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Research article

Valorization of soy waste through SSF for the production of compost enriched with *Bacillus 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 6.2×10^{11} CFU g^{-1} DM and 8.6×10^{10} spores g^{-1} DM after 6 days. Bt survived on solid culture under non-sterile conditions (3.8×10^{9} CFU g^{-1} DM and 1.3×10^{8} spores g^{-1} 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 (9.5×10^{7} CFU g^{-1} DM and 1.1×10^{8} spores g^{-1} 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).

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

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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, *B. 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 δ-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 ± 0.2 g O_2 kg^{-1} DM h^{-1} that indicates a high biodegradability (Ponsá et al., 2010), due to its N and C content and availability. A strain of *B. 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_2 content of input and output airflow per amount of dry matter present in the reactor, following Equation (1):

$$sOUR = F(0.209 - yO_2)\frac{P \times 60 \times 32}{R \times T \times DM} \quad (1)$$

where sOUR is the specific oxygen uptake rate (g O_2 kg^{-1} DM h^{-1}); F, the airflow in the reactor (L min^{-1}); yO_2, is the oxygen molar fraction in the exhaust gases (mol O_2 mol^{-1}); P, the pressure of the system assumed constant at 101,325 Pa; 32 is the oxygen molecular weight (g O_2 mol O_2^{-1}); 60 is the conversion factor from minute to hour; R, the ideal gas constant (8310 Pa L K^{-1} mol^{-1}); 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^{-1}.

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 h, then 1 mL of the mixture was transferred to a 500 mL Erlenmeyer flask with 100 mL of liquid medium until achieving 24 h 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 24 h at 500 mL scale (attaining viable cells and spore counts of $3.5 \cdot 10^9$ and $4.6 \cdot 10^9$ CFU g^{-1} DM) was used as inoculum.

2.3. Analytical methods

Viable cells were quantified by mixing 10 g of solid sample with 90 mL of Ringer solution (NaCl 0.225%, KCl 0.001%, $CaCl_2$ 0.012%, $NaHCO_3$ 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 18 h. 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