *Inoculum 1*, inoculum for ambient experiments (experiment 1); *Inoculum 2*, inoculum used for ambient vs mesophilic experiments (experiment 2); *ND*, not determined warm summer/autumn months in wine-producing countries. As a cost-effective option, it is feasible that PWWS could be pumped from existing settling tanks/deltas into simple digesters. These could be off-the-shelf polyethylene tanks, for example. Black containers exposed to sunlight could absorb heat and assist with increasing the temperatures of the reactor contents. Alternatively, specialized insulated reactors used in combination with solar heating could be employed.

Promising results were obtained at ambient temperatures (experiment 1). Good specific biogas (Fig. 1A) and $CH_4$ generation (Fig. 1B) were achieved within 40 days (Fig. 2). In terms of ISR and nutrient addition, both biogas and $CH_4$ generation increased with increased ISR and nutrient addition. In practice, reactor capacity is reduced at lower ISRs as less digestate needs to be retained between batches (for batch reactors), and more solids can be wasted in the case of continuous reactors. In this study, the comparatively low efficiency at ISR2 obtained during (ambient) experiment 1 would not support the use of this ISR for piloting a system to treat PWWS. Although reasonable absolute cumulative $CH_4$ generation was achieved at ISR2, the quantities were masked when corrected for the contribution by the inoculum control as prescribed in the standard method applied [45]. This led to some anomalous negative generation rates at ISR2. Although the inoculum had been pre-acclimated to a different batch of PWWS, it was hypothesized that the sensitive microbial populations needed to adjust to the slight change in substrate, especially when present in lower concentrations (ISR2). Many researchers do not pre-acclimate inocula to the intended substrate, which can reduce AD efficiency as acclimation then needs to take place during the experimental period [39]. This may lead to long lag phases, and in some cases, the microbial consortia may completely fail to adapt to the new substrate. In an attempt to standardize testing to allow inter- and intra-study comparisons, some standardized methodologies prescribe the use of inocula from municipal wastewater sludge digesters for all AD testing [45]. While

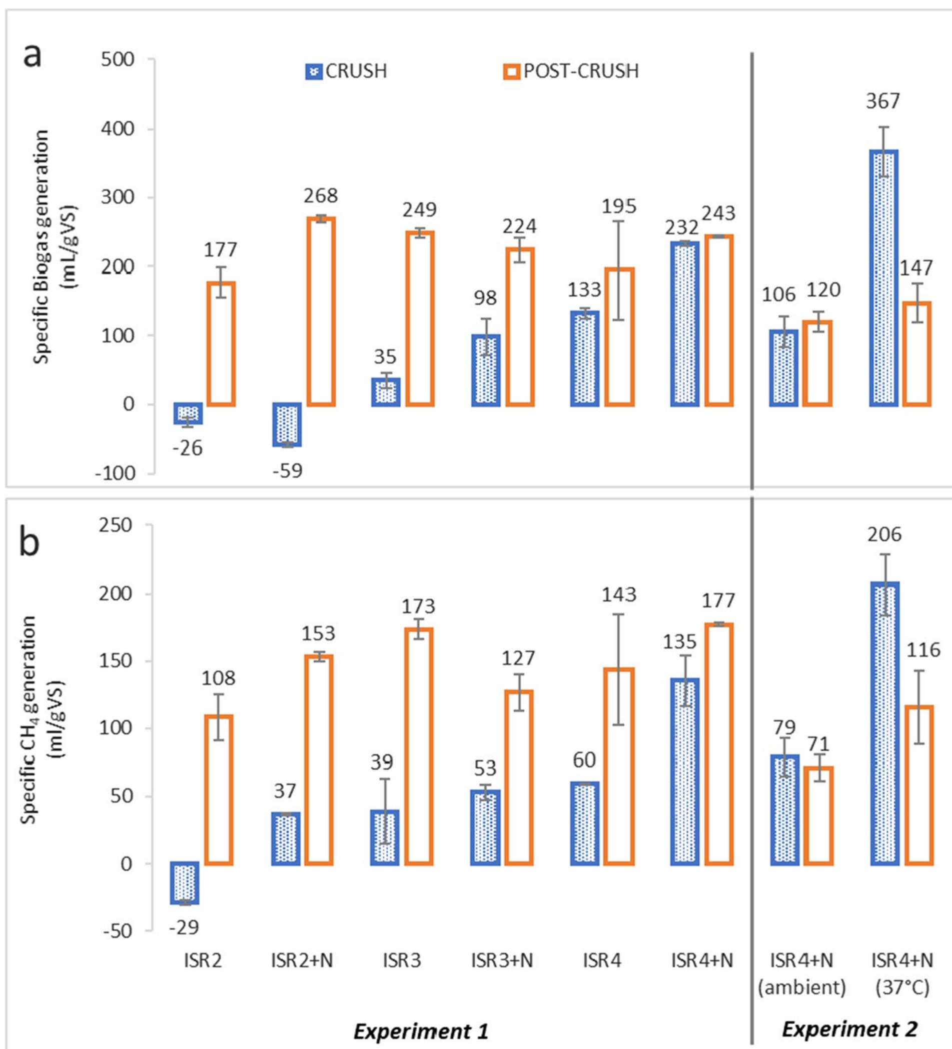
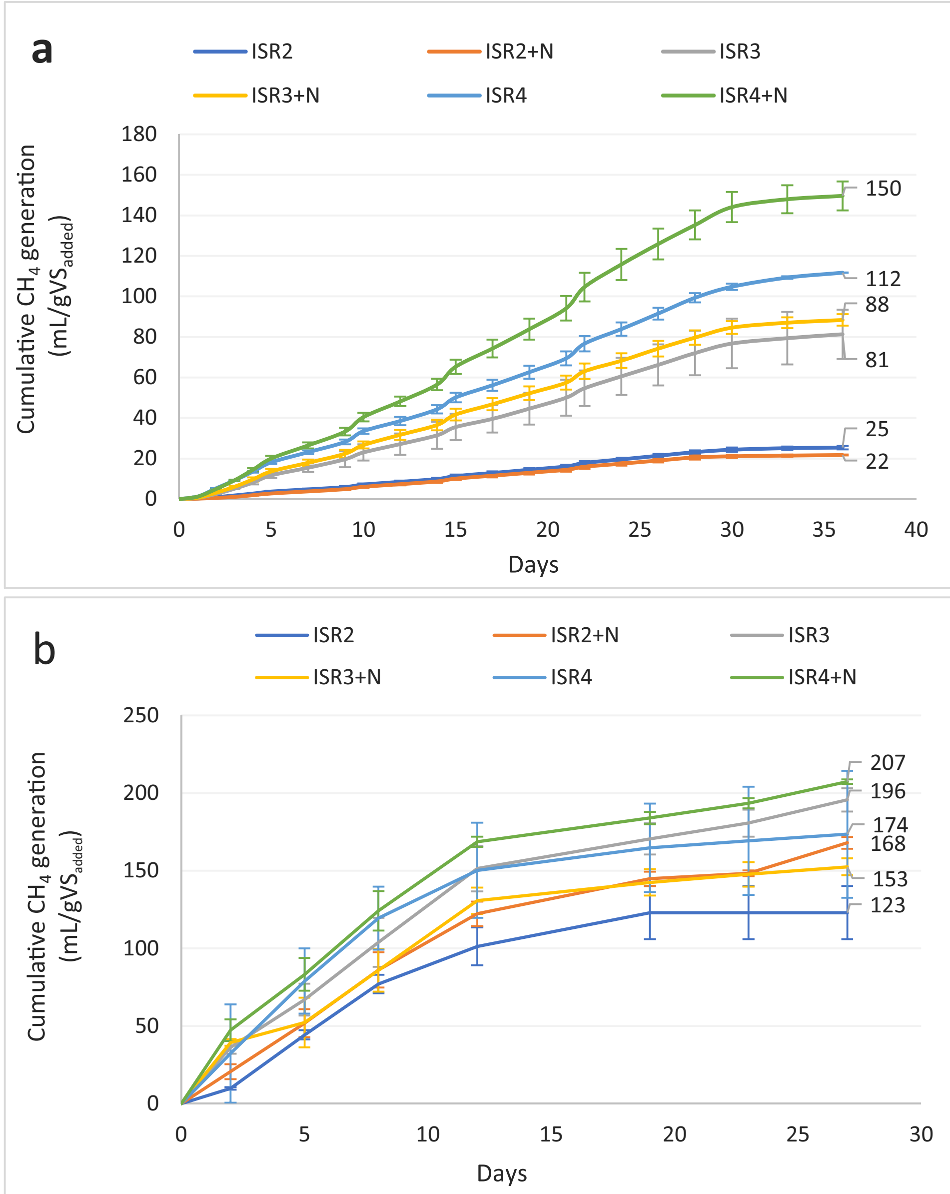



**Fig. 1** Corrected specific biogas (**A**) and methane (**B**) measured in reactors. ISR, inoculum to substrate ratio; N, (micro) nutrients (Co, Cu, Ni). Experiment 1 at ambient temperature and experiment 2 at ambient and 37 °C

**Fig. 2** Cumulative methane generation with crush season sludge (**A**) and post-crush season (**B**) primary winery wastewater sludge at ambient temperatures




this may be acceptable if a similar substrate is being tested, the sudden exposure of sensitive functional microbial species (such as methanogens) to completely different physicochemical environments may not be prudent. For example, it has been shown that methanogenesis was completely inhibited using a non-acclimated inoculum from digesters treating abattoir effluent [46], while good methanogenesis was obtained using a specifically pre-acclimated inoculum to digest the same tannery effluent [47].

In this study, the advantage of using acclimated inocula was jeopardized by the presence of residual substrate in the inoculum at the start of the experiments. Ideally, the substrate should be depleted in inocula used for BMP experiments [45]. The residual substrate was due to the nature of the PWWS that contained slowly biodegradable lignocellulosic material from the grape pomace, making substrate exhaustion impossible without compromising on the viability of the sensitive methanogenic populations via starvation. The presence of residual substrate resulted in biogas and $CH_4$ generation in the inoculum control. However, the origin of the substrate in the inoculum was the same as the experimental substrate, and the specific yields were corrected to include the biogas contribution from the inoculum in the results (Fig. 1). Notwithstanding the biogas contribution from the inoculum substrate, around 1.5 L $CH_4$ was generated from 1 L reactor contents at ISR2 during experiment 1 (Fig. 2A).

To assess the degree to which AD could be enhanced at under stable mesophilic conditions, the optimal $CH_4$ generation conditions (ISR4 with nutrient addition) were applied under both ambient and controlled mesophilic (37 °C) conditions simultaneously (experiment 2, Fig. 1). Under ambient conditions, the biogas and $CH_4$ yields were lower than those attained during experiment 1, once again demonstrating the variability in biogas yields that can be obtained when even slightly different inocula are used. In this case, all of the other experimental factors remained consistent between experiment 1 and experiment 2. The same inoculum had been maintained by feeding with the same PWWS. The results suggest that it is impossible to avoid temporal variations in the microbial community structure and function when maintaining inocula. However, the 2.6-fold higher specific $CH_4$ generation obtained at 37 °C clearly demonstrated that, as expected, performance efficiency could be increased significantly by increasing and controlling the temperature within an optimal range.

In comparison to the theoretical yield (530 $mLCH_4/gVS_{added}$ and 58% $CH_4$) calculated from the COD:VS ratio [41], the maximum yield (206 ± 2.7 $mLCH_4/gVS_{added}$) of the crush season PWWS was only 39% of the theoretical, but the $CH_4$ composition of the biogas (56%) was close to the theoretical value.

### 3.2.2 Anaerobic digestion of primary winery wastewater sludge from the post-crush season

In contrast to results obtained with crush season PWWS, no clear trend on the effect of ISR and nutrient addition on AD efficiency of post-crush PWWS was demonstrated (Fig. 1). However, in terms of absolute cumulative $CH_4$ generation (Fig. 2B), the highest yield was obtained at ISR2 with nutrient addition, and the lowest at ISR4, strongly suggesting that there were sufficient active functional microbes present at ISR2 for efficient methanogenesis. At ambient temperature (experiment 1), the yields attained were all higher with the post-crush season PWWS than with the crush season PWWS. This may have been related to the inoculum, and/or the presence of higher concentrations of readily biodegradable organics, including VFAs in the post-crush PWWS (COD 346 g/L, VFA 104 g/L) than in the crush PWWS (COD 149 g/L, VFA 29 g/L) and/or the use of a more robust inoculum.

The results of experiment 2 (ambient vs 37 °C) showed a similar trend to those obtained during AD of crush season PWWS in that substantially lower specific biogas and $CH_4$ yields were obtained at ambient temperature (ISR4 with nutrients) during experiment 2 than during experiment 1. In contrast to the results obtained with crush season PWWS, the amount of biogas was only negligibly higher (27 $mL/gVS_{added}$) at 37 °C than under ambient conditions during experiment 2. However, due to a promisingly high $CH_4$ contribution at 37 °C (79%) when compared to ambient conditions (59%), there was a greater difference in the specific $CH_4$ yield (45 $mL/gVS_{added}$). In comparison to the theoretical yield (496 $mLCH_4/gVS_{added}$) calculated from the COD:VS ratio [41], the maximum yield (177 ± 1.4 $mLCH_4/gVS_{added}$) was only 36% of the theoretical maximum, but the $CH_4$ composition of the biogas fell within the theoretical range (50–71%) under ambient conditions and was notably higher than the theoretical yield at 37 °C.

The differences in yields in experiments 1 and 2 with the same substrate at ambient temperatures could be explained by the fact that the average ambient temperatures during experiment 1 (20.7 ± 2.0 °C) were higher than those during experiment 2 (19.8 ± 1.87). However, greater temperature variations (range of temperatures) occurred during experiment 1 (16.8 to 27.1 °C) in comparison with experiment 2 (15.3 to 22.8 °C), which could theoretically lead to greater methanogenic community instability and reduced AD efficiency [48]. Furthermore, in comparison to lower temperatures, mesophilic and thermophilic temperatures typically improve reaction kinetics and promote methanogenesis [48–50]. Therefore, temperature differences and fluctuations do not appear to explain why the yields were also less at 37 °C than at ambient temperatures during experiment 2, even though higher temperatures can promote some reactor instability due to an increased rate of dissociation/solubilization of inhibitors as previously described [28].

Apart from the complexity of the physicochemical and biochemical reactions, these results appear to confirm that that the inoculum (as the only other variable) was less active during experiment 2, once again highlighting (i) the importance of having a suitable functional microbial consortium for BMP experiments and for larger scale reactor start-up and (ii) the inherent difficulty of comparing intra- and inter-study results. Typically, although BMP studies often include multiple variables, they do not account for temporal microbial variations as they are conducted as singular experiments.

Notwithstanding the inherent difficulties already alluded to regarding inter- and intra-study comparisons, results were compared with those reported in literature for AD of winery solid wastes under mesophilic conditions. All but one study used sludge from municipal wastewater treatment plants as the inoculum. Higher specific $CH_4$ yields (365 ± 20 $mLCH_4/gVS$) were obtained digesting pomace in 250-mL semi-continuous reactors [25]. However, the hydraulic retention time of 104 days was considerably longer than with PWWS where most biogas was generated within 30 days (Fig. 2). The highest specific $CH_4$ yields were obtained for batch AD of lees (876 ± 45 $mLCH_4/gVS$), grape must (838 $mLCH_4/gVS$), and SWWS (690 ± 25 $mLCH_4/gVS$) in 500 mL digesters with 57 days RT [21, 27]. These substrates have also been successfully co-AD at pilot scale in 230-L continuously stirred tank reactors, but only the overall biogas measurements were reported in this case (average 0.450 $m.^3/kg\ COD_{fed}$) [23]. It has been calculated that sufficient biogas could be generated from co-AD of these substrates to support winery operations [21]. The lowest yields (6.45 $mLCH_4/gVS$) were obtained with co-AD of dried spent grape pomace and cheese whey (100-mL digesters, 58 days), despite using a well-acclimated inoculum from a laboratory digester [24]. Similarly, the AD of stems, PWWS, and secondary WWS were stunted (< 30 $mLCH_4/gVS$) likely due to poor acclimation of inoculum to substrate [27]

Overall, this study demonstrated that PWWS is a suitable substrate for AD, with no lag phase and complete digestion taking place within 30–40 days even under ambient conditions. In general, nutrient addition and controlled higher temperatures (37 °C) enhanced biogas and $CH_4$ generation at higher ISRs, indicating the increased metabolic co-factor demand associated with higher concentrations and activities of the functional microbial communities (higher ISR).

#### 3.2.3 Evaluation of methane generation kinetic models

To evaluate the effectiveness of kinetic models for predicting the AD of PWWS, commonly applied models (cone, logistic, modified Gompertz, and first order) were fitted to the cumulative $CH_4$ production from experiment 1 for both crush and post-crush PWWS. In both cases, the cone, logistic, and first-order models fitted the experimental data with high precision (adjusted $R^2 > 0.958$). For optimal reactors (ISR4 + N), the models predicted the experimental data in the order: Logistic > Cone > First order > modified Gompertz according to AIC (Akaike information criterion) for the crush season PWWS, and predicted maximum $CH_4$ yields of 151.5, 142.0, 144.1, and 121.2 $mlCH_4/gVS_{added}$, respectively. For the post-crush PWWS, the model fit order was Cone > First order > Logistic > modified Gompertz, with predicted maximum methane yields of 199.4, 203.8, 195.2, and 155.7 $mlCH_4/gVS_{added}$, respectively. For reference, the complete set of calculated kinetic parameters has been provided in the Supplementary Material (Supplementary Tables 2 and 3).

### 3.3 Evaluation of digestate of crush and post-crush primary winery wastewater sludge as a soil conditioner/fertilizer

In-depth theoretical analyses of fertilizers need to account for a variety of different soil types and plants, ideally including pot and/or field experiments. Such analyses were beyond the scope of this study which was instead limited to a brief evaluation of the PWWS as a "proof of concept" to determine whether it may be suitable as a fertilizer. This was achieved by (i) comparing the macronutrient and micronutrient composition of the digestates with typical organic fertilizers and (ii) evaluating whether any elements were present in quantities that may be detrimental to plant and/or soil health.

The PWWS digestate composition was compared with a range of four commercial agricultural organic fertilizers based on composted chicken manure. Two of these were enriched, one with kelp and fishmeal, and the other with Ca and P. Both inorganic and organic forms of C are important as much C is being lost from the soil due to agriculture, other anthropogenic land use practices, and climate change across the globe [51, 52]. The total C concentrations were 2.0- to 2.6-fold higher in the PWWS than in the commercial fertilizers, indicating that would be a valuable source of C as a fertilizer. In terms of organic C, decayed organic material (humus) increases the water retention capacity and the cation exchange capacity of soils, assists with soil aeration, and provides a reservoir for gradual release of plant nutrients [53, 54]. All the PWWS digestates contained notable amounts of organic carbon (measured as TOC), and the amount was higher in the crush season PWWS digestate (average 261 g/kg) than in the post-crush season PWWS digestate (average 104 g/kg) (Table 3). Given the worldwide average soil organic carbon (SOC) concentration of 15.5 g/kg, even the post-crush PWWS could be a valuable contributor of humic material, especially in the case of sandy soils [55].

Different plants require macronutrients in different quantities, and some soils themselves contain sufficient elements. For example, as the name suggests, calcareous soils have high Ca concentrations, but have limited P and Zn availability [56]. To cater for different requirements, fertilizers contain variable ratios of the macronutrients N, P, and K, among other elements. The PWWS digestates contained similar amounts of N and K to the commercial fertilizers but were deficient in P, Ca, and S (Table 4). Depending on the soil type and crop, the PWWS may require supplementation with a source of these elements. Waste gypsum is an example of a sustainable source of Ca and S. In terms of micronutrients, both batches of PWWS digestates contained reasonable concentrations in comparison to the commercial organic fertilizers (Table 4), but both the commercial fertilizers and the PWWS contained high concentrations of Fe. Although Fe deficiency can be detrimental to plant growth, it can be toxic to plants in highly acidic or hypoxic soils (usually formed via waterlogging) as $Fe^{3+}$ is reduced to $Fe^{2+}$ which is more bioavailable than the oxidized form [26]. In well-aerated less acidic to alkaline soils, relatively high Fe concentrations are generally not problematic [26].

Unlike K, Na is not a plant nutrient and can negatively affect the soil structure by binding with negatively charged soil particles. This can be offset to some extent by the presence of divalent cations ($Ca^{2+}$ and $Mg^{2+}$) that have more than one binding site and replace $Na^+$ [57]. Although the Na

**Table 4** Concentrations of essential plant nutrients, sodium, and sodium adsorption ratio in the digestates from optimal anaerobic digestion at 37 °C and ambient temperatures

| | Crush PWWS | | Post-crush PWWS | | Commercial* |
|---|---|---|---|---|---|
| | 37 °C | Ambient | 37 °C | Ambient | |
| Macronutrients (g/kg dry weight) | | | | | |
| *Elemental CHNS analysis* | | | | | |
| C | 256.9 ± 8.1 | 277.8 ± 1.5 | 271.7 ± .9.1 | 229.2 ± 4.9 | 106–115 |
| H | 40.9 ± 3.7 | 43.8 ± 2.6 | 30.7 ± 1.1 | 51.0 ± 1.9 | - |
| N | 25.5 ± 1.4 | 25.6 ± 0.8 | 27.7 ± 0.1 | 21. ± 0.5 | 26–34 |
| S | 3.0 ± 0.2 | 3.0 ± 0.02 | 5.1 ± 0.5 | 3.6 ± 1.1 | 9–10 |
| *XRF analyses* | | | | | |
| Ca | 10.5 ± 0.24 | 9.8 ± 1.57 | 10.36 ± 0.83 | 9.69 ± 1.66 | 35–60 |
| K | 38.3 ± 2.11 | 37.9 ± 3.47 | 38.42 ± 1.91 | 27.67 ± 6.63 | 30–33 |
| Mg | 2.20 ± 0.07 | 2.00 ± 0.01 | 2.93 ± 0.09 | 2.38 ± 0.55 | 6–7 |
| P | 3.4 ± 0.01 | 3.3 ± 0.51 | 5.15 ± 0.43 | 4.47 ± 0.69 | 17–27 |
| Micronutrients (mg/kg dry weight, **g/kg dry weight) | | | | | |
| *XRF analyses* | | | | | |
| Fe** | 5.1 ± 0.01 | 5.0 ± 0.02 | 5.72 ± 0.04 | 4.76 ± 1.57 | 5 |
| B | 62 ± 11 | 57 ± 1.4 | 50 ± 0.1 | 40 ± 11 | 50 |
| Mn | 80 ± 1.1 | 70 ± 1.6 | 90 ± 1.0 | 80 ± 22 | 570–610 |
| Cu | 25 ± 2.2 | 23 ± 1.1 | 250 ± 17 | 180 ± 56 | 60 |
| Zn | 57 ± 2.8 | 49 ± 1.8 | 620 ± 7.9 | 450 ± 133 | 500–540 |
| Mo | 2.0 ± 0.1 | 4.0 ± 0.0 | ND | ND | 5 |
| Other (g/kg dry weight) | | | | | |
| TOC | 282 ± 13.0 | 241 ± 17.1 | 98.7 ± 10.6 | 110 ± 2.82 | ND |
| Na | 3.20 ± 0.17 | 3.10 ± 0.19 | 3.26 ± 0.08 | 2.28 ± 0.57 | ND |
| Ratios | | | | | |
| C:N | 10.08 | 10.87 | 9.82 | 10.66 | 3.73 |
| SAR | 1.3 | 1.3 | 1.2 | 0.9 | ND |

*Range of organic fertilizers based on composed chicken manure (Bio ganic® Bio ganic crumble®), enriched with kelp and fishmeal (Bio ocean®), enriched with calcium and phosphate (Bio rock®). *TOC*, total organic carbon; *SAR*, sodium adsorption ratio; *ND*, not determined

concentrations in the digestates were high (Table 4), the concentrations in the PWSS before AD were relatively low in comparison to the inocula (Table 4), clearly demonstrating that most of the Na in the digestates emanated from the inocula. The original starter culture was taken from AD of highly saline tannery effluent, and thereafter, the inoculum was fed constantly with WWW which also resulted in accumulation of Na over time. In reality, Na remains soluble in WWW, and is not expected to accumulate to high concentrations in PWWS [1, 38] as evidenced by the results in Table 3. Even with the anomalously high concentrations measured in the PWWS digestates, any sodicity risk was offset by high concentrations of Ca and Mg that reduced the sodium adsorption ratio (SAR) to ≤ 1.3. To put this in perspective, soils with and SAR > 13 are considered sodic [57, 58] and those with SAR < 5 pose a low risk [59]. Nevertheless, the Na levels will be closely monitored in future studies.

For reference, the character of the digestates from all of the reactor contents is included in the Supplementary Material (Supplementary Table 5).

Overall, the composition of the PWWS showed excellent potential for addition of C, N, and micronutrients to soils. Further work will be conducted using pot experiments to determine the effects of PWSS digestates on crop growth in different soils.

## 4 Conclusions

Concurrent AD of PWWS for bioenergy and biofertilizer supports a circular economy. During the warmer post-crush months, good biogas and $CH_4$ yields from AD may be feasible without the need for heating, making it economically viable for smaller wineries. The digestate is a promising agricultural fertilizer, especially for addition of C, N, and micronutrients to soils. To prevent potential Fe toxicity, it is recommended that it should not be used in highly acidic or waterlogged soils. Future studies will focus on conducting pot experiments with different batches of digestates and comparing the results.

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## Declarations

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## References

1. Welz PJ, Holtman GA, Haldenwang R, Le Roes-Hill M (2016) Characterisation of winery wastewater from continuous-flow settling basins and waste stabilisation ponds over the course of one year:implications for biological wastewater treatment and land application. Water Sci Technol 74:2036–2049. https://doi.org/10.2166/wst.2016.226
2. Ahmed N, Yadav V, Yadav A, Rahman MU, Yuan WZ, Li Z, Wang X (2020) Integrated biorefinery approach to valorize winery waste: A review from waste to energy perspectives. Sci Tot Env 719:137215. https://doi.org/10.1016/j.scitotenv.2020.137315
3. Beres C, Costa GNS, Cabezudo I, da Silva-James NK, Teles ASC (2017) Towards integral utilization of grape pomace from winemaking process: A review. Waste Manag 68:581–594. https://doi.org/10.1016/j.wasman.2017.07.017
4. Devesa-Rey R, Vecino X, Varela-Alende JL, Barral MT, Cruz JM, Moldes AB (2011) Valorization of winery waste vs. the costs of not recycling. Waste Manag 18:2327–2336. https://doi.org/10.1016/j.wasman.2011.06.001
5. Filippi K, Papapostolou H, Alexandri M, Vlysidis A, Mytsi ED, Ladakis D, Pateraki C, Haroutounian SA, Koutinas A (2022) Integrated biorefinery development using winery waste streams for the production of bacterial cellulose, succinic acid and value-added fractions. Bioresour Technol 343:125989. https://doi.org/10.1016/j.biortech.2021.125989
6. Ilyas T, Chowdary P, Chaurasia D, Gnansounou E, Pandey A, Chaturvedi P (2021) Sustainable green processing of grape pomace for the production of value-added products: A review. Environ Technol Innov 23:101592. https://doi.org/10.1016/j.eti.2021.1015
7. Sirohi R, Tarafdar A, Singh S, Negi Y, Gaur VK, Gnansounou E, Bharathiraja B (2020) Green processing and biotechnological opportunities of grape pomace: Current trends for sustainable biorefinery. Bioresour Technol 314:123771. https://doi.org/10.1016/j.biortech.2020.123771
8. Zhang N, Hoadley A, Patel J, Lim S, Li C (2017) Sustainable options for the utilization of solid residues from wine production. Waste Manag 60:173–183. https://doi.org/10.1016/j.wasman.2017.01.006
9. Benetto E, Jury C, Kneip G, Vazquez-Rowe I, Huck V, Minette F (2015) Life cycle assessment of heat production from grape marc pellets. J Cleaner Prod 87:149–158. https://doi.org/10.1016/j.jclepro.2014.10.028
10. Muhlack RA, Potumarthi R, Jeffery DW (2018) Sustainable wineries through waste valorisation: A review of grape marc utilisation for value-added products. Waste Manag 72:99–118. https://doi.org/10.1016/j.wasman.2017.11.011
11. Pinga L, Brosse N, Chrusciel L, Navarrete P, Pizzi A (2011) Extraction of condensed tannins from grape pomace for use as wood adhesives. Indust Crops Products 33:253–257. https://doi.org/10.1016/j.indcrop.2010.10.007
12. Schönnenbeck C, Trouvé G, Valente M, Garra P, Brilhac JF (2016) Combustion tests of grape marc in a multi-fuel domestic boiler. Fuel 180:324–331. https://doi.org/10.1016/j.fuel.2016.04.034
13. Zabaniotou A, Kamaterou P, Pavlou AA, Panayiotou C (2018) Sustainable bioeconomy transitions: Targeting value capture by integrating pyrolysis in a winery waste biorefinery. J Cleaner Prod 172:3387–3397. https://doi.org/10.1016/j.clepro.2017.11.077
14. Bertran E, Sort X, Soliva M, Trillas I (2004) Composting winery waste: sludges and grape stalks. Bioresour Technol 95:203–208. https://doi.org/10.1016/j.biortech.2003.07.012
15. Gómez-Brandón M, MartÍnez-Cordeiro H, DomÍnguez J (2021) Changes in the nutrient dynamics and microbiological properties of grape marc in a continuous-feeding vermicomposting system. Waste Manag 135:1–10. https://doi.org/10.1016/j.wasman.2021.08.004
16. Hungría J, Gutierrez MC, Siles JA, Martín MA (2017) Advantages and drawback of OFMSW and winery waste co-composting. J Cleaner Produc 164:1050–1057. https://doi.org/10.1016/j.jclepro.2017.07.029
17. Majbar Z, Lahlou K, Abbou MB, Ammar A et al (2018) Co-composting of olive mill waste and wine processing waste: an application of compost as soil amendment. J Chem:1–9. https://doi.org/10.1155/2018/7918583
18. Mtimkulu Y, Meyer AH, Mulidzi AR, Shange PL, Nchu F (2017) Assessing and monitoring the effects of filter material amendments on the biophysicochemical properties during composting of solid winery waste under open field and varying climatic conditions. Waste Manag 59:59–69. https://doi.org/10.1016/j.wasman.2016.10.042
19. Flores L, Garcia J, Pena R, Garfi M (2019) Constructed wetlands for winery wastewater treatment: a comprehensive life cycle assessment. Sci Tot Environ 659:1567–1576. https://doi.org/10.1016/j.scitotenv.2018.12.348
20. Oliveira M, Duarte E (2010) Treatment and use of non-conventional organic residues in agriculture. RAMIRAN International Conference, Lisboa, pp 12–15
21. Montalvo S, Martinez J, Castillo A, Huilñir C, Borja R, GarcÍa V, Salazar R (2020) Sustainable energy for a winery through biogas production and its utilization: A Chilean case study. Sustain Energ Technol Assessments 37:100640. https://doi.org/10.1016/j.seta.2020.100640

22. Lin L, Xu F, Ge X, Li Y (2018) Improving the sustainability of organic waste management practices in the food-energy, water nexus: A comparative review of anaerobic digestion and composting. Renew Sustain Energ Rev 89:151–167. https://doi.org/10.1016/j.rser.2018.03.025
23. Da Ros C, Cavinato C, Pavan P, Bolzonella D (2017) Mesophilic and thermophilic anaerobic codigestion of winery wastewater sludge and wine lees: an integrated approach for sustainable wine production. J Environ Manag 203:745–752. https://doi.org/10.1016/j.jenvman.2016.03.029
24. Kassongo J, Shahsavari E, Bali AS (2022) Substrate-to inoculum ratio drives solid-state anaerobic digestion of unamended grape marc and cheese whey. PLOS One 17:e0262940. https://doi.org/10.1371/journal.pone.0262940
25. Pellera FM, Gidarakos E (2017) Anaerobic digestion of solid agro industrial waste in semicontinuous mode: Evaluation of mono-digestion and co-digestion systems. Waste Manag 68:103–1009. https://doi.org/10.1016/j.wasman.2017.06.026
26. Delias DS, Da-Silva CJ, Martins AC, de Oliveira DSC, do Amarante L (2022) Iron toxicity increases oxidative stress and impairs mineral accumulation and leaf gas exchange in soybean plants during hypoxia. Env Sci Pollut Res 29:22427–22438. https://doi.org/10.1007/s11356-021-17397-3
27. Guerini Filho M, Lumi M, Hasan C, Marder M, Leite LCS, Konrad O (2018) Energy recovery from wine sector wastes: A study about the biogas generation potential in a vineyard from Rio Grande do Sul, Brazil. Sustain Energy Technol Assess 29:44–49. https://doi.org/10.1016/j.seta.2018.06.006
28. Mpofu AB, Welz PJ, Oyekola OO (2020) Anaerobic digestion of secondary tannery sludge:optimization of initial pH and temperature and evaluation of kinetics. Waste Biomass Valoriz 11:873–885. https://doi.org/10.1007/s12649-018-00564-y
29. Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: A review. Bioresour Technol 99:4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057
30. Schmidt T et al (2014) Trace element supplementation in the biogas production from wheat stillage - Optimization of metal dosing. Bioresour Technol 168:80–85. https://doi.org/10.1016/j.biortech.2014.02.124
31. Berhe S, Leta S (2018) Anaerobic co-digestion of tannery wastewater and tannery solid waste using two-stage anaerobic sequencing batch reactor: focus on performances of methanogenic step. J Mater Cycles Waste Manag 20:1468–1482. https://doi.org/10.1007/s10163-018-0706-9
32. Gao S, Zhao M, Chen Y, Yu M, Ruan W (2015) Tolerance response to in situ ammonia stress in a pilot-scale anaerobic digestion reactor for alleviating ammonia inhibition. Bioresour Technol 198:372–379. https://doi.org/10.1016/j.biortech.2015.09.044
33. Welz PJ, Ramond JB, Cowan DA, Prins A, Burton SG (2011) Ethanol degradation and the value of incremental priming in pilot scale constructed wetlands. Ecol Eng 37:1453–1459. https://doi.org/10.1016/j.ecoleng.2011.09.003
34. Buyukkamaci N, Filibeli A (2004) Volatile fatty acid formation in an anaerobic hybrid reactor. Process Biochem. 39:1491–1494. https://doi.org/10.1016/S0032-9592(03)00295-4
35. Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Progress Energy Combust Sci 34:755–781. https://doi.org/10.1016/j.pecs.2008.06.002
36. O'Flaherty V, Lens P, Leahy B, Colleran E (1998) Long-term competition between sulphate reducing and methane-producing bacteria during full-scale anaerobic treatment of citric acid production wastewater. Water Res 32:815–825. https://doi.org/10.1016/S0043-1354(97)00270-4
37. Kibangou VA, Lilly M, Mpofu AB, de Jonge N, Oyekola OO, Welz PJ (2022) Sulfate-reducing and methanogenic microbial community responses during anaerobic digestion of tannery effluent. Bioresour Technol 347:126308. https://doi.org/10.1016/j.biortech.2021.126308
38. Holtman GA, Haldenwang R, Welz PJ (2018) Biological sand filter system treating winery wastewater for effective reduction in organic load and pH neutralisation. J Water Process Eng 25:118–127. https://doi.org/10.1016/j.jwpe.2018.07.008
39. Mpofu AB, Oyekola OO, Welz PJ (2021) Anaerobic treatment of tannery wastewater in the context of a circular bioeconomy for developing countries. J Cleaner Prod 296:126490. https://doi.org/10.1016/j.jclepro.2021.126490
40. Thanh PM, Ketheesan B, Yan Z, Stuckey D (2016) Trace metal speciation and bioavailability in anaerobic digestion: A review. Biotechnol Adv 34:122–136. https://doi.org/10.1016/j.biotechadv.2015.12.006
41. Angelidaki I, Sanders W (2004) Assessment of the anaerobic biodegradability of macropollutants. Rev Env Sci Biotech 3:117–129. https://doi.org/10.1007/s11157-004-2502-3
42. Bayard R, Benbelkacem H, Gourdon R, Buffière P (2017) Characterization of selected municipal solid waste components to estimate their biodegradability. J Env Manag 87:1–9. https://doi.org/10.1016/j.jenvman.2017.04.087
43. Buswell AM, Neave SL (1930) Laboratory studies of sludge digestion. Illinois State Water Survey 30(85). https://hdl.handle.net/2142/94553
44. Kesharwani N, Bajpai S (2021) Pilot scale anaerobic co-digestion at tropical ambient temperature of India: Digester performance and techno-economic assessment. Bioresour Technol Reports 15:100715. https://doi.org/10.1016/j.biteb.2021.100715
45. Holliger C, Alves M, Andrad D, Angelidaki I, Astals S, Wierinck I et al (2016) Towards a standardization of biomethane potential tests. Water Sci Technol 74:2515–2522. https://doi.org/10.2166/wst.2016.336
46. Horn EJ, Oyekola OO, Welz PJ, van Hille RP (2022) Biological desulfurization of tannery effluent using hybrid linear flow channel reactors. Water 14:32. https://doi.org/10.3390/w14010032
47. Mpofu AB, Kaira WA, Oyekola OO, Welz PJ (2022) Anaerobic co-digestion of tannery effluents:Process optimization for resource recovery, recycling and reuse in a biocircular economy. Process Safety Env Protec 158:547–559. https://doi.org/10.1016/j.psep.2021.12.027
48. Yu Q, Feng L, Zhen X (2021) Effects of organic loading rate and temperature fluctuation on the microbial community and performance of anaerobic digestion of food waste. Env Sci Pollut Res 28:13176–13187. https://doi.org/10.1007/s11356-020-11548-8
49. Kai Y, Gallegos D, Zheng Z, Stinner W, Wang X, Pröter J, Schäfer F (2021) Exploring the combined effect of total ammonia nitrogen, pH and temperature on anaerobic digestion of chicken manure using response surface methodology and two kinetic models. Bioresour Technol 337:125328. https://doi.org/10.1016/j.biortech.2021.125328
50. Rahman MA, Shahazi R, Nova SNB, Uddin MR, Hossain MS, Yousuf A (2022) Biogas production from anaerobic co-digestion using kitchen waste and poultry manure as substrate – part 1: substrate ration and effect of temperature. Biomass Conv Biorefin 9:1–11. https://doi.org/10.1007/s13399-021-01604-9
51. Dash PK, Bhattacharyya P, Roy KS, Neogi S, Nayak AK (2019) Environmental constraints' sensitivity of soil organic carbon decomposition to temperature, management practices and climate change. Ecol Indicators 107:105644. https://doi.org/10.1016/j.ecolind.2019.105644
52. Lin H, Zhou M, Zheng F, Xu P, Ma S, Zhang B, Li Z, Wang Y, Zhu B (2022) How do soil organic carbon pool, stock and their stability respond to crop residue incorporation in subtropical

calcareous agricultural soils? Agric Ecosys Env 332:107927. https://doi.org/10.1016/j.agee.2022.107927
53. Moore R, Clark WD, Stern KR, Vodopich D (1995) Botany. Wm. C. Brown Publishers, Dubuque
54. Zhang J, Chi F, Wei D, Zhou B, Cai S, Li Y, Kuang E, Sun L, Li L-J (2019) Impacts of long-term fertilization on the molecular structure of humic acid and organic carbon content in soil aggregates in black soil. Sci Reports 9:11908. https://doi.org/10.1038/s41598-019-48406-8
55. Deluz C, Nussbaum M, Sauzet O, Gondret K, Boivin P (2020) Evaluation of the potential for soil organic carbon content monitoring with farmers. Front Env Sci 8:113. https://doi.org/10.3389/fenvs.2020.00113
56. Akhtar M, Yousaf S, Sarwar N, Hussain S (2019) Zinc biofortification of cereals – role of phosphorus and other impediments in alkaline calcareous soils. Environ Geochem Health 41:2365–2379. https://doi.org/10.1007/s10653-019-00279-6(0123456789
57. Jesus JM, Danko AS, Fiuza A, Borges M-T (2018) Comparison of vegetative bioremediation and chemical amendments for non-calcereous highly saline-sodic soil remediation. Water Air Soil Pollut 229:274. https://doi.org/10.1007/s11270-018-3928-5
58. Seeling BD (2000) Salinity and sodicity in North Dakota soils, EB-57 edn. North Dakota State University, Fargo
59. Re MZ et al (2022) Managing Salt-affected Soils for Crop Production. Pacific Northwest Extension Publication 601:1–24