Physicochemical, Microbial, and Microbiome Dynamics in Winery Waste Composting

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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. Until recently, such wastes have been generally used for distillation, landfilling, incineration, and/or land-spreading. The last decades, composting has attracted considerable attention as a sustainable and environmentally-friendly alternative for the treatment of agro-industrial waste. Composting is the natural process of transforming organic matter to fertilizers, rich in essential nutrients for plant growth by microorganisms under controlled conditions. 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 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). Noticeably, the effectiveness of the final products was further verified in a pilot scale cultivation of vineyards (proof-of-concept study).

Material and methods: 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. Samples from the composting system were collected every 10 days and for up to 60 days for physicochemical, enzymatic, microbial, and microbiome analyses. Physicohemical analysis included the determination of moisture content, pH, temperature, conductivity, total carbon and nitrogen content (facilitating the calculation of the C/N ratio), oxygen uptake rate, carbonate content, as well as essential nutrients and heavy metals, such as phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), zinc (Zn), iron (Fe), and copper (Cu). Dehydrogenase activity was determined using a colorimetric method. Total Aerobic Counts, *Lactobacillus* spp., Enterobacteriaceae, coliforms, *Clostridium* spp., *Escherichia coli*, *Salmonella* spp., and yeasts/molds were also enumerated. For assessing microbiome dynamics, DNA extraction from compost samples at the beginning (day 0) and at the end of the process (day 60) was followed by the amplification of specific DNA regions (bacterial 16S rRNA, fungal ITS1/ITS2, and archaeal 16S rRNA) and application of MiSeq sequencing technology allowed for next-generation sequencing. Subsequent analysis of the sequencing data identified operational taxonomic units (OTUs) and facilitated taxonomic classification through established databases. Additionally, the potential phytotoxicity of the compost final product was evaluated by seed germination and calculation of germination index. Specifically, an aqueous extract of the compost product was used to soak barley seeds (50 barley seeds in 50 mL aqueous extract). After 24h, 15 barley seeds were incubated in petri dishes, lined with filter paper soaked with extract for germination at 28°C for 5 days. Deionized water was used as a control sample. The number of germinated seeds and the length of the roots were determined to calculate the germination index (GI). Subsequently, the suitability of the final compost product as a substrate in grapevine growth was studied (proof-of-concept study). In this vein, the compost product was mixed with a commercial substrate (soil) in 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 variety/treatment, 2 stumps/pot, 1 year old stumps). On a daily basis, shoot growth was determined and after 35 days all leaves from each stump were collected, dried at 65°C for 72h, and their exact weight before and after drying was calculated. 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).

Results & Discussion: 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. The pH was weakly acidic (6.3) on the first day, while at the end of the process it varied from weakly acidic to weakly alkaline values (6.88-7.25). 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. 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. 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. 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. 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. 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. 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. 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. 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. 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 metals ion levels were significantly ($p < 0.05$) decreased compared to day 40. According to the literature (Pinter *et al*., 2019; Paradelo *et al*, 2009), 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.

Enzymatic activity is a useful tool to provide information about the potential of the compost to perform biochemical reactions and is related to compost characterization and maturity. Determination of dehydrogenase activity is related to the metabolic status of the microbial feedstock. On the first 40 days, the activity of dehydrogenase increased significantly ($p < 0.05$) and decreased as mature and stable compost was produced. Dehydrogenases play an important role in the biological oxidation of soil organic matter, transferring hydrogen from organic substrates to inorganic receptors. The activity of dehydrogenases increases under anaerobic conditions, due to the prevalence of anaerobic microorganisms responsible for producing these enzymes. 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. Microbial populations were also determined at regular intervals during the process. Significant increase in the cellular levels of all microbial species were observed on day 20 and a significant reduction ($p < 0.05$) was observed on day 60. *Clostridium* spp. levels decreased significantly ($p < 0.05$) on day 60 compared to day 0. The Next-Generation DNA Sequencing analysis revealed significant changes in the microbial communities between the beginning and the end of the composting process. In total, eight bacterial and six fungal phyla were detected. *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Candidatus saccharibacteria*, *Chloroflexi*, *Cyanobacteria*, *Planctomycetes*, and *Proteobacteria* were identified. *Proteobacteria* were the most abundant at the beginning of the process, representing 97.57% of the total identified sequences. At day (60), *Proteobacteria* decreased significantly ($p < 0.05$), while *Bacteroidetes* increased significantly ($p < 0.05$). The *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Entomophthoromycota*, *Glomeromycota*, and *Mucoromycota* fungal phyla were also detected. The predominant fungal phylum was *Ascomycota* (97,43%). However, the changes in the percentage of fungal phyla observed were not significant. The next step was to test the final product for potential phytotoxicity in barley seeds; a Germination Index (GI) equal to 133.99 was estimated. Of note, GI values > 80 indicate no phytotoxicity. Finally, 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 the percentage yields ≥ 90% were determined.

Conclusion: Analyzing the microbiome diversity at the beginning and at the end of the composting process of winery waste provided valuable insights about the bioprocess. Excess enzymatic activities, physicochemical analysis results (low C/N, total N increased, neutral pH), along with 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.

References

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