Incorporation of substrates and inoculums as operational strategies to promote lignocellulose degradation in co-composting of green waste

E.R. Oviedo-Ocaña[1], J. Soto-Paz[1], V. Sanchez-Torres[2]

[1]Escuela de ingeniería civil, Universidad Industrial de Santander, Bucaramanga, Santander, 680002. Colombia
[2]Escuela de ingeniería química, Universidad Industrial de Santander, Bucaramanga, Santander,680002. Colombia
Keywords: Green waste, co-composting, bacterial, inocula, lignocellulose.
Presenting author email: eroviedo@uis.edu.co

Abstract

Green waste (GW) represents a management challenge due to its heterogeneous composition and predominance of lignocellulosic compounds such as cellulose (40%), hemicellulose (20-30%) and lignin (20-30%) [1]. Although composting can be used to valorise GW by transforming it into a product with potential agricultural value. The difficulty to degrade lignocellulose increases the processing time and generates low quality products [2,3]. To optimise GW composting, several strategies have been developed to accelerate the biodegradability of lignocellulosic compounds [2,4]. The incorporation of co-substrates such as sawdust (SW) and rock phosphate (PR) have been used to provide porosity and phosphorus [5,6], but few studies have evaluated the addition of food waste (FW) as an amendment, which in developing countries constitutes more than 70% of municipal solid waste [7]. The incorporation of bacterial inocula during the cooling phase is other strategy that represents a key operational change to improve the process [8]. Despite this, some studies reported no significant effects associated with inoculation on the composting process due to the potential competition and antagonistic relationship between microbial species. [9,10]. This study evaluated the incorporation during the cooling phase of a bacterial inocula obtained in a previous study consisting of the bacterial strains *Bacillus sp.* and *Paenibacillus sp.* in the co-composting of GW with FW on the efficiency of the process and the quality of the final product. The search for options to improve the composting of GW can contribute to a greater implementation of this technology for the urban management of these wastes.

The study was carried out with the GW generated at the Universidad Industrial de Santander, located in the city of Bucaramanga (Colombia). The FW were obtained from a market place where separation at source is carried out and were made up of PFW and UPFW [11]. Sawdust (SW) and rock phosphate (PR) were incorporated as bulking agents. SW provided carbon, porosity and adjusted the moisture content. On the other hand, PR provided phosphorus and also porosity and the amount was defined according to [6]. The substrates were manually mixed at a ratio of 50% GW, 32.5% UFW, 2.5% PFW, 13% SW and 2%PR and the C/N ratio was higher than 25. The experiments were conducted in reactors with a volume of 120 L according to [12]. Three treatments were considered: i) Treatment A: (substrate mix + inocula); ii) Treatment B (substrate mix only); and iii) Treatment C (GW only). Before starting the experiment, the GW, UFW and PFW were manually crushed to a particle size between 30 and 50 mm [4]. Table 1 presents the physicochemical characteristics of the substrates and treatments.

Table 1. Physico-chemical characteristics of substrates and treatments

Treatment	Moisture (%)	pH	EC (mS/cm)	TOC (%, db)	TN (%, db)	C/N ratio	Lignocellulose (%, db)
PFW	75.5 ± 7.6	4.9 ± 0.4	4.9 ± 0.1	22.2 ± 7.4	1.9 ± 0.3	11.7± 0.5	17.0 ± 2.6
UFW	79.1 ± 8.3	5.1 ± 0.3	3.1 ± 0.4	33.5± 5.9	1.2 ± 0.6	27.9 ±2.8	17.8± 3.3
***TA, TB**	58.2 ± 2.5	6.3 ± 0.2	3.5 ± 0.4	47.7 ± 3.1	1.7 ± 0.3	27.6± 1.8	23.8 ± 1.9
TC	27.3 ± 4.9	6.9 ± 0.1	3.0 ± 0.3	26.6 ± 5.8	1.2 ± 0.5	22.2± 0.4	35.1 ±6.1

* Characteristics of TA, TB includes SW and RP.

The bacterial inocula consisted of *Bacillus sp* and *Paenibacillus sp* strains with concentrations$4.85*10^5$UFC mL^{-1} y $1.44*10^5$ UFC mL^{-1} respectively**:** The inocula was prepared prior to the onset of the cooling phase (i.e. when the temperature was close to 45°C). The lignocellulose concentration was determined according to the protocol of the National Renewable Energy Laboratory (NREL) considering the moisture, ash content at 550°C and aqueous and organic extractives of the sample [13].

Figure 1a shows the temperature profiles of each treatment during the process. The mesophilic phase in TA and TB occurred until day 1 of the process, while for TC it lasted until day 4. Longer time in TC is associated with a higher presence of slowly biodegrading organic carbon [14,2]. In the thermophilic phase, TA reached the highest temperature of (64°C) after 8 days of processing. In contrast, TB reached (i.e. 60.7°C) at 9 days, showing no significant difference with respect to that found in TC (p <0.05). The results indicated that the incorporation of FW, SW and PR had a significant ($p<0.05$) and synergistic effect on GW composting [15,11]. During the cooling stage, the incorporation of the bacterial inoculum influenced the temperature profile of AT, increasing the temperature gradient (i.e. day 19) after its incorporation and extending until day 27 of the process. This is associated with the fact that the inoculum stimulated the degradation of OM and lignocellulose, possibly by secretion of lignolytic enzymes [16]. The results found in this study are similar to those reported by Wang et al. [2] in GW composting with eggshells and rice husks. Then, the temperature decreased gradually in all treatments, with TA being the treatment that reached room temperature (i.e. day 38) the fastest, demonstrating the synergistic effect of inoculation. In contrast, TC required 47 days to reach room temperature, showing significant differences with respect to TA and TB (p =0.19). In addition, it showed that the incorporation of co-substrates also had an impact on reducing the processing time.

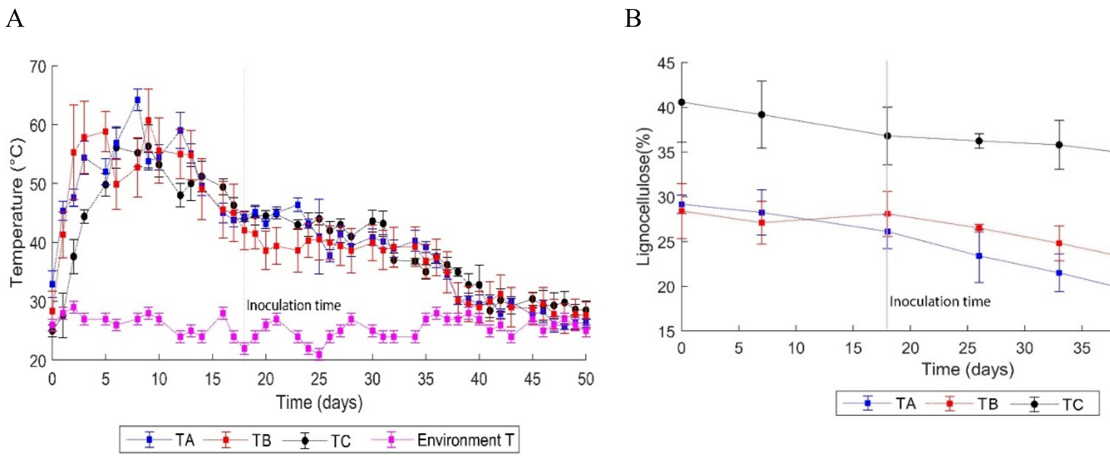

Figure1. Temperature and lignocellulose profiles

Figure 1b shows the degradation of lignocellulose over time. At the beginning of the process (i.e. mesophilic phase), lignocellulose degradation was limited and did not release lower molecular weight organic compounds to satisfy the needs of the microorganisms [1]. This was mainly observed in TC, where the predominance of difficult-to-degrade carbon may have limited biological activity and prolonged the duration of the process. In contrast, in TA and TB, biological activity was stimulated, and rapidly degradable carbon was consumed, which increased the temperature, an aspect that may have favoured the secretion of xylose and cellulose. According to different authors, part of the cellulose and hemicellulose present in lignocellulose can be degraded when the temperature exceeds 60°C. [17,18].

LBR increased in all treatments after the start of the composting process. During the cooling phase, LBR increased to values of 31.7%, 23.1% and 15.5% for TA, TB and TC respectively. These results are consistent with those observed by Yu et al. (2018) due to mineralisation and humification processes. The higher LBR for TA is associated with higher TN content which promotes the enzymatic activity of cellulose, xylanase and phenol oxidase among other ligninolytic enzymes [19,20]. Likewise, pH values closer to neutrality in AT could stimulate microbial activity [21]. Fuerthermore, Kausar et al. [22] report that delignification takes place more rapidly at natural pH than at acidic or alkaline pH. The results found here were higher than those reported byYu et al. [23] on green waste composite with an exogenous inoculum (27.81%).

The study showed that the best TA treatment with inoculation during the cooling phase reduced the processing time by 4 to 13 days compared to the treatment without inoculation (TB) and control (TC) with a lignocellulose degradation of 31.7%. In addition, the incorporation of co-substrates such as UFW, PFW, AS and RP are useful to improve GW composting.

Bibliography

1. Chen, X., Cheng, W., Li, S., Tang, X., Wei, Z.: The “quality” and “quantity” of microbial species drive the degradation of cellulose during composting. Bioresource Technology **320**, 124425 (2021). doi:https://doi.org/10.1016/j.biortech.2020.124425
2. Wang, W., Zhang, L., Sun, X.: Improvement of two-stage composting of green waste by addition of eggshell waste and rice husks. Bioresource Technology **320**, 124388 (2021). doi:https://doi.org/10.1016/j.biortech.2020.124388

3. Zhang, L., Sun, X.: Changes in Physical, Chemical, and Microbiological Properties During the Two-Stage Composting of Green Waste due to the Addition of β-cyclodextrin. Compost Science & Utilization **27**(1), 46-60 (2019). doi:10.1080/1065657X.2019.1585304
4. Zhang, L., Sun, X.: Addition of fish pond sediment and rock phosphate enhances the composting of green waste. Bioresource Technology **233**, 116-126 (2017). doi:https://doi.org/10.1016/j.biortech.2017.02.073
5. Reyes-Torres, M., Oviedo-Ocaña, E.R., Dominguez, I., Komilis, D., Sánchez, A.: A systematic review on the composting of green waste: Feedstock quality and optimization strategies. Waste Management (2018). doi:https://doi.org/10.1016/j.wasman.2018.04.037
6. Hu, C., Yang, O., WANG, L.-x., YAN, B.-x., LI, Y.-x., DING, D.-w.: Phosphate rock reduces the bioavailability of heavy metals by influencing the bacterial communities during aerobic composting. Journal of Integrative Agriculture **20**(5), 1137-1146 (2021).
7. Usmani, Z., Sharma, M., Karpichev, Y., Pandey, A., Chander Kuhad, R., Bhat, R., Punia, R., Aghbashlo, M., Tabatabaei, M., Gupta, V.K.: Advancement in valorization technologies to improve utilization of bio-based waste in bioeconomy context. Renewable and Sustainable Energy Reviews **131**, 109965 (2020). doi:https://doi.org/10.1016/j.rser.2020.109965
8. Li, J., Wang, X., Cong, C., Wan, L., Xu, Y., Li, X., Hou, F., Wu, Y., Wang, L.: Inoculation of cattle manure with microbial agents increases efficiency and promotes maturity in composting. 3 Biotech **10**(3), 128 (2020). doi:10.1007/s13205-020-2127-4
9. Lei, F., VanderGheynst, J.S.: The effect of microbial inoculation and pH on microbial community structure changes during composting. Process Biochemistry **35**(9), 923-929 (2000). doi:https://doi.org/10.1016/S0032-9592(99)00155-7
10. Rastogi, M., Nandal, M., Khosla, B.: Microbes as vital additives for solid waste composting. Heliyon **6**(2), e03343 (2020). doi:https://doi.org/10.1016/j.heliyon.2020.e03343
11. Hernández-Gómez, A., Calderón, A., Medina, C., Sanchez-Torres, V., Oviedo-Ocaña, E.R.: Implementation of strategies to optimize the co-composting of green waste and food waste in developing countries. A case study: Colombia. Environmental Science and Pollution Research, 1-7 (2020).
12. Rawoteea, S.A., Mudhoo, A., Kumar, S.: Co-composting of vegetable wastes and carton: Effect of carton composition and parameter variations. Bioresource technology **227**, 171-178 (2017).
13. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D.: Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure **1617**(1), 1-16 (2008).
14. Wang, H., Zhao, Y., Wei, Y., Zhao, Y., Lu, Q., Liu, L., Jiang, N., Wei, Z.: Biostimulation of nutrient additions on indigenous microbial community at the stage of nitrogen limitations during composting. Waste Management **74**, 194-202 (2018). doi:https://doi.org/10.1016/j.wasman.2017.12.004
15. Oviedo-Ocaña, E., Dominguez, I., Komilis, D., Sánchez, A.: Co-composting of Green Waste Mixed with Unprocessed and Processed Food Waste: Influence on the Composting Process and Product Quality. Waste and Biomass Valorization, 1-12 (2019).
16. Zeng, G., Yu, M., Chen, Y., Huang, D., Zhang, J., Huang, H., Jiang, R., Yu, Z.: Effects of inoculation with Phanerochaete chrysosporium at various time points on enzyme activities during agricultural waste composting. Bioresource Technology **101**(1), 222-227 (2010).
17. Song, C., Li, M., Wei, Z., Jia, X., Xi, B., Liu, D., Zhu, C., Pan, H.: Effect of inoculation with multiple composite microorganisms on characteristics of humic fractions and bacterial community structure during biogas residue and livestock manure co-composting. Journal of Chemical Technology & Biotechnology **91**(1), 155-164 (2016).
18. Yang, L., Jie, G., She-Qi, Z., Long-Xiang, S., Wei, S., Xun, Q., Man-Li, D., Ya-Nan, Y., Xiao-Juan, W.: Effects of adding compound microbial inoculum on microbial community diversity and enzymatic activity during co-composting. Environmental Engineering Science **35**(4), 270-278 (2018).
19. Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y.: Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global change biology **20**(7), 2356-2367 (2014).
20. Vrsanska, M., Buresova, A., Damborsky, P., Adam, V.: Influence of different inducers on ligninolytic enzyme activities. Journal of Metallomics and Nanotechnologies **3**, 64-70 (2015).
21. Duan, M., Zhang, Y., Zhou, B., Qin, Z., Wu, J., Wang, Q., Yin, Y.: Effects of Bacillus subtilis on carbon components and microbial functional metabolism during cow manure–straw composting. Bioresource Technology **303**, 122868 (2020). doi:https://doi.org/10.1016/j.biortech.2020.122868
22. Kausar, H., Ismail, M.R., Saud, H.M., Othman, R., Habib, S.: Use of lignocellulolytic microbial consortium and pH amendment on composting efficacy of rice straw. Compost Science & Utilization **21**(3-4), 121-133 (2013).
23. Yu, Sun, X., Li, S., Cai, L., Zhang, P., Kang, Y., Yu, Z., Tong, J., Wang, L.: Application of quadratic regression orthogonal design to develop a composite inoculum for promoting lignocellulose degradation during green waste composting. Waste Management **79**, 443-453 (2018). doi:https://doi.org/10.1016/j.wasman.2018.08.018