# Physicochemical, Microbial, and Microbiome Dynamics in Winery Waste Composting

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## Abstract

**Purpose:** The aim of the present study was to investigate the physicochemical, microbial, and microbiome dynamics during the microbial composting of winery waste and assess the suitability of the resulting compost as a biofertilizer for grapevine growth.

**Methods:** A pilot-scale composting system was utilized and samples were collected over a 60-day period to study the physicochemical parameters, enzymatic activity, microbial counts, and microbiome composition. Additionally, the potential phytotoxicity of the compost was evaluated using a germination index test with barley seeds and its effectiveness as a substrate for grapevine growth was assessed in a proof-of-concept study.

**Results:** Throughout the microbial composting process, moisture content, pH, temperature, conductivity, and C/N ratio were monitored, indicating dynamic microbial activity. Enzymatic activity increased initially and decreased as compost matured. Microbial populations showed shifts with significant changes in bacterial and fungal communities over time. The compost exhibited no phytotoxicity and supported grapevine growth with percentage yields of leaf dry matter comparable to commercial substrates.

**Conclusion:** Winery waste microbial composting led to stable biofertilizer production, evidenced by physicochemical stability, lack of phytotoxicity, and effectiveness in promoting grapevine growth. These findings suggest the potential of composting as a sustainable waste management solution in the winemaking industry, though further research is required to fully understand the mechanism of the bioprocess and optimize composting protocols.

**Keywords:** composting, winery waste, biofertilizer, microbiome dynamics

## Introduction

Annually, the winemaking industry generates 0.3–0.5 kg of wine by-products/L, including winter pruning by-products that can be toxic if disposed of without pretreatment, due to high content of organic load and phytotoxic compounds along with high acidity [1]. Until recently, such wastes have been generally used for distillation, landfilling, incineration, and/or land-spreading [1,2]. The last decades, composting has attracted considerable attention as a sustainable and environmentally-friendly alternative for the treatment of agro-industrial wastes.

Composting is the natural process of transforming organic matter to fertilizers, rich in essential nutrients for plant growth by microorganisms under controlled conditions [3]. During the bioprocess, organic matter is decomposed with the help of various microbes and it is divided into three phases, based on the temperature of the biomass: (1) the mesophilic phase (20-40°C), which typically lasts a few days (5-10 days), (2) the thermophilic phase (35-65°C), which can last from a few days to several months, and (3) the ripening phase [4]. The high temperatures that develop are the result of intense microbial activity during the first two phases and are responsible for eliminating the pathogenic microorganisms. During the different temperature phases, diverse microbial species dominate. In the first stage, soluble and easily degradable compounds, such as sugars and proteins, are broken down and a sharp rise in temperature is observed. In the second stage, once the temperature exceeds 40°C, mesophilic are replaced by thermophilic microorganisms [5]. In the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats, and complex carbohydrates like cellulose that is the main structural molecule in plants. As the concentration of these compounds is depleted, the temperature gradually decreases and the

mesophilic microorganisms take over the final phase (ripening phase). Usually, in this phase, the fungal population increases, while the bacterial population decreases. Aerobic microorganisms prevail when oxygen levels are greater than 5% and their growth is sought, as they are the most important initiators of the process [5, 6].

Microbial composting systems are divided into open and closed systems. In the open-type systems, the biomass to be composted is stacked in long parallel rows, the height, width, and shape of which are adjusted according to the type of the material to be processed and the type of equipment used for stirring. In closed systems, composting takes place in special bioreactors [7, 8].

The organic substrate used in the composting process affects microbial populations, reflecting thus the dynamics of enzymatic activity, decomposition of organic matter, and nitrogen transformations. However, the microbiome associations during the whole process are still unexplored. Thus, the aim of the present study was to investigate the microbiome dynamics along with the physicochemical, enzymatic, and microbial changes during the process of winery wastes composting (plant biomas, vine shoots, grape stalks, grape pomace, and wine lees from vineyards) in a windrow pile system aerated by agitation and potential phytotoxicity of the compost was evaluated using a germination index test with barley seeds. Noticeably, the effectiveness of the final product was further verified in a pilot scale cultivation of vineyards (proof-of-concept study).

## Material and methods

### Windrow pile system and sample collection

A pilot scale windrow pile with a 3:1width/height ratio loaded with winery waste (plant biomas, vine shoots, grape stalks, grape pomace, and wine lees from vineyards) was used and mechanical agitation was applied every 3 days with a suitable compost stirrer. The windrow pile was loaded with 70% carbon-rich materials (5 kg vine stems and branches and 15 kg grape marc), 30% nitrogen -rich materials (6 kg grass), and 1.5 kg of soil, according to Barros et al. [9]. Branches and grass were shredded using an electrical shredder (STIHL, GHE 355) before loading to the windrow pile.

Samples (~120 g) were collected every 10 and for up to 60 days and stored at -20 ℃ until analysis [10].

### Chemical analysis

### Moisture Content, pH, Conductivity, and Temperature

The samples were dried at 121°C for 12 h and the moisture content was assessed by measuring the weight loss. Temperature and conductivity were measured using a stainless steel composting waterproof thermometer with a temperature range 0–250°C and a portable conductivity meter (CON150EUTECH, Thermoscientific, Singapore), respectively. The samples were diluted with water at a ratio of 1:10 (weight/volume) and the pH of the resulting supernatant was determined using a pH meter (HANNA, Limassol, Cyprus).

### Total Carbon (C) and Nitrogen (N), C/N Ratio, and $O_2$ Uptake Rate

Total carbon (C) and total nitrogen (N) were assessed, as described before by Yeomans et al. [11] and Bremner et al. [12], respectively (minimum detection limit for C was 3% and 0.75% for N). The oxygen ($O_2$) uptake rate was measured directly using a portable oximeter linked to a 1.5-meter catheter equipped with an $O_2$-measuring sensor (HANNA, Limassol, Cyprus).

### Macro- and Micronutrients

The carbonate content was measured using a calcimeter (HANNA, Cyprus). Phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu) levels were determined via atomic absorption spectrophotometry using a General Electric photometer (Agrosoil hardware, Bedfordshire, UK), following the protocol described in EN ISO 9001:2000 [13]. The minimum detectable limit was 0.05 ppm.

### Enzymatic Activity

Dehydrogenase activity was assessed using a colorimetric method, as previously described [1, 14]. Briefly, compost samples were mixed with $CaCO_3$ and a solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) and then incubated at 37°C for 24h. After incubation, 25mL of methanol was added and the solution was filtered and diluted again in 50mL of methanol. Absorbance was measured at 485 nm using a microplate reader and the dehydrogenase activity was calculated based on the following equation: [(S−C) × 100] / (6 × % dm), where S is the mean value of the samples (μg TPF), C is the value of the control (μg TPF), 6 is the initial soil weight (g) and % dm is the percentage of soil dry matter of each sample, expressed as g TPF/g (dry matter)/24 hours.

**Microbiological Analysis**

Samples (25 g) were homogenized with sterile ¼ Ringer's solution (225 mL), followed by serial dilutions and plate counting. The microbial populations were determined as follows: (a) Total Aerobic Count (TAC) by plating on Plate Count agar (Condalab, Madrid, Spain) at 30°C for 72 h; (b) *Lactobacillus* spp. (Lactic Acid Bacteria, LAB) by plating on acidified MRS agar at pH 5.7 (Condalab) at 37°C for 72 h under anaerobic conditions (Merck Millipore Anaerobic Jar 2.5 L, Merck Millipore 2.5 L Sachets); (c) *Enterobacteriaceae* by plating on Violet Red Bile Glucose agar (VRBG) (Condalab) at 37°C for 24 h; (d) Coliforms by plating on Violet Red Bile agar (VRBA) (Condalab) at 37°C for 24 h; (e) *Clostridium* spp. by plating on Tryptose Sulfite Cycloserine agar (Condalab) at 37°C for 48 h under anaerobic conditions (Merck Millipore Anaerobic Jar 2.5 L, Merck Millipore 2.5 L Sachets); (f) *Escherichia coli* by plating on Harlequin™ Tryptone Bile Glucuronide agar (TBX) (LABM, Heywood, UK) at 37°C for 24 h; (g) *Salmonella* spp. by plating on Xylose Lysine Deoxycholate agar (VWR International GmbH, Darmstadt, Germany) at 37°C for 24 h; and (h) Yeasts and molds by plating on Malt Extract agar (Condalab) at 30°C for 72 h. Cell levels were expressed as log cfu/g.

**DNA Extraction, PCR Amplification, and 16S and ITS rRNA Sequencing**

Samples were collected at the beginning (day 0) and at the end (day 60) of the process. Total DNA extraction was conducted using the NucleoSpin® Soil kit (MACHEREY-NAGEL GmbH & Co., KG, Düren, Germany), according to the manufacturer's instructions. Next-generation sequencing (NGS) was carried out by MR DNA using MiSeq sequencing (http://www.mrdnalab.com, Shallowater, TX, USA), as previously described [1]. In brief, for bacterial analysis, the V1-V3 region of the 16S rRNA gene was amplified with 27F and 519R primers (5'-AGRGTTTGATCMTGGCTCAG-3' and 5'GTNTTACNGCGGCKGCTG-3', respectively). Fungal analysis involved amplification of the highly variable Internal Transcribed Spacer (ITS) regions with ITS1 and ITS4 primers (5'-TTGGTCATTTAGAGGAAGTAA-3' and 5'-TCCTCCGCTTATTGATATGC-3', respectively). Archaeal analysis targeted the 16S rRNA gene region using Arch2A519F and Arch1017R primers (5'-CAGCMGCCGCGGTAA-3' and 5'-GGCCATGCACCWCCTCTC-3', respectively). PCR amplification consisted of 30–35 cycles, followed by purification of the amplicons using Ampure XP beads. Prepared samples were then processed for Illumina DNA library creation and sequencing data was analyzed using MR DNA's proprietary pipeline. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence, taxonomically classified using BLASTn against curated databases, and low-abundance OTUs (< 0.01) were removed. Raw data analysis and calculation of a- diversity were conducted using the Rhea platform [15].

**Germination Index**

The potential phytotoxicity of the final product was evaluated by seed germination and calculation of the germination index, according to Paradelo et al. [16]. An aqueous extract [prepared by mixing the compost product with deionized water (1/10 w/v), followed by incubation at 20°C under agitation (60 rpm/min) for 2h, then filter paper and sterilized by a 0.22 μm filter] was used to soak barley seeds (50 barley seeds in 50 mL aqueous extract) and after 24h, 15 barley seeds were incubated in petri dishes, lined with filter paper soaked with extract at 28°C for 5 days. Deionized water was used as the control sample. The number of germinated seeds and the length of the roots were determined and the germination index was calculated by the equitation: Germination Index (GI) = 100 x [(G x L) / (Gc x Lc)], where G refers to germination percentage of the compost sample, Gc refers to germination percentage of the control sample, L refers to the root size of the compost product, and Lc refers to radicle size of the control sample.

**Proof-of-consent study**

The compost product was mixed with a commercial substrate (soil) at 25:75 or 50:50 (product : commercial substrate) percentage ratios and the mixture was used to plant vines in pots [10 stumps of Moschato Alexandrias (Muscat of Alexandria) variety / treatment, 2 stumps / pot, 1 year old stumps]. The commercial substrate used was Mikskaar 250 L (Mikskaar, Estonia), which is a mixture of a black-blonde peat with a ratio of 30%-70%. The volume of the substrate (product : commercial substrate) per pot was 18 L and the planting of the stumps took place in February 2023. On a daily basis, shoot growth was determined and after 35 days all leaves from each stump were collected, dried at 65°C for 72 h, and their exact weight before and after drying was determined. The results were expressed as a percentage of fresh or dry leaf mass yield in the tested compost product compared to the fresh or dry leaf mass yield observed in the control samples (100% commercial substrate).

**Statistical analysis**

All experiments were performed at least in triplicate and data is expressed as average values ± STDEV. Results were analyzed by one-way ANOVA and significant differences were determined by Tukey's and the Bonferroni post hoc test. ANOVA tables and significance levels ($p < 0.05$) were calculated with Statistica v.12 software.

**Results & Discussion**

**Physicochemical analysis**

**Moisture content, pH, Conductivity, and Temperature**

The moisture content of the organic material during composting was maintained at 40-60%, as it plays a pivotal role for the smooth operation of the system. Excess water can create anaerobic conditions and lead to the production of unpleasant odors. Conversely, a lack of water results in dehydration, halting the biological processes and yielding a biologically unstable product [17, 18]. Measurements were made every 10 days and in case a moisture content under 40% was observed, the pile system was wetted manually with water, until the appropriate humidity was reached.

The pH was weakly acidic (6.30) on the first day, while at the end of the process it varied from weakly acidic to weakly alkaline values (6.88-7.25) (Figure 1). This change suggested the potential use of the product as a soil conditioner, since crops respond more favorably when the soil pH ranges from a weakly acidic to a weakly alkaline level [19].

Electrical conductivity was significantly ($p < 0.05$) increased from 1.17 mmhos/cm to 2.25 mmhos/cm from day 0 to day 40 and then decreased significantly ($p < 0.05$) to 1.85 mmhos/cm at day 60 (Figure 2), remaining within the upper limit of 3 mmhos/cm proposed for the use of the compost product as a soil conditioner [20]. Electrical conductivity depends on the type of the composting material and is related to the concentrations of the ions. The decrease in electrical conductivity was associated with the stabilization of the product produced [21].

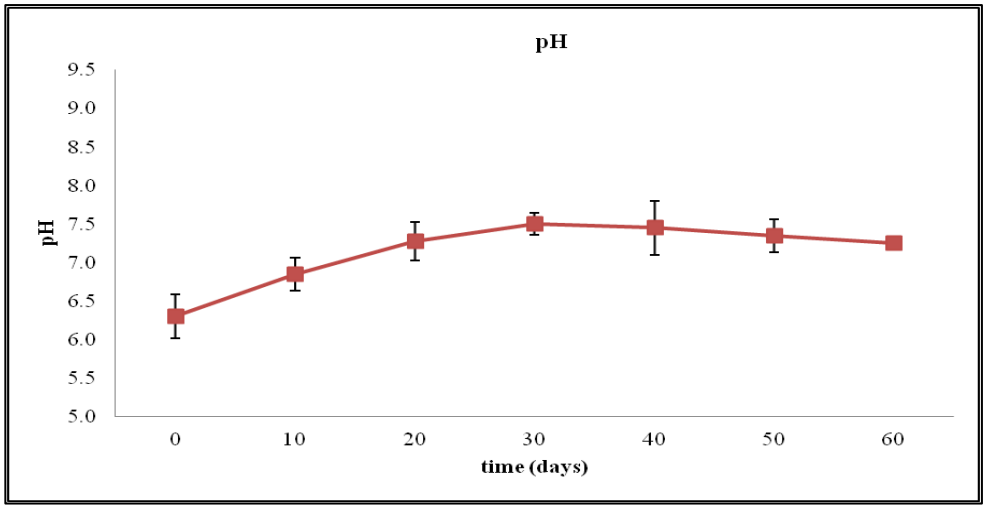


**Fig. 1** pH changes during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days.

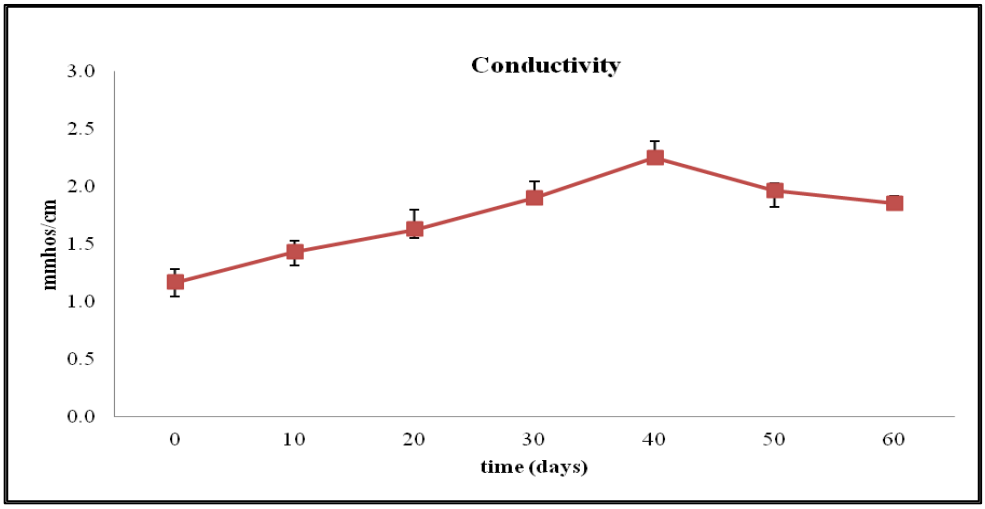


**Fig. 2** Changes in electrical conductivity expressed as mmhos/cm at 25°C, during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days

The temperature of the system increased significantly ($p < 0.05$) during the initial 15 days (> 30°C) and ranged between 31.0 - 47.5 °C from day 15 to day 40 (Figure 3). Temperature is an indicator of the microbial activity during the process and levels close to ambient temperature at the end of the process is a good indicator of the end of the bio-oxidative phase [22]. Of note, no fluctuations of the temperature to higher values were noticed.

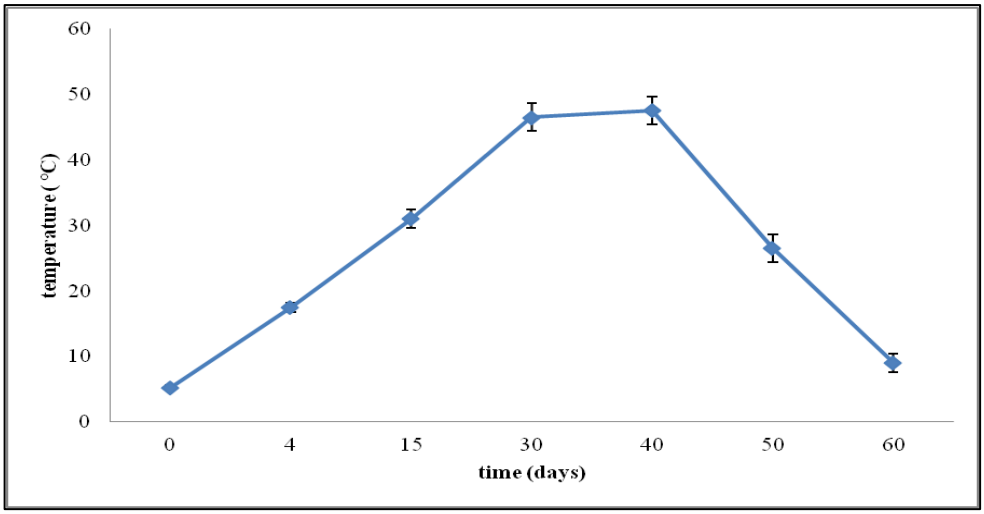


**Fig. 3** Changes in temperature during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days

**Total C, Total N, C/N Ratio, and $O_2$ Uptake Rate**

Total C decreased significantly ($p < 0.05$) from 50.53 % of the dry weight (day 0) to 22.61 - 31.65% (day 60), but no significant ($p > 0.05$) variation in total N between day 0 and day 60 was noted. Hence, the C/N ratio decreased significantly ($p < 0.05$) from 27.5 on the day of the system initiation to 11.39 on day 60 (Figure 4). The C/N ratio is used as a factor of stability and maturity of the compost and its reduction is an important indicator of rapid mineralization and decomposition of the initial raw material [9]. In this context, the ideal C/N ratio at the beginning of the process is between 25 – 30 : 1 and tends to be much lower at the end of the process [9]. Consistent with the literature [9, 23], the C/N ratio was significantly reduced at day 60 in our system.

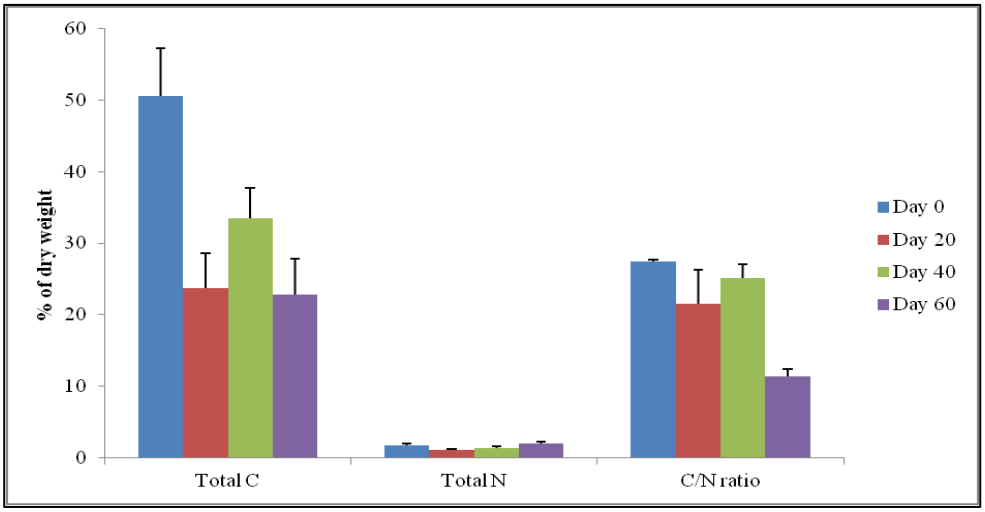


**Fig. 4** Changes in C/N ratio during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days

Oxygen uptake rate refers to the biological activity of a material and its reduction at the end of the process is an indicator of the final product stability, as it estimates the readily biodegradable organic matter still present in composting material [24]. The oxygen uptake rate was 8.1 g $O_2$/kg at the beginning of the composting process, when biodegradable organic matter was in high amounts, but significantly ($p < 0.05$) lower (6.85 g $O_2$/kg) compared to mature and stable compost produced at the end (day 60) (Figure 5).

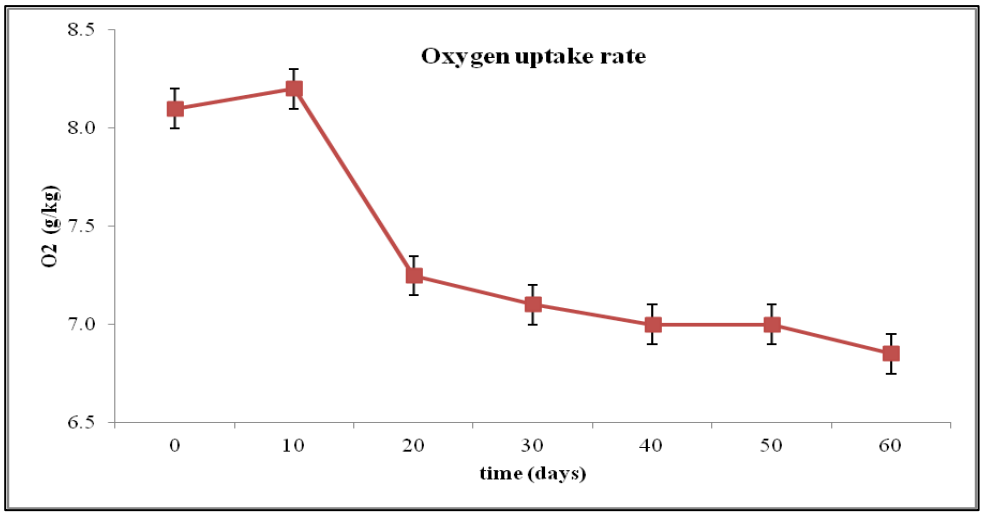


**Fig. 5** Oxygen uptake rate during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days, expressed as $O_2$ g/kg

**Macro- and Micronutrients**

The levels of heavy metals and important trace elements of the produced product were determined and the results are presented in Table 1. In the first 40 days, a significant ($p < 0.05$) increase in the Ca, Mg, K, P, and Fe concentrations was observed, while on day 60, the metal ions levels were significantly ($p < 0.05$) decreased compared to day 40. According to the literature [13, 25], both increase and decrease in metal ions have been observed in composting products from different types of waste. Importantly, levels of Mn and Cu ranged within the limits suggested by the European legislation [26].

**Table 1:** Concentration of important trace elements and metals during composting winery wastes with a windrow pile composting system.

| Trace elements/ metals | Day 0 | Day 10 | Day 40 | Day 60 |
|---|---|---|---|---|
| Total Ca (% dry weight) | 0.10±0.06 | 1.60±0.60 | 5.60±0.70 | 1.50±0.50 |
| Active Ca (% dry weight) | 1.40±0.32 | 0.94±0.18 | 1.09±0.02 | 0.13±0.02 |
| CaCO3 (% dry weight) | 1.40±0.21 | 2.50±0.90 | 6.70±0.70 | 1.60±0.50 |
| P (ppm) | 0.32±0.04 | 0.26±0.05 | 0.93±0.25 | 0.25±0.02 |
| K (ppm) | 0.45±0.001 | 0.36±0.001 | 4.05±0.14 | 0.40±0.16 |
| Mg (ppm) | 3.92±0.09 | 1.00±0.27 | 1.73±0.53 | 2.00±0.64 |
| Zn (ppm) | 580.00±14.10 | 78.00±39.60 | 48.90±15.30 | 83.50±23.30 |
| Mn (ppm) | 331.50±2.12 | 119.25±44.62 | 40.63±13.61 | 237.50±24.75 |
| Fe (ppm) | 16.50±2.12 | 44.50±4.95 | 43.40±4.10 | 12.00±2.83 |
| Cu (ppm) | 32.00±1.41 | 12.50±0.71 | 11.50±2.12 | 33.50±2.12 |

**Enzymatic activity**

Maturity of the final product can be also assessed by the determination of enzymatic activity which is related to the biochemical activity of the compost [27]. In specific, determination of dehydrogenase activity is related to the metabolic status of the microbial feedstock [28]. Dehydrogenase activity was higher at the beginning of the process and decreased on day 60 ($p > 0.05$) (Table 2). Dehydrogenases play an important role in the biological oxidation of soil organic matter, transferring hydrogen from organic substrates to inorganic receptors [29]. The activity of dehydrogenases increases under anaerobic conditions, due to the prevalence of anaerobic microorganisms, which are enzyme producers [30]. Microbial composting is an aerobic process and the decrease in the activity of dehydrogenases indicates

the successful biodegradation of the organic matter, as well as the sufficient presence of oxygen in the systems under study.

**Table 2:** Dehydrogenase activity during composting of winery waste in a pilot scale windrow pile operated for 60 days.

| Dehydrogenase activity (μg TPF/g dm/24h) | Day 0 | Day 20 | Day 40 | Day 60 |
|---|---|---|---|---|
| | 96.48±18.29 | 37.41±10.01 | 41.85±15.64 | 30.94±4.84 |

**Microbial Populations**

Microbial populations were also determined at regular intervals during the process (Figure 6). Significant increase in the cellular levels of all microbial species, except *Clostridium* spp., was observed on day 20, due to the high amount of biodegradable organic matter available to microbes and a significant reduction ($p < 0.05$) was noticed on day 60, as mature compost was produced. *Clostridium* spp. levels decreased significantly ($p < 0.05$) during the process. According to the European Regulation (EU) 2019/10097 [26], *Salmonella* spp. should be undetectable in the final product, while *Escherichia coli* levels should range < 3 logcfu/g. Importantly, the above criteria were met in our case. Subirats et al. [31] indicated the suitability of aerobic composting process as a method to reduce the levels of spore –forming or pathogenic bacteria in solid wastes, such as *Clostridium* spp. and fecal coliforms, which is confirmed in our study, as the sanitization of the final product was accomplished. Of note, TAC, *Enterobacteriaceae*, and yeasts/molds levels ranged between 3.66 – 8.76 logcfu/g.

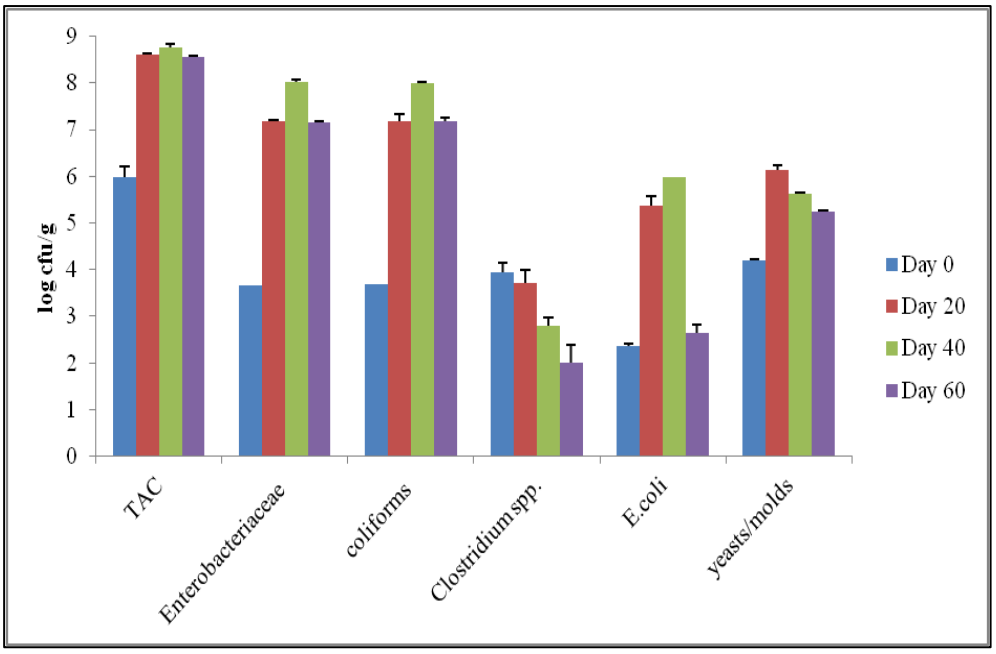


**Fig. 6** Microbial population levels during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days, expressed as logcfu/g of compost. TAC: Total Aerobic Counts

**Next-generation sequencing DNA analysis**

Bacteria and fungi play an important role in the decomposition of organic matter. The Next-Generation DNA Sequencing analysis identified 206 bacterial, 417 fungal, and no archaeal OTUs and revealed significant changes in the microbial communities between the beginning and the end of the composting process ($p < 0.05$). Diversity indices provide important information about the rarity, but also the probability that the species of a microbial community are common [32]. In this vein, Shannon and Simpson indices (a-diversity) were used [1, 32]. The Shannon index increased significantly ($p < 0.05$),

while the Simpson index decreased significantly ($p < 0.05$) for bacteria, while the changes observed for fungi were not significant ($p > 0.05$) (data not shown).

In total, 8 bacterial phyla were detected. Specifically, *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Candidatus saccharibacteria*, *Chloroflexi*, *Cyanobacteria*, *Planctomycetes*, and *Proteobacteria* were the bacterial phyla identified. *Proteobacteria* were the most abundant at the beginning of the process, representing 99.37% of the total identified sequences. At day 60, *Proteobacteria* decreased significantly ($p < 0.05$), while *Acidobacteria*, *Armatimonadetes*, and *Bacteroidetes* increased significantly ($p < 0.05$) (Table 3).

**Table 3:** Changes in bacteria phylum abundances (%) during microbial composting of winery waste (grapes, lees, etc.) in a pilot scale windrow pile operated for 60 days.

| Bacterial phyla | Day 0 | Day 60 |
|---|---|---|
| *Acidobacteria* | 0.13±0.05 | 1.98±0.92 |
| *Armatimonadetes* | 0.03±0.001 | 0.28±0.12 |
| *Bacteroidetes* | 0.37±0.16 | 16.62±1.36 |
| *Candidatus saccharibacteria* | 0.01±0.001 | 0.04±0.00 |
| *Chloroflexi* | 0.01±0.001 | 0.06±0.02 |
| *Cyanobacteria* | 0.02±0.001 | 0.02±0.001 |
| *Planctomycetes* | 0.03±0.001 | 0.03±0.001 |
| *Proteobacteria* | 99.37±0.26 | 80.97±0.32 |

In genus level, 65 bacterial genera were detected at the beginning and the end of the bioprocess (Figure 7). The most abundant genus on day 0 was *Acetobacter* (94.70%), while the remaining genera ranged < 1%. At day 60, the most abundant genera were *Brevundimonas* (27.46%), *Sphingomonas* (8.12%), *Sphingopyxis* (7.24%), *Moheibacter* (6.86%), *Devosia* (5.83%), *Asticcacaulis* (3.79%), *Pedobacter* (3.62%), *Novosphingobium* (3.00%), *Dyadobacter* (2.99%), *Chitinophaga* (2.78%), *Hephaestia* (2.27%), *Pseudaminobacter* (2.05%), *Caulobacter* (1.93%), *Shinella* (1.76%), *Phenylobacterium* (1.62%), *Agrobacterium* (1.37%), *Nitratireductor* (1.18%), *Blastocatella* (1.18%), *Ochrobactrum* (1.16%), *Roseomonas* (1.08%), while the remaining genera ranged < 1%. At day 60, the bacterial genera abundance of *Agrobacterium*, *Altererythrobacter*, *Aminobacter*, *Asticaucallis*, *Blastocatella*, *Blastomonas*, *Bosea*, *Brevyndimonas*, *Caulobacter*, *Chitinophaga*, *Devosia*, *Dokdonella*, *Dyadobacter*, *Fimbriimonas*, *Hephaestia*, *Hoeflea*, *Hyphomicrobium*, *Kaistia*, *Mesorhizobium*, *Methylocela*, *Moheibacter*, *Mycoplana*, *Nitratireductor*, *Novosphingobium*, *Ochrobactrum*, *Paracoccus*, *Pedobacter*, *Phenylobacterium*, *Phyllobacterium*, *Pseudaminobacter*, *Pseudorhodobacter*, *Rhodopseudomonas*, *Rhodovarius*, *Roseomonas*, *Shinella*, *Sphingobium*, *Sphingomonas*, *Sphingopyxis* and *Sphingosinicella* increased significantly ($p < 0.05$), while *Acetobacter* and *Methylosinus* decreased significantly ($p < 0.05$), while no significant changes were detected for the rest of the bacterial genera ($p > 0.05$).

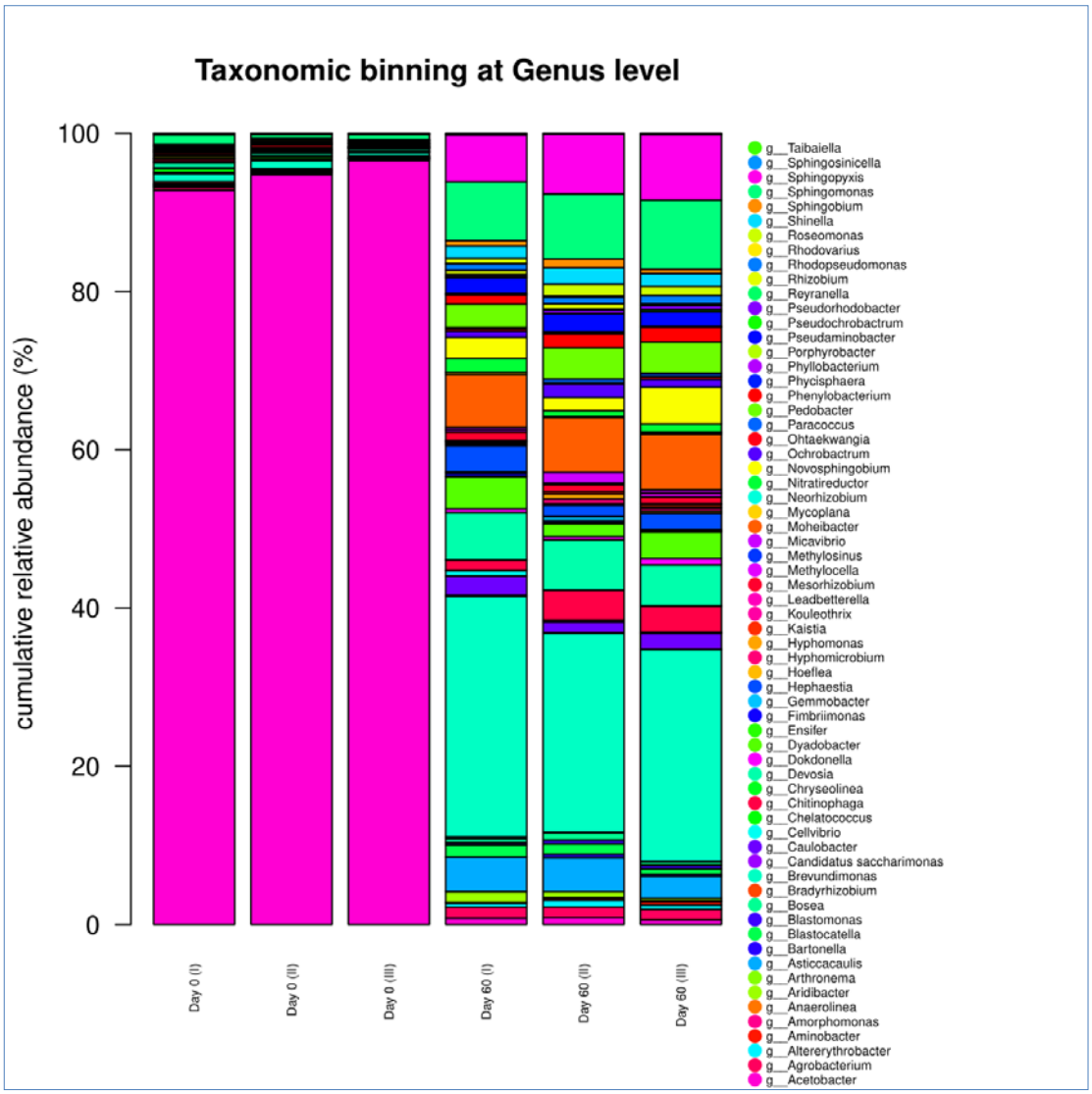


**Fig. 7** Bar plot of the mean relative genus abundances of the predominant bacteria during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days. The I, II, III refers to repetitive samples.

The genus *Brevundimonas* has been isolated from a wide range of environmental conditions, and it often promotes plant growth [33]. The genera *Sphingopyxis*, *Sphingomonas*, and *Novosphingobium* are well known for their role in environmental nutrient cycling and they produce beneficial phytohormones that promote plant growth [34, 35]. *Moheibacter* species are known for colonization of soils rich in organic matter [36, 37, 38, 39] and are commonly found in compost products, suggesting that *Moheibacter* species can decompose and utilize organic material [39]. *Phenylobacterium* has the ability to degrade complex carbohydrates, such as cellulose and possibly lignin [40], grape seeds, and grape stalks that have a high content of carbohydrates and lignocellulosic materials [41]. *Asticcacaulis*, *Caulobacter*, *Devosia*, *Ochrobactrum*, *Pedobacter*, and *Shinella* genus belong to the group of plant growth promoting bacteria named Rhizobia [42 - 44]. Their main function is to fix atmospheric nitrogen together with plants and they have been widely studied as symbiotic plant growth promoters [42, 45]. The diversity in the percentage abundance in bacteria genera reveals the successful biotransformation performed, resulting in a final product rich in plant growth promoting bacteria.

Regarding the fungal phyla, *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Entomophthoromycota*, *Glomeromycota*, and *Mucoromycota* were detected. The predominant fungal phylum was *Ascomycota* (97.43%) and its percentage decreased significantly ($p < 0.05$) after 60 days of microbial composting, while *Basidiomycota* OTUs increased significantly ($p < 0.05$) at the end of the process. The changes observed for the rest fungal phylum were not significant (Table 4).

**Table 4:** Changes in fungal phylum abundances (%) during microbial composting of winery waste (grapes, lees, etc.) in a pilot scale windrow pile operated for 60 days.

| Fungal phyla | Day 0 | Day 60 |
|---|---|---|
| *Ascomycota* | 97.43±0.64 | 90.28±3.05 |
| *Basidiomycota* | 2.35±0.64 | 8.16±2.57 |
| *Chytridiomycota* | 0.01±0.001 | 0.01±0.001 |
| *Entomophthoromycota* | 0.01±0.001 | 0.01±0.00 |
| *Glomeromycota* | 0.03±0.01 | 0.86±0.40 |
| *Mucoromycota* | 0.18±0.01 | 0.68±0.09 |

At genus level, the most abundant fungi genera on day 0 were *Saccharomyces* (32.21%), *Ophiocordyceps* (30.19%), *Wickerhamomyces* (9.70%), *Pichia* (6.92%), *Diplodia* (3.66%), *Cladosporium* (1.57%), *Aspergillus* (1.54%), *Aureobasidium* (1.29%), and *Myceliophthora* (1.20%). At day 60, the most abundant fungi genera were *Nectria* (21.20%), *Penicillium (17.10%), Fusarium (12.21%), Doratomyces* (10.19%), *Galactomyces* (5.68%), *Parascedosporium* (4.38%), *Clonostachys* (4.10%), *Gibellulopsis* (3.75%), *Coprinopsis* (3.41%), *Trichosporon* (2.02%), *Plectosphaerella* (1.50%), *Guehomyces* (1.34%), and *Psora* (1.20%) (Figure 8). The fungal genus abundance of *Amanita*, *Clonostachys*, *Coprinopsis*, *Galactomyces*, *Gibellulopsis*, *Graphium*, *Parascedosporium*, *Penicillium*, *Pesotum*, *Petriella*, *Plectosphaerella*, *Verticillium*, and *Volutella* increased significantly at day 60 ($p < 0.05$), while the fungal genus abundance of *Acremonium*, *Aspergillus*, *Aureobasidium*, *Babjeviella*, *Blastomyces*, *Coltricia*, *Curvularia*, *Dioszegia*, *Epicoccum*, *Filobasidium*, *Hydnellum*, *Mortierella*, *Microdochium*, *Myceliophthora*, *Ophiocordyceps*, *Saccharomyces*, *Saccharomycodes*, *Spencermartinsia*, and *Wallemia* decreased significantly ($p < 0.05$).

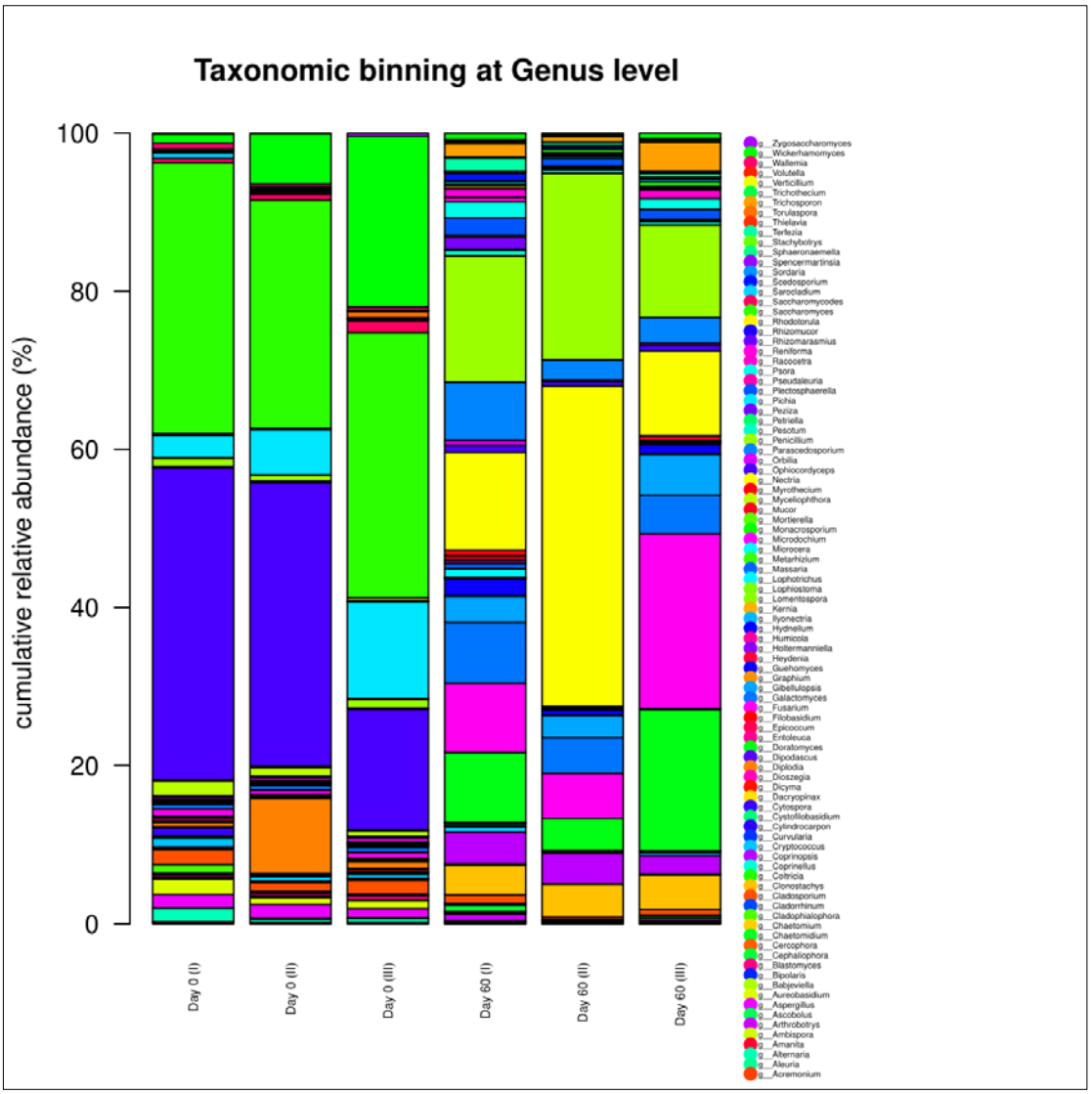


**Fig. 8** Bar plot of the mean relative genus abundances of the predominant fungi during microbial composting of winery waste in a pilot scale windrow pile operated for 60 days. The I, II, III refers to repetitive samples.

*Parascedosporium* is a fungus that produces enzymes active against carbohydrates (carbohydrate-active), which when grown on a substrate with lignocellulose (stems are rich in lignocellulose), enhances its decomposition [46]. *Guehomyces* is associated with the activity of laccase, an oxidase of starches and possibly degrades organic matter [47]. *Gibellulopsis* has been reported in composting products by other studies, but also in rotting root system and it is related to the carbohydrate content of the substrate [48, 49]. According to the literature, the genus *Galactomyces* has been associated with competitive mechanisms against phytopathogenic fungi and can be used as a biocontrol agent [50]. The presence of the genus *Penicillium* is considered beneficial, since it promotes the solubilization of phosphorus from organic matter, through the action of phosphatases, acting as a biostimulator [52]. Some species of the genus *Fusarium* are pathogenic and the phytotoxicity of the final product should be evaluated before field application.

**Germination Index**

Majlessi et al. [52] indicated that even if the final product is stable (low C/N ratio, neutral pH, and low electrical conductivity), there is a possibility that it contains phytotoxic compounds and in order to avoid environmental risks, it is mandatory to check the potential phytotoxicity of compost products prior application to soil. Hence, the final product was tested for potential phytotoxicity in barley seeds. Germination and radical growth were measured, and the GI was equal to 133.99. Noticeably, GI values > 80 indicate no phytotoxicity [16, 53].

**Proof-of-consent study**

The final product was mixed with a commercial substrate at 25 and 50%. A ratio greater than 50% was not evaluated, as according to Paradelo et al. [16], 50% is considered the upper limit for using winery waste compost products for use as a plant growth substrate. The effectiveness of the final product was verified by estimating the percentage yield of grapevine leaf dry matter compared to the control samples, leading to values 110 ± 1.3 and 90 ± 1.8 for 25 : 75 and 50 : 50 (product : commercial substrate), respectively. Hence, the final product was considered suitable as a substrate in grapevine growth, as percentage yields ≥ 90% were determined. Of note, the 25 : 75 (product : commercial substrate) ratio resulted in significant higher percentage yield of grapevine leaf dry matter compared to 50 : 50 ratio.

**Conclusion**

Our study highlighted the potential of winery waste composting as a sustainable waste management solution and soil amendment for vineyard cultivation. The comprehensive analysis of physicochemical, microbial, and enzymatic parameters, coupled with microbiome profiling and plant growth studies, provided a robust assessment of compost quality and performance. Analyzing the microbiome diversity at the beginning and at the end of the composting process of winery wastes provided valuable insights of the biobioprocess. Excess enzymatic activities, physicochemical analysis results (low C/N, increased total N, and neutral pH), along with the lack of phytotoxicity indicated the suitability of the final product and its effectiveness as a substrate for vine growth was confirmed. However, more research is still required to fully understand the underlying mechanisms of winery waste biotransformation into efficient biofertilizer and verify its efficiency in real plant culture conditions.

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