SUSTAINABLE AND INTEGRAL EXPLOITATION OF AGAVE

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Accelerated vermicomposting with *Bjerkandera adusta* pre-treatment of agave bagasse

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ABSTRACT

The *Agave tequilana* Weber is used to generate products such as tequila and fructans, in which agave bagasse is generated as solid waste. The principal use of bagasse is to produce composts in a traditional process of 7-month average. The aim of this study was to improve the agave bagasse degradation with an accelerated vermicomposting during 90 days, which consisted of a pre-treatment of fractionated or whole bagasse with *Bjerkandera adusta* UAMH 8258 or native fungi followed by a vermicomposting with *Eisenia fetida*. Also, the stability and maturity of obtained vermicomposts were evaluated. Results indicated that the fractionated bagasse favoured degradation either with native fungi or with *B. adusta*. The pre-treatment accelerated the degradation of bagasse when it was subjected to a vermicomposting process. Degradation of hemicellulose, cellulose and lignin in pre-treatment (with native or *B. adusta* fungi) were greater than in vermicomposting. The better indexes of stability and maturity were obtained with the vermicompost from bagasse fractionated, indicating that a minor particle size favoured the degradation. In this work, with an accelerated vermicomposting it was possible to reduce degradation time to 3 months.

Keywords: *Eisenia fetida*, lignin, lignocellulosic enzymes, white rot fungi

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INTRODUCTION

High quantities of blue agave crops are used almost exclusively for tequila production, due to the existence of the appellation of origin (AO). However, blue agave is cultivated in other states, outside the AO such as Zacatecas, Sinaloa and Durango among others. Recently, these crops outside the AO have been used to generate other products such as fructans and inulin to diversify the utilization of *A. tequilana* Weber (Waleckx et al., 2008). In 2013, 226.5 million litters of tequila were produced from 776.9 thousand tons of *A. tequilana*, which generated a large amount of bagasse and vinasses as waste from tequila production process (CRT, 2014). Iñiguez et al. (2001) estimated that bagasse represents 40% of the total weight of the agave heads (wet weight). An estimation with this base indicated that 310.76 thousand tons of bagasse were generated in 2013 by tequila production. Other estimations indicated that in the fructans production process 73% of agave heads (wet weight) are generated as bagasse (Personal communication). Some tequila factories use the agave bagasse to generate compost in windrow systems where they irrigate the vinasses. However, the degradation process is slow with a process time from 6 to 8 months due to the lignocellulosic composition of the bagasse (Iñiguez et al., 2011). The high generation of bagasse and the long composting process time makes insufficient treatment and causes environmental issues due to improper disposition. The main objective of this study was to accelerate the degradation of the agave bagasse using a pre-treatment with *Bjerkandera adusta* fungus followed by vermicomposting with *Eisenia fetida*. At the end of the process the stability and maturity of obtained vermicomposts were evaluated.

MATERIALS AND METHODS

We collected samples of fractional (< 4 mm) and whole bagasse (6-8 cm) of an agave from the state of Zacatecas and derived from fructans production. The bagasse was pre-treatment in a solid-state fermentation with the fungus *B. adusta* and/or with native fungi, during 45 days in different treatments (Table 1). We measured the degradation of hemicellulose, cellulose, lignin (Van Soest and Wine, 1967) and total carbohydrate (Dubois et al., 1956) at 0, 30 and 45 days. In addition, we evaluated the enzymatic activity of lignin peroxidase (LnP), manganese peroxidase (MnP) and Laccase (Lac) (Leonowicz and K, 1981). After the pre-treatment, we carried out a vermicomposting process with *E. fetida* complementing the input of nitrogen with a sewage sludge obtained from a plant of treatment of domestic wastewater, which was free of pathogens and with an heavy metals content below to permissible maximum limits of NOM-004-SEMARNAT-2000 and the USEPA, with 146 mg kg^{-1} of inorganic N and 1525 mg kg^{-1} available phosphorus. At 0, 30 and 45 days were evaluated the content of hemicellulose, cellulose, lignin. The final vermicomposts were determined for inorganic nitrogen (NO_3^-, NO_2^-, NH_4^+), germination index (Mathur et al., 1993), breathing index (Bartha and Pramer, 1965), humic and fulvic acids, and the content of *Salmonella spp*., and faecal coliforms according to NMX-AA-042-1987. All parameters evaluated were subject to an analysis of variance (ANOVA) using a PROC GLM with the SAS statistical program (2009) to analyse the significant differences among treatments in the pre-treatment with *B. adusta* and in the vermicomposting process with Tukey's test and $P < 0.05$.

Table 1 Combinations of treatments with whole (W) and fractionated (F) bagasse for the pre-treatment with and without *B. adusta* UAMH 8258 fungi (B) under sterile (s) and non-sterilized (ns) conditions.

	Bagasse	
Treatments in pre-treatment [a]	Whole	Fractionated
B. adusta with Native fungi, (non-sterile)	BNWns	BNFns
B. adusta (sterile)	BWs	BFs
Native fungi, (non-sterile)	NWns	NFns
Biotic controls	CWs	CFs
Treatments in vermicomposting [a]	Whole	Fractionated
B. adusta with Native fungi, (non-sterile) plus earthworm	BNWns+ E	BNFns+E
B. adusta (sterile) plus earthworm	BWs+E	BWs+E
Native fungi (non-sterile) plus earthworm	NWns+E	NFns+E
Controls plus earthworm	CWs+E	CFs+E
B. adusta (sterile) without earthworm	BWs-E	BFs-E
Native fungi (non-sterile) without earthworm	NWns-E	NFns-E

[a] All treatments were in triplicated (n = 3).

RESULTS AND DISCUSSION

The native fungi of bagasse and *B. adusta* consumed the residual sugars (carbohydrates totals) from bagasse in 30 days, without significant differences between the treatments (Figure 1).

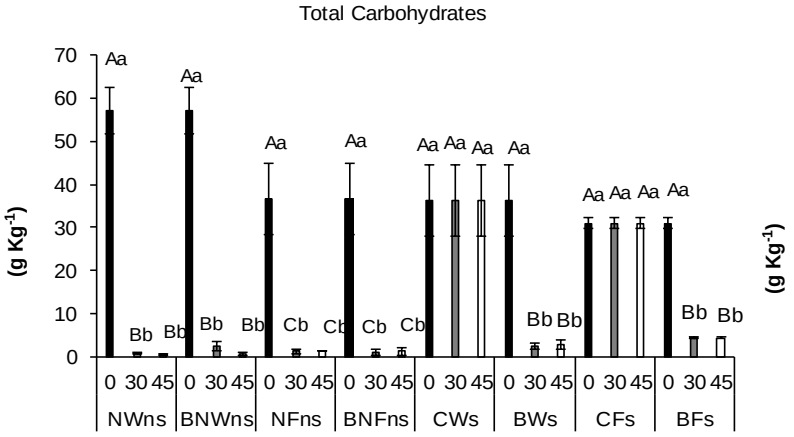

Figure. 1. Degradation of total carbohydrates, during pre-treatment of fractionated bagasse (F) or whole (W) with *B. adusta* (B) or native fungi (N) in sterilized (s) or non-sterilized (ns) conditions.

The bagasse fractional favoured the degradation of lignin by the mixture with fungi native, plus *B. adusta* and *B. adusta* only (NFNs, BFNs and BFs), where the degradation of lignin was BFE > CFNe > BFNe without significant differences between them ($P > 0.05$) (Table 2). Similar degradation have been found by other authors using bagasse with fungi as *Lenzites betulin*, *Daedalea elegans*, *Polyporus giganteus* on sugarcane with a degradation of lignin of the 47%, 59% and 65% respectively in 90 days (Oluseyi and Isola, 2009). The treatments of the native fungi and *B. adusta* presented activities of MnP and Lac to 30 days. The treatment in bagasse whole with native fungi plus *B. adusta* (BWNs) showed enzymatic activities for the day 30 of MnP (7.65×10^{-6} U g^{-1}) and Lac (44×10^{-6} U g^{-1}), while in fractional bagasse (BFNs) only showed activity of MnP of 155.5×10^{-6} g^{-1}. Treatments with *B. adusta* in whole bagasse (BWs) only had activity of Lac (34.5×10^{-6} U g^{-1}) on 30 days and for the day 45 showed both MnP (12.4×10^{-6} U g^{-1}) and Lac (84.5×10^{-6} U g^{-1}). The same treatment with fractional bagasse (BFs) presented only activity of MnP (812.1×10^{-6} U g^{-1}) at 30 day with a 72% of lignin degradation, and for the day 45 presented the two activities of MnP (77.5×10^{-6} U g^{-1}) and Lac (60.5×10^{-6} U g^{-1}) with a lignin degradation 74%.

Table 2. Total degradation of lignocellulosic components in the different stage of process.

Treatment	Pre-treatment degradation (%)			Vermicomposting degradation (%)[a]			Total degradation (%)		
	H*	C*	L*	H	C	L	H	C	L
BWs+E	54	32	62	40	60	12	94	92	74
BNWns+E	51	58	61	43	34	12	94	92	73
BFs+E	65	49	72	0	0	0	65	49	72
BNFns+E	71	43	71	22	50	5	93	93	76
CWs+E	0	0	0	21	29	15	21	29	15
NWns+E	64	51	56	15	37	0	79	88	56
CFs+E	0	0	0	0	29	24	0	29	24
NFns+E	67	43	74	27	43	17	94	86	91
BWs-E	54	32	62	17	13	4	71	45	66
BFs-E	65	49	72	3	0	1	68	49	73
NWns-E	40	0	33	12	24	14	52	24	47
NFns-E	41	0	29	5	26	25	46	26	54

[a] The percentage of degradation in the vermicomposting was calculated by difference of final degradation minus the degradation in the pre-treatment; * H = hemicellulose C = cellulose L = lignin.

This showed that the presence of *B. adusta* favoured the production of these enzymes to get the breaking of lignin as biological pre-treatment to degrade the bagasse agave. Also, it was observed that the enzyme activity and the degradation of lignin were greater in the bagasse fractionated than whole. García-Torres y Torres-Sáe (2003), found in sugarcane bagasse (0.08 cm particle size) with the fungi *Trametes versicolor* and *Pleurotus floridae* a Lac concentration of 0.11 U g^{-1} and 0.05 U g^{-1}; MnP concentration of 0.03 U g^{-1}, 0.05 U g^{-1} and 0.01 U g^{-1} respectively, at 21 days.

On the other hand, degradation was greater in the vermicomposting of fractional bagasse and pre-treated with *B. adusta* + fungi native (BFNs) (hemicellulose 93%, cellulose 93% and lignin 76%) than native fungi (NFNs) (hemicellulose 94 %, cellulose 86% and lignin 91%). The treatments with whole bagasse (NWNs, NWs, BWNs, BWs) had a lower degradation that the fractional bagasse (NWNs, NWs, BWNs, BWs) (Table 2). This suggested that particle size has an unimportant role in the colonization of the fungus and that a smaller particle size favoured the degradation of the bagasse in an accelerated vermicomposting with a pre-treatment with fungi of the white rot. In a similar study, Kumar et al. (2010) reported degradation of 69% lignin, 32% hemicellulose and 62% cellulose in sugarcane bagasse pre-treated with a mixture of fungi (*Pleurotus sajor-caju, Asperigillus niger* and *Trichoderma viridae Asperigillus*) and followed by vermicomposting with earthworms *Drawida wills* during 40 days.

The vermicompost obtained from the treatment of *B. adusta* + fungi native (BWNs) had characteristics of maturity and stability very close to or within the indices reported by other authors, such as 200 mg NH_4^+ kg^{-1}, 8 mg NO_2^- kg^{-1}, 175 mg NO_3^- kg_{-1}, 1.3 NH_4^+ /NO_3^-, 1.4 HA/FA and 175 mg CO_2 kg^{-1} h^{-1}. Followed by the NFNs treatments and BFNs that had values of these indices adequate. All treatments had values >100% of germination index which indicated that they were not phytotoxic (Table 3).

Table 3 Maturity and stability indices for vermicomposts obtained with accelerated vermicomposting process.

Tratamiento	N-NH4 (mg kg-1)	N-NO3 (mg kg-1)	N-NO2 (mg kg-1)	NH4/NO3	HA/FA	CO2 (mg kg-1 h-1)
NWNs+E	45 ± 49	1322 ± 676	16 ± 0	0.033 ±0.05	1.24 ±0.14	143.3 ± 14.1
BWNs+E	200 ± 99	175 ± 42	8 ± 0	1.300 ± 0.9	1.37 ± 0.08	57.60 ± 8.98
NFNs+E	186 ± 0	772 ± 0	24 ± 0	0.241 ± 0.1	0.41 ±0.00	15.56 ± 0.00
BFNs+E	40 ± 0	532 ± 0	8 ± 0	0.075 ± 0.04	1.07 ±0.28	65.02 ± 0.00
NWs+E	30 ± 14	312 ± 66	10 ± 8	0.093 ± 0.05	0.53 ±0.27	45.28 ± 17.57
BWs+E	40 ± 0	347 ± 211	15 ± 2	0.163 ± 0.1	0.88 ±0.17	57.06 ± 6.24
NFs+E	50 ± 0	1936 ± 0	8 ± 0	0.026 ± 0.01	0.53 ±0.0	15.21 ± 0.00
Indices	75-5002	>402	<52	0.5-32	>1.91	≤1203

[1] (Raj y Antil, 2011); [2] (Wichuk y McCartney, 2010); [3] (Hue y Liu, 1995)

CONCLUSIONS

The agave bagasse pre-treatment either with *B. adusta* UAMH 8258 or native fungi accelerated degradation when it was subjected to a vermicomposting process, which was higher in fractionated than in whole bagasse. Degradation of hemicellulose, cellulose and lignin in pre-treatment were greater than those attained in the vermicomposting process. An accelerated vermicomposting (pre-treated with *B. adusta* plus native fungi) could reduce the degradation time of agave bagasse to 3 months compared to a traditional composting performed in the tequila factories by 7 months. The vermicomposts obtained were stable and mature according to most of the standards and limits established to composts.

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