Valorisation of organic waste through solid-state fermentation for sustainable agriculture: Biostimulant production by *Trichoderma harzianum*

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1. Introduction

The exponential increase in the production of urban, industrial, and agricultural waste driven by the continuous expansion of the population has become a major environmental problem for a society, which is based on a linear economic model, characterized by resource extraction, utilization, and subsequent disposal. To drastically reduce waste production and mitigate the associated adverse environmental effect, requires coordinated efforts to facilitate the transition to a circular economic model in which waste is redefined as a resource for bioenergy and/or bioproducts.

Another important concern in developed societies is the use of chemical fertilizers, which are causing soil degradation, water sources pollution, and atmosphere contamination (Diacono & Montemurro, 2012). To reduce this pollution is important to promote sustainable agricultural practices. In this sense, crop inoculation with beneficial microorganisms is a sustainable agricultural technique that promotes plant growth through mechanisms like the production of phosphorus-solubilizing enzymes, phytases, and indole-3-acetic acid (IAA) (Acuña et al., 2011). Microbial inoculants, primarily bacteria and fungi, serve as biofertilizers to replace chemical fertilizers, as biopesticides for pest and disease control, and as agents for bioremediation to improve soil health (O'Callaghan et al., 2022). In this context, Solid-State Fermentation (SSF) is emerging as a promising technology for the valorisation of organic waste and enabling the production of high value-added bioproducts, including biosurfactants, biostimulants (Ghoreishi et al., 2023) and biopesticides (Sala et al., 2020).

The objective of this work is to test the feasibility of growing *Trichoderma harzianum* on an organic complex residue such as the source-selected organic fraction of municipal solid waste (OFMSW) and analyse the production of IAA by this fungus. According to Ghoreishi et al. (2023), the presence of tryptophan induces the synthesis of IAA. Therefore, in this work, we will evaluate the effect of tryptophan on *T. harzianum* growth and IAA production. With this approach, OFMSW is valorised through SSF to produce biostimulants to promote a sustainable agriculture within the paradigm of circular bioeconomy.

2. Materials and methods

2.1. Microorganism and organic waste

The fungal strain used in this study was *Trichoderma harzianum* CECT 2929. For use, the fungus was cultivated as explained in Sala et al. (2020). The spore concentration was determined using a Neubauer chamber. The OFMSW was obtained from a local composting treatment plant (Mancomunitat La Plana, Malla, Barcelona, Spain), containing 24 % of dry matter (DM), 91 % of organic matter (on a dry basis), and a pH of 5.6. For the SSF, the substrate was mixed with wood chips as a bulking agent, to prevent compaction and ensure a proper porosity. Before SSF, the mixture of substrate and bulking agent was autoclaved at 121 ºC for 1 h for sterilisation.

2.2. Solid-State Fermentation (SSF)

SSF tests were conducted in 0.5 L packed bed bioreactors, filled with 100 grams of total sample of which 25% (wet weight) corresponded to the bulking agent. The mixture was manually mixed, inoculated with an initial concentration of 10^7 spores per gram of DM (Sala et al., 2020), and the temperature was maintained at 25 ºC. A constant humidified airflow was provided and controlled by a mass airflow meter (Bronkhorst, The Netherlands) at 0.2 mL min^{-1} g^{-1} total wet weight for ensuring aerobic conditions. The oxygen content in the outlet gases was assessed using an electrochemical oxygen sensor O_2-A_2 (Alphasense, UK) and the specific Oxygen Uptake Rate (sOUR) was calculated as elsewhere (Ghoreishi et al., 2023). This oxygen consumption is a direct measure of the biological activity of microorganisms, and it is expressed in g O_2 kg DM^{-1} h^{-1}. Two SSF were simultaneously carried out with the same inoculum and monitored for 6 days, one with 15 mL of tryptophan (12 g/L) and the other without tryptophan, as a control, with 15 mL of water to maintain the same moisture. Fermentations were conducted in triplicate, and the results at the end of the process are presented as a mean and standard deviation of this triplicate. Tukey test was used for statistics.

2.3 Analytical procedures

Each 24 hours the fermented extract was analysed in terms of pH, spores of *T. harzianum* (CFUs), IAA and tryptophan. The initial and final samples were also characterized for dry and organic matter. For the *T. harzianum* spores counting of the fermented samples, a solid-liquid extraction was performed with 10 g of sample and 90 mL of Tween 80 (0.05% v/v). The mixture was agitated for 20 minutes at 25 ºC. Then, the extracts were diluted and plated on potato dextrose agar containing 0.03 g L^{-1} of Rose Bengal. After 2 days at 30 ºC, colonies

were counted. The number of colonies was considered equivalent to the number of spores, as was demonstrated in a previous test (data not shown). For the pH, IAA, and tryptophan analysis, the extraction and further centrifugation was done according to Ghoreishi et al. (2023). The supernatants were later analysed by HPLC to determine the IAA and tryptophan.

3. Results and discussion

Figure 1 shows the sOUR profiles for *T. harzianum* with the peak of maximum oxygen consumption (sOUR) before day 3, as reported before (Ghoreishi et al., 2023; Sala et al., 2020). The sOUR is a direct measure of microbial activity, therefore, the peak of maximum oxygen consumption is related to the maximum growth of *T. harzianum.*

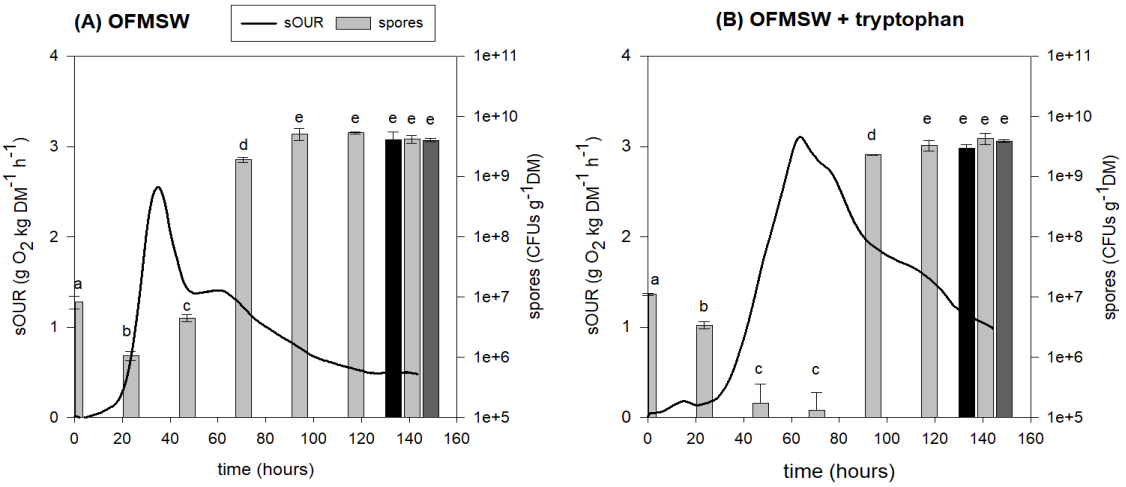

Figure 1. Spore production and sOUR over time during the SSF with the OFMSW (A) and the OFMSW with tryptophan (B). Triplicate was analysed at the end of the experimentation for statistical differences among fermentations (Tukey test).

In the assay with tryptophan (B), growth is delayed about 48 hours compared to the control without tryptophan (A). In both cases, there is practically no spore production during the maximum oxygen consumption. The increase in spore production can be observed two days after the sOUR peak. In the control assay (A), there were no significant differences in spore production as of 96 hours, so it can be concluded that the maximum production had already been reached. Whereas, in the SSF with tryptophan, the maximum spore production is reached after 120 hours. Similar results can be seen in Sala et al. (2020), where the maximum spore production was $2 \cdot 10^9$ spores per gram of DM after 6 days. The results of the analysis of the IAA production are ongoing.

4. Conclusions

Results have demonstrated the viability of *T. harzianum* to grow on OFMSW as sole substrate, as well as a considerable increase in spore production. In the SSF without tryptophan, a production of $5 \cdot 10^9$ spores per gram of DM was obtained after 4 days. In the other case, with tryptophan, $3 \cdot 10^9$ spores per gram of DM was reached after 5 days. The effect of tryptophan on the production of IAA seems essential and it is currently being studied.

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6. References

Acuña, J. J., Jorquera, M. A., Martínez, O. A., Menezes, D., Blackburn, −, Fernández, M. T., Marschner, P., Greiner, R., & Mora, M. L. (2011). Indole acetic acid and phytase activity produced by rhizosphere bacilli as affected by pH and metals. *Journal of Soil Science and Plant Nutrition*, *11*(3), 1–12.

Diacono, M., & Montemurro, F. (2012). Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development 2009 30:2*, *30*(2), 401–422.

Ghoreishi, G., Barrena, R., & Font, X. (2023). Using green waste as substrate to produce biostimulant and biopesticide products through solid-state fermentation. *Waste Management*, *159*, 84–92.

O'Callaghan, M., Ballard, R. A., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use and Management*, *38*(3), 1340–1369.

Sala, A., Artola, A., Sánchez, A., & Barrena, R. (2020). Rice husk as a source for fungal biopesticide production by solid-state fermentation using B. bassiana and T. harzianum. *Bioresource Technology*, *296*.