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**Valorization of soy waste through SSF for the production of compost enriched  
with *B. thuringiensis* with biopesticide properties**

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## Abstract

There is a growing generation of biodegradable wastes from different human activities from industrial to agricultural including home and recreational activities. On the other hand, agricultural and horticultural activities require significant amounts of organic amendments and pesticides. In this framework, the present study evaluates the viability of soy fiber residue valorization as organic soil amendment with biopesticide properties through aerobic solid-state fermentation (SSF) in the presence of *Bacillus thuringiensis* (Bt). The experiments were performed first under sterile and non-sterile conditions at lab scale using 115 g of sample and controlled temperature (30°C). Bt growth was successful in sterile conditions, obtaining  $6.2 \times 10^{11}$  CFU g<sup>-1</sup> DM and  $8.6 \times 10^{10}$  spores g<sup>-1</sup> DM after 6 days. Bt survived on solid culture under non-sterile conditions ( $3.8 \times 10^9$  CFU g<sup>-1</sup> DM and  $1.3 \times 10^8$  spores g<sup>-1</sup> DM). Further, the valorization process was scaled-up to 10 L reactors (2300 g) under non-sterile conditions obtaining a final stabilized material with viable Bt cells and spores ( $9.5 \times 10^7$  CFU g<sup>-1</sup> DM and  $1.1 \times 10^8$  spores g<sup>-1</sup> DM in average) after 9 days of SSF. These results confirm the possibility of managing biodegradable wastes by their transformation to a waste derived soil amendment with enhanced biopesticide effect, in comparison to traditional compost using a valuable and low-cost technique (SSF).

**Keywords:** waste valorization; soy fiber residues; *B. thuringiensis*; soil amendment; solid state fermentation; compost.

## 1. Introduction

Organic wastes are worldwide produced in increasing amounts from different sources. Many industrial wastes contain an organic fraction or are mainly organic in nature. From those wastes, some are biodegradable. In that case, preferred management options include their valorization to obtain biogas, valuable products or a soil organic amendment among others (Murthy and Naidu 2012). A good example are wastes from agro and food industries that are hardly contaminated by substances that can prevent them from being recycled into valuable products such as fertilizers, closing in this manner the cycle: raw materials for agro and food products come from agriculture and agricultural soils, thus valorization of organic wastes from these industries into soil will contribute to maintain soil fertility and compensate the loss of nutrients (Chiew et al. 2015; Paradelo et al. 2013). Specifically, a local factory produces soy milk and tofu and generates 20 t per week of soy residues following grain processing. These soy residues are currently treated by composting and/or used for livestock feed. Furthermore, these soy residues are rich in water-insoluble ingredients including fiber in its majority, but also protein, fat, starch and sugar. Thus, they can be potentially used as high quality media for fermentation (Hiesh and Yang 2004). About 1.1 kg of fresh residue has been reported to be produced from every kilogram of soybeans processed into soy milk or tofu (Khare et al., 1995). In fact, different researchers explored the possibilities of soy wastes as raw material in the production of organic acids, acetone, butanol, ethanol or enzymes from fermentable sugars (Karki et al., 2011).

There is also the novel possibility of obtaining a soil amendment with added biopesticide properties through solid-state fermentation processes in presence of *Bacillus thuringiensis* (Bt). Solid-state fermentation (SSF) has been defined as the

fermentation process that involves a solid matrix and it is performed in the absence or near absence of free water on a substrate possessing enough moisture to ensure microorganisms' growth and metabolism (Pandey 2003). The SSF process has been used in different studies for the production of high value added products such as enzymes, biofuel, biosurfactants and biopesticides from many residues, mostly working at laboratory scale (Murthy and Naidu 2012; Singhania et al. 2009). Thus, SSF is also presented as a viable technique for waste valorization as a source of valuable products to be considered in a biorefinery scheme (Forster-Carneiro et al. 2013).

On the other hand, *Bacillus thuringiensis* (Bt) is a spore former, facultative anaerobic gram-positive bacterium present in soil, water and plant surfaces. It is a producer of a paraesporal crystal protein also called  $\delta$ -endotoxin. The toxin has a great potential to cause mortality to insects belonging to different orders such as Diptera, Coleoptera and Lepidoptera, pests that destroy more than 40% of the world's food, forage, and fiber production. Conversely, these toxins are innocuous for plants, animals and human beings. The biopesticides used in biological control of plagues are an environmentally safe alternative to synthetic pesticides. They have been used worldwide for many years for food crops and forestry pests (Chandler et al. 2011).

The production of Bt based-biopesticides has been studied mainly by submerged fermentation and applied at industrial scale, with few studies in solid-state fermentation. In these cases, different wastes have been used as substrates, such as soy residues, wastewater treatment sludge, kitchen waste, wheat bran, among others (Devi et al. 2005; Zhang et al. 2013; Zhuang et al. 2011). So far, all the SSF studies have been performed under sterile conditions and mesophilic temperatures (Pham et al. 2010; Smitha et al.

2013).

Taking advantage of the ability of Bt to produce spores in adverse conditions, the aim of this study is to valorize soy fiber residue (from food industry) by SSF to obtain a soil amendment with the biopesticide effect of Bt. In this sense, the challenge is to make this specific bacterium grow in soy fiber residue under SSF process without sterilization and under self-heating conditions (which is the case of SSF at real scale with significant amounts of waste) including two scales (500 mL and 10 L reactors). This is, to our knowledge, the first study conducted under these conditions, as a viability waste valorization test for future application at industrial scale as a management option for different biodegradable organic residues including industrial, agricultural and municipal wastes.

## **2 Materials and Methods**

### **2.1 Materials**

Soy fiber residue (95.9% organic matter, C/N 12.2, pH 7.35) from a local food industry in Barcelona (Spain) was used as substrate in valorization tests. Wood sticks were mixed with soy fiber (1:1, v:v) to add structure to the solid matrix and compensate the soy waste high moisture content (83.78 %). Soy fiber residue presented a dynamic respiration index (DRI) of  $4.7 \pm 0.2 \text{ g O}_2 \text{ kg}^{-1} \text{ DM h}^{-1}$  that indicates a high biodegradability (Ponsá et al. 2010), due to its N and C content and availability. A strain of *Bacillus thuringiensis subsp. kurstaki* (ATCC 35866) was used in this study.

## 2.2 SSF at 500 mL and 10 L reactors

Both 500 mL and 10 L reactors were equipped with temperature, airflow and oxygen monitoring and online calculation of the specific oxygen uptake rate, sOUR. This value was calculated as the difference in O<sub>2</sub> content of input and output airflow per amount of dry matter present in the reactor, following Equation 1:

$$\text{sOUR} = F (0.209 - y_{\text{O}_2}) \frac{P \times 60 \times 32}{R \times T \times \text{DM}} \quad (\text{Eq. 1})$$

Where sOUR is the specific oxygen uptake rate (g O<sub>2</sub> kg<sup>-1</sup> DM h<sup>-1</sup>); F, the airflow in the reactor (L min<sup>-1</sup>); y<sub>O<sub>2</sub></sub>, is the oxygen molar fraction in the exhaust gases (mol O<sub>2</sub> mol<sup>-1</sup>); P, the pressure of the system assumed constant at 101,325 Pa; 32 is the oxygen molecular weight (g O<sub>2</sub> mol O<sub>2</sub><sup>-1</sup>); 60 is the conversion factor from minute to hour; R, the ideal gas constant (8310 Pa L K<sup>-1</sup> mol<sup>-1</sup>); T, the temperature at which F is measured (K) and DM, the dry matter present in the reactor (kg).

The experiments at 500 mL scale were performed at constant temperature (30°C) in triplicate both in sterile and non-sterile conditions as well as control test (without Bt inoculation, in triplicate) for a period of 6 days, using 100 g of soy residue with 5-10% Bt as inoculum and properly mixed with wood sticks as bulking agent (15 g). Constant aeration was provided at 15 mL min<sup>-1</sup>.

Experiments at 10 L scale were performed with 2300 g of mixture operating under near-adiabatic conditions (no temperature control). Aeration was provided following a control strategy with sOUR as the control parameter in order to maximize biological activity as detailed in Puyuelo et al. (2010). Runs were undertaken for 20 days, taking

samples in days 0, 6, 9, 13, 16 and 20. The mixture was prepared by first mixing soy fiber and Bt inoculum (10%), and adding in a second step wood sticks as bulking agent in a wet weight ratio of 1:1.

Inoculum for SSF was prepared by culturing Bt in 5 mL nutrient broth (Oxoid®, Powder 1%, Peptone 1%, NaCl 0.5%) at 30°C and 130 rpm for 3 hours, then 1 mL of the mixture was transferred to a 500 mL Erlenmeyer flask with 100 mL of liquid medium until achieving 24h of growing. Biomass was centrifuged (3500 rpm) and the concentrated inoculum was applied to the substrate in the 500 mL reactors. In the case of 10 L reactors, a Bt-soy fiber mixture obtained by SSF in 24h at 500 mL scale (attaining viable cells and spore counts of  $3.5 \cdot 10^9$  and  $4.6 \cdot 10^9$  CFU g<sup>-1</sup> DM) was used as inoculum.

### **2.3 Analytical methods**

Viable cells were quantified by mixing 10g of solid sample with 90 mL of Ringer solution (NaCl 0.225%, KCl 0.001%, CaCl<sub>2</sub> 0.012%, NaHCO<sub>3</sub> 0.005%) in a shaker at 130 rpm for 30 min. Serial dilutions were prepared from this mixture, then plated on nutrient agar petri dishes and incubated at 30°C during 18h. Manual counting of viable cells was performed afterwards. To quantify the spore's content, the diluted sample was maintained at 80°C during 10 min, followed by 5 min in a cold bath (iced bath) (Zhuang et al. 2011). After this, the same procedure for viable cells was applied. In both cases, results were expressed as the mean value of triplicates. The viable cells are expressed as CFU (colony forming units) per gram of dry matter of the solid matrix. Gram stain was used to differentiate and identify Bt in the mixture and malachite green (Brock et al. 1984) was used as a differential stain for bacterial endospores. Scanning electron



microscopy (SEM) was used (Evo® MA10, Carl Zeiss) in order to identify the toxin morphologically.

The dynamic respiration index (DRI) of initial mixtures and final products was measured in triplicate using a dynamic respirometer (Ponsá et al. 2010) to evaluate the degree of biological stability and final material stability. In this analysis, 100 g waste sample was placed in an Erlenmeyer flask, containing a plastic net to support the organic waste and provide an air distribution chamber, placed in a water bath at 37°C (Barrena et al., 2005). Airflow in the reactors was manually adjusted by means of an air flow controller (Bronkhorst Hitec, the Netherlands) to provide constant airflow, and modified when necessary to ensure minimum oxygen content in exhaust gases of 10% v/v. Exhaust air from the flasks was sent to an oxygen sensor prior dehumidification in a water trap. Both air flow meters and oxygen sensors were connected to a data acquisition system to continuously record these values for DRI calculation. Equation 1 is also valid for DRI in the conditions of the analysis.

Germination tests were performed as proposed in Komilis and Tziouvaras (2009) using radish seeds. 10 g (ww) of the final material obtained in the 10L reactors were mixed with deionized water in an Erlenmeyer flask at a ratio of 10:1 (water volume, in ml, to dry weight, in g) for 30 min at room temperature using a magnetic stirrer. The suspension was then filtered under vacuum using a 0.45-µm filter. 10 radish seeds were plated in a Petri dish where also 10 mL of filtrate were added. A control test was performed adding 10mL of deionized water instead of filtrate. Tests were undertaken in triplicate. Petri dishes were left at room temperature (22°C) for seven days. After this period germinated seeds were counted and root length measured. Germination

percentage and root length were expressed as a percentage of the corresponding values of the control (Komilis and Tziouvaras, 2009).

MacConkey agar medium N°2 (Oxoid®) as described by Brock et al. (1984) was used to evaluate the presence of *Enterobacteriaceae* in initial and final samples. As Bt toxicity has been related to protease activity, this parameter was also determined. The extraction and quantification of protease activity was realized as described previously by Abraham et al. (2013). One unit of alkaline protease activity was defined as 1 µg of tyrosine released per minute and per g of DM of residue under the assay conditions. Moisture content, organic matter content, total organic carbon (TOC), total nitrogen Kjeldahl (TNK) and pH were determined according to the standard procedures recommended by the Test Methods for the Examination of Composting and Compost (2001).

### **3 Results and Discussion**

In a first step, the viability of Bt growth on soy fiber residue was tested at lab scale (500 mL Erlenmeyer reactors) under sterile and non-sterile conditions. Afterwards, to attain the main objective of this work, experiments working under non-sterile and self-heating conditions as well as at a pilot scale (10 L reactors) were undertaken, simulating the conditions performed at industrial scale.

#### **3.1 SSF experiments at 500 mL scale**

SSF experiments were first performed in sterile conditions to check the capability of Bt to grow in soy fiber residue. sOUR profiles clearly showed the development of Bt in the solid matrix. The maximum rate of oxygen consumption (sOUR max) was  $2.8 \pm 0.2$  g O<sub>2</sub>

$\text{kg}^{-1} \text{DM h}^{-1}$ , reached at day 2, after a lag phase of  $16 \pm 8 \text{ h}$  (Table 1). The cumulative oxygen consumption for four days ( $\text{COC}_4$ ) was  $236 \pm 2 \text{ g O}_2 \text{ kg}^{-1} \text{DM}$ .

Viable cells and spore count were performed in one of the triplicates. Gram and malaquite green stains were used to confirm the presence of Bt during the fermentation using microscopy ( $\times 100$ ). CFU at the moment of maximum sOUR were higher than the initial inoculum ( $6.2 \times 10^{11}$  compared to  $1.5 \times 10^9 \text{ CFU g}^{-1} \text{DM}$ ) and remained at that order of magnitude. Besides, the amount of spores counted at final stage (6 days) was  $8.6 \times 10^{10} \text{ spores g}^{-1} \text{DM}$ . Both parameters showed the adequate development of Bt in the selected solid matrix and agreed with previous SSF studies. Devi et al. (2005) found that the production of Bt at lab scale on sterile wheat bran medium was  $6.6 \times 10^{10} \text{ spores g}^{-1} \text{DM}$  and  $1 \times 10^{11} \text{ spores g}^{-1} \text{DM}$  when enriching medium with C and N sources. Brar et al. (2007) and Yezza et al. (2006) found a correlation between the toxicity of Bt (spore formation), the cell count and the protease activity produced in submerged fermentation experiments on Bt growth on sterile conventional media, wastewater and wastewater sludge. Thus, protease activity was also determined in this work. Protease activity values obtained in days 3 and 6 were  $1282 \pm 32$  and  $1357 \pm 18 \text{ U g}^{-1} \text{DM}$  respectively. The protease yield was much higher than that obtained in SmF at the same scale in sterile conditions ( $336 \pm 8 \text{ U g}^{-1} \text{DM}$ ) and in previous SSF experiments using the same residue in non-sterile conditions without the addition of Bt ( $310 \pm 9 \text{ U g}^{-1} \text{DM}$ , Abraham et al. 2013).

Table 1. Parameters obtained in sterile and non-sterile SSF experiments of soy fiber residue with Bt inoculum (6 days of fermentation).

	sOURmax (g O <sub>2</sub> kg <sup>-1</sup> DM h <sup>-1</sup> )	COC <sub>4</sub> (g O <sub>2</sub> kg <sup>-1</sup> DM)	Lag phase (h)	Final Spore count (CFU g <sup>-1</sup> DM)	Final Viable Cells (CFU g <sup>-1</sup> DM)	Protease Activity (U g <sup>-1</sup> DM)
Sterile Test						
Soy + Bt (1.5x10 <sup>9</sup> CFU g <sup>-1</sup> DM)	2.8±0.2	236±2	16±8	8.6x10 <sup>10</sup>	6.2x10 <sup>11</sup>	1282 ± 32 (3d) 1357 ± 18 (6d)
Test 1 (non-sterile)						
Soy	6.5±0.7	325±1	6.5±1.0	n.a.	n.a.	n.a.
Soy + Bt (4.3x10 <sup>9</sup> CFU g <sup>-1</sup> DM)	4.8±0.7	292±13	5.2±0.6	1.3x10 <sup>8</sup> ±2.83x10 <sup>7</sup>	3.8x10 <sup>9</sup> ±7.7x10 <sup>7</sup>	616 ± 18 (3d) 497 ± 17(6d)
Test 2 (non-sterile)						
Soy	7.0±0.9	319±51	4.6±1.5	n.a.	n.a.	n.a.
Soy + Bt (4.3x10 <sup>9</sup> CFU g <sup>-1</sup> DM)	5.9±1.6	277±20	1.4±0.5	n.a.	n.a.	n.a.

sOUR: specific oxygen uptake rate; COC<sub>4</sub>: cumulative oxygen consumption in 4 days; n.a. not analyzed. Results are a mean ± SD of three values

With the aim of checking the viability of valorizing the waste as obtained from the industrial process, a second experiment was performed in non-sterile conditions. The amount of inoculum was increased from 5 to 10% due to the possibility of major competition with autochthonous microorganisms. The experiment was performed twice (Test 1 and Test 2) in triplicate using soy fiber collected at the food industry in two different days. The results of the fermentation are summarized in Table 1. Values for sOUR<sub>max</sub> and COC<sub>4</sub> obtained in SSF in presence of Bt were lower than the values obtained in the reactors without Bt addition. Total oxygen consumed in Bt reactors was 13% and 10% less than in non-inoculated soy fiber reactors. Bt growth can induce a reduction of biodiversity in the solid matrix, implying lower rates of oxygen consumption compared to the native microbial population. Contrarily, the lag phase was shorter in Bt experiments (21% and 69% respectively), suggesting a fast adaptation of Bt during the initial phase of SSF. Values of sOUR<sub>max</sub> under non-sterile conditions are higher than those obtained in the tests with the sterilized soy residue while the lag phase is shorter when non-sterilized waste is used. This comparison suggests that the activity of Bt progresses in parallel to the activity of the microorganisms originally present in soy fiber residue contributing to organic matter degradation and stabilization.

Protease activity values obtained after 3 and 6 days of experiment were  $616 \pm 18$  and  $497 \pm 17 \text{ U g}^{-1} \text{ DM}$  respectively, values clearly lower than those obtained on sterile soy waste and higher than those reported in Abraham et al. (2013) for non-sterile soy residue without Bt addition. Thus, the presence of Bt increases the production of proteases from non-sterile soy fiber waste although better conditions for the production of these enzymes seem to be found under sterile conditions.

Viable cells and spore counts were performed only in one of the replicates. The results showed a slight decrease in the number of viable cells during the experiment (from an initial value of  $4.3 \times 10^9$  to a value of  $3.6 \times 10^8$  CFU g<sup>-1</sup> DM at day 6) probably due to the consumption of the nutrients and to the ending of the exponential Bt growth, or due to competition with other microorganisms. Finally, at 6 days of SSF, a production of spores due to adverse conditions was detected ( $1.3 \times 10^8$  spores g<sup>-1</sup> DM). Viable cells and spores in the final material indicate that Bt is able to survive in a non-sterile soy fiber waste after 6 days of a SSF process. SEM (Scanning Electron Microscopy) image in Figure 1a reveals the presence of the toxin produced by Bt since this toxin has rhomboidal shape and has been previously recognized using this technique (Swiecicka et al. 2008).

### **3.2 10 L Reactors scale-up**

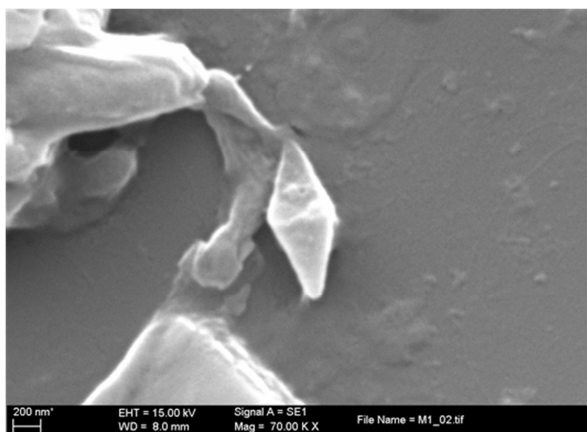
Bt growth was investigated under non-sterile conditions and with no temperature control (self-heating of the material) in 10 L adiabatic reactors simulating the real conditions that would be found in a full-scale SSF, composting-like, process. The metabolic heat generated during waste degradation was accumulated and a dynamic profile of temperature was developed. This allowed determining the effect of temperature on Bt growth and the possibility of waste hygienization. Figure 2 shows temperature and sOUR profiles for a control reactor without inoculation (R1) and for the Bt inoculated reactors in duplicate (R2 and R3). Viable cells and spore counts are also shown for the latest (figures 2b and 2c). Table 2 summarizes the initial and final values of other process parameters (pH, water content and DRI) as well as initial and final total cell count, total oxygen consumption along the SSF processes and results of the germination tests. As stated above, aeration was provided according to a sOUR

control strategy to maximize biological activity. There was a moisture increase in the material due to the use of completely closed reactors since a significant part of the water produced during SSF is retained inside the reactor as condensate in vessels' cover affecting moisture content of the upper material layers.

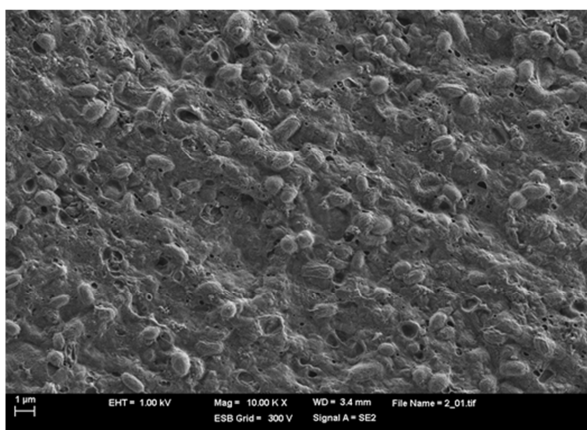
As can be seen in Figure 2, temperature reached the thermophilic range ( $>45^{\circ}\text{C}$ ) in the first day of the process. Maximum temperatures achieved were  $60.1^{\circ}\text{C}$  (R1),  $63.3^{\circ}\text{C}$  (R2) and  $65^{\circ}\text{C}$  (R3) occurring in day 3 of process in the case of control reactor and in day 2 for reactors R2 and R3 containing Bt. Temperatures over  $45^{\circ}\text{C}$  were maintained during 6.3 days in R1, 3.6 and 4.7 days in R2 and R3 respectively. This difference can be due to the presence of Bt inoculum previously acclimated to soy waste and active in degrading organic matter at initial temperatures. Higher temperatures and higher sOUR values in R2 and R3 when compared to R1 could lead to a lower amount of degradable organic matter in Bt inoculated reactors to support a longer thermophilic phase. Lag phase in R2 and R3 is almost non-existent compared to R1 (12 h). Temperature and sOUR profiles show a peak after material sampling and mixing (days 5 and 8) due to the enhancement of organic matter availability as observed by Abraham et al. (2013). Total oxygen consumed (Table 2) shows no real difference between Bt inoculated reactors and control. Final DRI values are characteristic of a very stable material (Ponsá et al. 2010). Germination index over 100% in all samples indicate no phytotoxic effects of the material. Thus, the compost obtained in the three reactors can be considered as a mature and stable material adequate for soil application (Komilis and Tziouvaras, 2009).

Figure 1. SEM images of the parasporal Bt crystal and spores: a) product obtained at lab scale (10kX) and samples taken after 60h of process at 10 L reactors (70 kX) b) R2 and c) R3.

a)



b)



c)

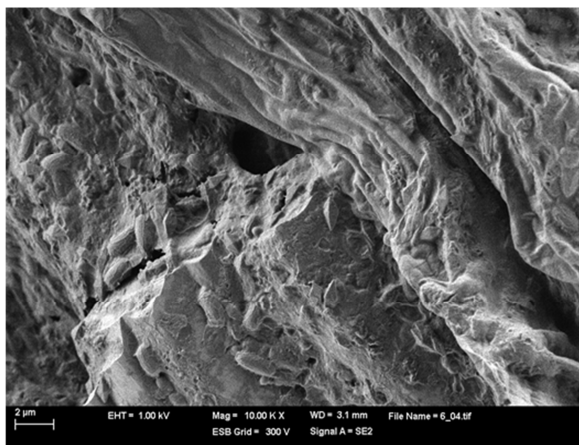
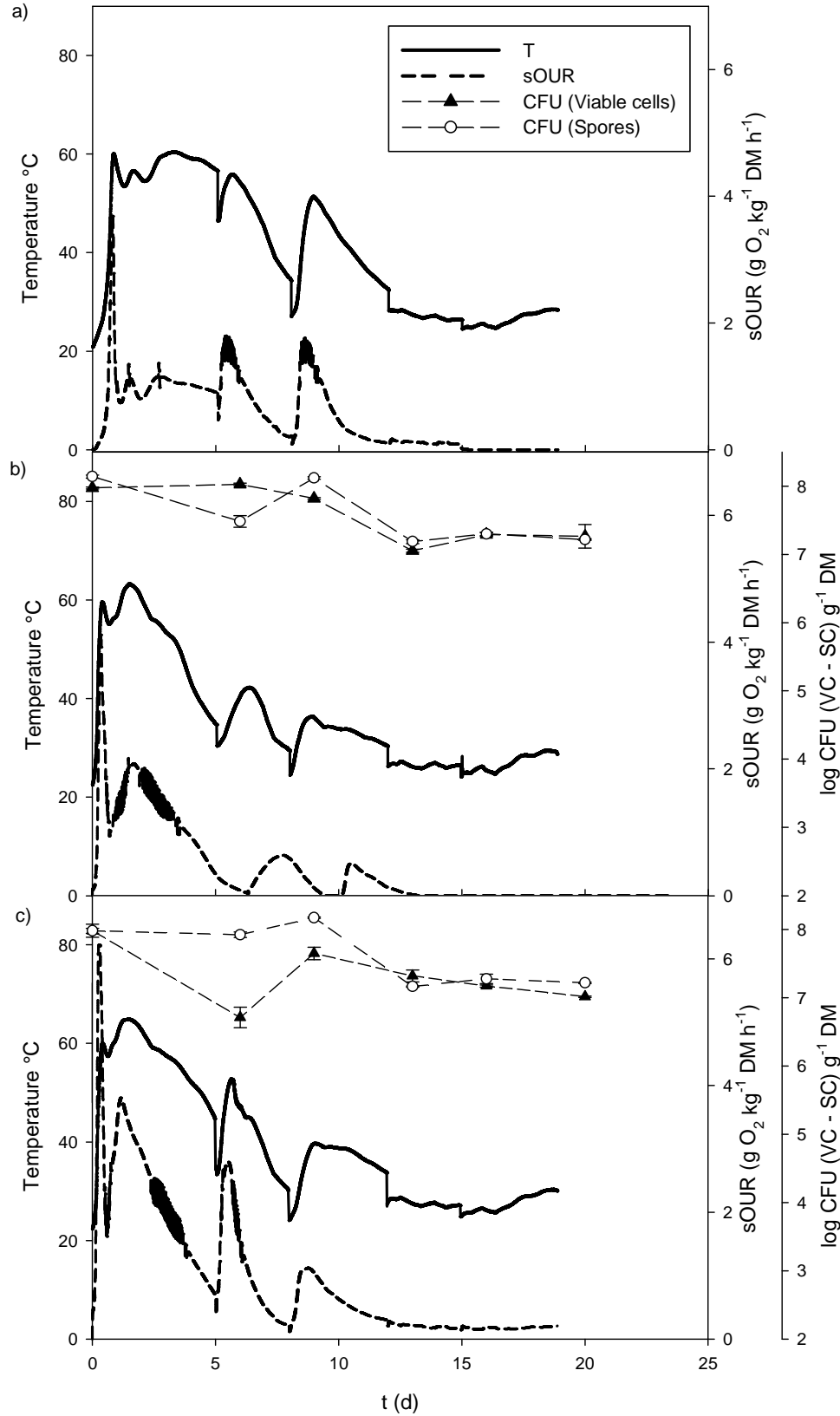




Figure 2. Temperature profile (solid line), sOUR evolution (dashed line), viable cells (VC) (triangle) and spore counts (SC) (circle) for SSF test 10 L Reactors: a) R1 (control, no Bt); b) R2 (Bt inoculated) and c) R3 (Bt inoculated).



Values of sOUR in Figure 2 indicate significant microbial activity until day 10 of the process approximately in the three reactors. In this period, viable Bt cells were maintained almost constant in R2 and R3 to decrease afterwards. Spore count decreased after temperature peak in both reactors to rise again in day 8 before the final descent to  $1.7 \times 10^7$  CFU g<sup>-1</sup> DM and  $1 \times 10^7$  CFU g<sup>-1</sup> DM in R2 and R3 respectively. The duration of process must be optimized to the highest spore and viable cells concentration with enough stability of final product. In any case, results evidence that Bt can resist thermophilic temperatures that would be achieved in a full-scale waste treatment plant during waste processing and is capable of competing with microorganisms already present in soy waste without addition of supplementary nutrient or applying any pretreatment to waste. This fact will enhance the possibilities of industrial scale utilization of soy fiber residue for Bt enriched soil amendment production. Zhuang et al. (2011) obtained values over  $10^{10}$  for viable Bt cells and spore counts working with sterilized sewage sludge in 8L SSF reactors operating at constant temperature, enhancing Bt growth with the addition of wheat bran and straw powder. Zhang et al. (2013) attributed to the difficulty of maintaining process temperature at desired values the decreased in spore count from  $10^{10}$  working with 4 kg to  $10^5$  working with 50 kg of sterilized kitchen waste.

Finally, SEM images of the final material in R2 and R3 (Figure 1b and 1c) show Bt spores and the characteristic rhomboidal shape  $\delta$ -endotoxin crystals, confirming presence of Bt in the fermented solid matrix. The protease activity values obtained in R2 and R3 were 392 and 290 U g<sup>-1</sup> DM respectively and correspond to the period of maximum biological activity and maximum temperature achieved in the SSF process. These values were lower than the obtained at 500 mL scale at the same conditions but

30°C. Temperature rise in 10 L reactors due to scale up could affect the production of these enzymes.

In addition, hygienization tests of final product were performed. While enterobacteriaceae were present in fresh waste samples, they were absent in the final material from R1, R2 and R3 (Table 2). Thus, a sanitized material was obtained with a significant content of Bt cells and spores in the cases of R2 and R3.

In conclusion, the results obtained at different scales, mainly in 10 L reactors, confirm the possibility of soy fiber waste valorization by means of SSF process through a product that can serve as soil amendment with enhanced biopesticide effect. This process can be also explored as potential valorization route for other type of organic wastes as obtained in the industrial plant or collection system, including organic fraction of municipal solid wastes, wastewater sludge or other agro or food industrial wastes.

Table 2. Initial and final values of pH, moisture, DRI, total O<sub>2</sub> consumed, viable, spores and total cell count in 10 L reactors.

Parameters	R1		R2		R3	
	(Control, no Bt)		(Bt)		(Bt)	
	Initial	Final	Initial	Final	Initial	Final
Moisture (% wb*)	48.9	57.3	50.1	56.4	45.7	54.7
pH	6.69	9.10	7.33	9.01	7.25	8.94
DRI (g O <sub>2</sub> kg <sup>-1</sup> DM h <sup>-1</sup> )	2.1±0.5	0.19±0.02		0.27±0.01		0.33±0.03
Total O <sub>2</sub> consumed (g)		418.2		317.4		516.2
Germination index (%)		134±21		115±20		146±22
Bt Viable cell count	0	0	9.3x10 <sup>7</sup>	1.8x10 <sup>7</sup>	9.6x10 <sup>7</sup>	1.7x10 <sup>7</sup>
(CFU g <sup>-1</sup> DM)			±0.3x10 <sup>7</sup>	±0.7x10 <sup>7</sup>	±1.6x10 <sup>7</sup>	±0.1x10 <sup>7</sup>
Bt Spore count	0	0	1.4x10 <sup>8</sup>	1.7x10 <sup>7</sup>	9.6x10 <sup>7</sup>	1.0x10 <sup>7</sup>
(CFU g <sup>-1</sup> DM)			±0.1x10 <sup>8</sup>	±0.1x10 <sup>7</sup>	±0.8x10 <sup>7</sup>	±0.1x10 <sup>7</sup>
Enterobacteriaceae	7·10 <sup>2</sup>	nd <sup>+</sup>	4·10 <sup>2</sup>	nd	2·10 <sup>2</sup>	nd
(CFU/g)						

\*wb: wet basis; <sup>+</sup>nd: not detected.

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