DOI 10.1016/j.wasman.2017.09.041

A novel strategy for producing compost with enhanced biopesticide properties through solid-state fermentation of biowaste and inoculation with *Bacillus thuringiensis*

Cindy Ballardo, Raquel Barrena, Adriana Artola*, Antoni Sánchez

Composting Research Group

Department of Chemical, Biological and Environmental Engineering

Escola d'Enginyeria, Universitat Autònoma de Barcelona

Cerdanyola del Vallès, 08193 Barcelona, Spain

*Corresponding author: +34 935814480; adriana.artola@uab.cat

Abstract

In the framework of a circular economy, organic solid wastes are considered to be resources useful for obtaining value-added products. Among other potential uses, biodegradable wastes from agricultural, industrial, and domestic sources are being studied to obtain biopesticides through solid-state fermentation (SSF), mainly at the laboratory scale. The suitability of biowaste (source-selected organic fraction of municipal solid waste) for use as a substrate for *Bacillus thuringiensis* (Bt) growth under non-sterile conditions in a 10 L SSF reactor was determined in this study. An operational strategy for setting up a semi-continuous process yielding a stabilised organic compost-like material enriched with Bt suitable for use as a soil amendment was developed. Concentrations of $1.7 \cdot 10^7$-$2.2 \cdot 10^7$ and $1.3 \cdot 10^7$-$2.1 \cdot 10^7$ CFU g^{-1} DM for Bt viable cells and spores, respectively, were obtained in the final material. As the results confirmed, Bt-enriched compost-like material with potential biopesticide properties can be produced from non-sterile biowaste.

Keywords: *Bacillus thuringiensis*; biopesticide potential; enriched compost; non-sterile biowaste; solid-state fermentation

1. Introduction

Worldwide population growth has two consequences, among others, that require immediate actions and consensus plans: increasing production of all types of residues and increasing demand for agricultural products, mainly as direct or indirect human food sources. A significant portion of the waste produced from domestic, agricultural, and industrial activities is organic in nature, for example, food waste, yard waste, straw and other agricultural wastes, animal manures, food industry wastes, and wastewater sludge.

In the framework of a circular economy, the potential of solid state fermentation (SSF) for converting organic solid wastes into resources to obtain value-added products is gaining attention (Sánchez et al., 2015). SSF is defined as a fermentation process that is carried out in the absence or near absence of free water on a substrate that possesses sufficient moisture to support the growth of microorganisms and their metabolic activity (Thomas et al., 2013). SSF has been presented as an alternative to submerged fermentation to obtain enzymes, surfactants, biofuels, flavours, and other metabolites that can be used as raw materials in the chemical or pharmaceutical industry or as a direct product of these industrial activities. In fact, various studies have reported results obtained at the lab scale using organic wastes as feedstocks for the production of the aforementioned substances (Abraham et al., 2014; Bansal et al., 2012; Murthy and Naidu, 2012; Singhania et al., 2009; Zulkeflee et al., 2016). SSF has been included as a viable technique for waste valorisation in a biorefinery scheme (Forster-Carneiro et al., 2013).

Biopesticides have also been produced through SSF. Chandler et al. (2011) summarised the definition of a biopesticide as "a mass-produced agent manufactured from a living microorganism or a natural product and sold for the control of plant pests". This is the case for *Bacillus thuringiensis* (Bt), the most widely used microbial biopesticide, which is a gram-positive bacterium naturally present in soil that produces a crystal protein known as d-endotoxin. This parasporal crystal presents toxicity to many insect pests of the orders Diptera, Coleoptera, and Lepidoptera by ingestion, causing gut cell lysis (Chandler et al., 2011). Bt has traditionally been produced by submerged fermentation and applied by spraying onto crops, but the cost of production by this method is high due to the cost of the production medium. However, agricultural and industrial by-products with an adequate content of nutrients have been proposed as alternative growing media including wastewater and wastewater sludge (Prabakaran and Balaraman, 2006; Vidyarthi et al., 2002; Yezza et al., 2006).

In the case of Bt production through SSF, soy residues, wastewater sludge, kitchen waste, food waste, and wheat bran are some of the wastes that have been investigated (Devi et al., 2005; Zhang et al., 2013; Zhuang et al., 2011; Zou et al., 2016). To date, all such SSF studies have been performed under sterile conditions, and the majority of them have been done at the scale of a few grams (Pham et al., 2010; Smitha et al., 2013). In addition to sterilisation, other pre-treatments have been applied to substrate wastes to enhance Bt production (Ozcan et al., 2010). During SSF, the substrate is degraded to some extent. Previous works have demonstrated the ability of Bt to survive in an SSF process without temperature control using non-sterilised residual soy fibre as the substrate (Ballardo et al., 2016). During this process, thermophilic temperatures were attained and stabilisation and hygienisation of the substrate were reached. The final material could be considered as a soil organic amendment (compost-like)

containing Bt with potential biopesticide properties. The presence of Bt can enhance the effect of some bio-protective agents found in some composts (Suarez-Estrella et al., 2013). From a circular economy point of view, organic wastes from an agricultural source (in this case, soy waste) can be returned to agricultural activities in the form of crop protection and amendment agents. It is also possible that this practice could be extended to other wastes from agriculture and the agro and food industries and even to municipal organic food wastes (biowaste).

The objective of the present work was to determine the suitability of the source selected organic fraction of municipal solid waste (OFMSW) for use as substrate for Bt growth through SSF under non-sterile conditions at the scale of 10 L. A compost-like material with added biopesticide properties can be obtained as the final product of the SSF process. Different operational strategies have been tested to maximise Bt growth, and these strategies have focused on the suitability of the process for full-scale plant implementation in a semi-continuous mode and self-sustained inoculation procedure. The use of a high production waste, such as OFMSW, as received in the treatment plant (without pre-treatment or addition of supplementary nutrient sources) and the working scale represent a further step in biowaste valorisation through SSF.

2. Materials and Methods

2.1 Materials

Source selected OFMSW (25 kg) already mixed with wood chips as a bulking agent in a 1:1 volumetric ratio was collected from a composting plant in Manresa (Barcelona, Spain). The main characteristics of the mixture are summarized in Table 1 and presented as average and standard deviation values of at least three samples

collected during the study. Once collected, the OFMSW was separated into 5 kg bags and stored at −20ºC. The waste was used for the various tests after defrosting during 1 day at room temperature.

A commercial strain of Bt subsp. *kurstaki* (CECT 4497) was used in this study (Spanish Type Culture Collection, University of Valencia).

2.2 SSF reactors

The adiabatic 10 L reactors used for SSF 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 the O_2 content of the input and output airflow per amount of dry matter present in the reactor, following Equation 1:

$$sOUR = F\,(0.209 - yO_2)\,\frac{P \times 60 \times 32}{R \times T \times DM}, \qquad \text{(Eq. 1)}$$

where sOUR is the specific oxygen uptake rate (g O_2 kg^{-1} DM h^{-1}); F is the airflow entering and exiting the reactor (L min^{-1}); yO_2 is the oxygen molar fraction in the exhaust gases (mol O_2 mol^{-1}); P is the pressure of the system, assumed constant at 101,325 Pa; 32 is the molecular weight of diatomic oxygen (g O_2 mol O_2^{-1}); 60 is the conversion factor from minute to hour; R is the ideal gas constant (8,310 Pa L K^{-1} mol^{-1}); T is the temperature at which F is measured (K); and DM is the dry matter present in the reactor (kg).

SSF reactors were filled with non-sterile OFMSW and operated under near-adiabatic conditions (no temperature control). Aeration was provided continuously at a variable

airflow following a control strategy with sOUR as the control parameter to maximise biological activity, as detailed in Puyuelo et al. (2010). Samples of the material were withdrawn from the reactor for moisture and organic matter determination and cell count at different process days. Bt inoculum was added to OFMSW at a 10 % volume to weight ratio either at the beginning of the SSF process or after reaching thermophilic conditions according to the strategy followed. Reactors were run in duplicate. Control reactors were set-up in all cases following the same procedure and containing OFMSW without Bt inoculum (results not reported). The presence of Bt in the control reactors was checked periodically with negative results.

Inoculum for SSF was prepared by culturing Bt in 5 mL of nutrient broth (Oxoid®, powder 1 %, peptone 1 %, NaCl 0.5 %) at 30°C, 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 20 h of growth under the same agitation conditions. Scale-up was continued to 2 L reactors, while maintaining the 10 % v:v Bt inoculum:nutrient broth ratio. Compressed air was provided to the vessels by means of a plastic pipe at 2 NL h^{-1} flow, measured with a rotameter, to ensure adequate oxygen content for Bt growth. Another plastic pipe was installed for output flow of gases. Reactors were agitated mechanically and placed in a water bath for temperature control at 30ºC. Finally, the biomass was centrifuged (3,500 rpm) and the concentrated inoculum was mixed with the OFMSW.

Three operational strategies were tested in SSF 10 L reactors:

Strategy 1: Bt inoculation from the beginning of the process

Strategy 2: Inoculation of Bt after the thermophilic phase of the process (process temperature around 40ºC).

Strategy 3: Use of Bt containing solid mixture resulting from a previous SSF process for reactors' inoculation. It consisted of the following processes:

- A 10 L reactor, R1, was operated following Strategy 2, i.e. starting with OFMSW and inoculating Bt to the solid matrix after the thermophilic phase (approximately day 15 of the process). Day 1 of the total process was established as the first day of R1 operation.
- Inoculated R1 (named R1′) was run for 7 days, until day 21 of the total process, for Bt acclimation and growth.
- After the 7 days of R1 operation, a second reactor (R2) for OFMSW SSF was set up. It proceeded identically to R1 (thermophilic phase followed by return to mesophilic temperatures after 15 days, at approximately day 21 of the total process).
- At day 21 of the total process, with mesophilic temperatures in R2 (after the temperature peak), the contents of R2 were mixed completely with the contents of R1′, which acted as inoculum in a 1:1 w:w ratio. The resulting mixture was placed in two new 10 L reactors, RA and RB.
- RA and RB were operated for 30 additional days. Periodic mixing of the solid matrix was performed to promote a homogeneous moisture and organic matter distribution.

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 30ºC for 18 h. Manual counting of viable cells was performed subsequently. To quantify the spore content, the diluted sample was maintained at 80°C for 10 min, then placed in a cold (iced) bath for 5 min (Zhuang et al. 2011). After this process, the same procedure used for viable cells was applied. In both cases, the results were expressed as average value and standard deviation of triplicates in colony forming units (CFU) per gram of dry matter of 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) (Evo® MA10, Carl Zeiss) was used to identify Bt toxin morphologically.

Moisture content, organic matter content, and pH were determined following the standard procedures recommended by the Test Methods for the Examination of Composting and Compost (2001).

3. Results and Discussion

3.1 Previous results

Previous experiments using soy fibre residue as substrate have demonstrated the feasibility of this media for sustaining Bt development under non-sterile conditions at the 10 L reactors scale (Ballardo et al., 2016). Other authors have obtained satisfactory results using sterilised food and kitchen waste as economic substrates for Bt-based biopesticides production (Zhang et al., 2013; Zou et al., 2016). In light of the mentioned references, the suitability of OFMSW as a Bt growth medium was explored. First, tests performed at the 500 mL scale using sterilised and non-sterilised OFMSW under

continuous aeration and constant temperature (30ºC) were undertaken and achieved satisfactory results (not shown). To examine the viability of the process at a larger scale, 10 L adiabatic reactors were operated for SSF under non-sterile conditions following different operational strategies.

3.2 Operational strategies at the 10 L reactor scale

Three operational strategies were investigated to overcome the difficulties that occur when the process is performed at the 10 L scale.

The results obtained in **Strategy 1** are shown in Figure 1. The initial solid matrix (3.2 kg of OFMSW and wood chip mixture) had a moisture content of 59 %, organic matter content of 79 % (dry basis), and pH of 5.45. As expected, temperature increased immediately, reaching its maximum (69ºC) during the second day of the process (heat generation due to microorganism metabolism in the adiabatic reactors). Values higher than 45ºC (thermophilic range) were maintained for 7.5 days (from day 1 to day 8). The temperatures achieved and maintained in the reactor ensure the sanitation of the final product which is free of pathogenic microorganisms. Viable cells decreased from $5.8 \cdot 10^6$ CFU g^{-1} DM to $3 \cdot 10^5$ CFU g^{-1} DM until day 15 and showed a further increment on day 28 in coincidence with reactor temperatures being maintained in the mesophilic range (less than 45ºC). Temperature could have influenced Bt viable cell growth and development in the solid mass, although competition with indigenous OFMSW microbial populations could also have been present. Spore counts were almost constant during reactor operation at around $3 \cdot 10^6$ CFU g^{-1} DM. Considering sOUR values (and thus microbial activity during the process), a maximum of 5.1 g O_2 kg^{-1} DM h^{-1} was reached during the first day of the process, which is in the range reported previously for

OFMSW (Barrena et al., 2011). The peak observed in sOUR values should be attributed to thermophiles, adapted to the temperature conditions and mainly responsible (as higher values of sOUR demonstrate) for organic matter degradation during the process. A lower peak of sOUR can be observed on day 6 (Fig. 1) due to reactor mixing for representative sampling. From day 8 onward, mesophilic microorganisms regain prevalence. sOUR values are lower during the beginning of this phase, but they do not become negligible until the end (1.2 g O_2 kg^{-1} DM h^{-1} on day 8 to 0.3 g O_2 kg^{-1} DM h^{-1} on day 20). Thus, a stabilised material is obtained. Gonzalez et al. (2016) reported a decrease in the abundances of mesophilic and thermophilic bacteria and fungi after the thermophilic period during sewage sludge composting and established a relationship among total concentration of microorganisms and volatile solids content. This circumstance may have contributed to the Bt growth observed after day 15.

After 28 days of reactor operation, the stabilised solid was kept in an open container at room temperature for an additional 15 days (maturation phase) to determine Bt behaviour in the product obtained from SSF.

Taking into account the decrease of total Bt population registered by applying Strategy 1, a second operational strategy, **Strategy 2,**was tested inoculating Bt when process temperature descended to 40ºC. Two factors were expected to influence Bt growth under this strategy: the amount of remaining easily bioavailable organic matter in the solid matrix and the presence of indigenous mesophiles competing for that fraction. The results, presented in Figure 2, show Bt growth from day 15 of the process (when inoculation took place) until day 30, in both viable cells and spores, reaching $9 \cdot 10^6$ CFU g^{-1} DM (initial values were $2.5 \cdot 10^6$ and $4.3 \cdot 10^6$ CFU g^{-1} DM for viable cells and spores,

respectively). In this case, the maximum temperature reached was 64.6ºC, and thermophilic conditions were maintained for 7 days. The initial conditions were 3.2 kg of OFMSW and wood chip mixture (1:1, volume basis) containing 65% moisture (w:w, wet basis), 75% organic matter (w:w, dry basis), and having a pH of 5.23. The maximum sOUR, with a value of 4.5 g O_2 kg^{-1} DM h^{-1}, was obtained during the first day of the process and was in the range registered under Strategy 1 and reported previously for OFMSW (Barrena et al., 2011), confirming the adequacy of the solid matrix for process development. Because no loss in Bt viable cells and spores but rather a 4-fold increment of viable cells was observed using Strategy 2, this operation mode was considered satisfactory. The evolution of sOUR values confirmed the stabilisation of the material through the process.

The main restriction when operating at the 10 L scale was the preparation of the inoculum. As explained in the Materials and Methods section, the inoculum was prepared using a commercial medium with Bt growing in submerged fermentation. Prior to solid waste inoculation, the liquid inoculum was centrifuged to avoid excessive moisture content of the final solid mixture. All these steps can pose economic and operational difficulties when operating at a larger scale. **Strategy 3** was an attempt to overcome these aspects. As explained in Materials and Methods, a 10 L SSF reactor, R1, inoculated following Strategy 2 (becoming R1') was used as inoculum for R2 producing two new 10 L SSF reactors, RA and RB. These reactors were then operated for 30 days.

The whole process (12 days of R1 operation prior to Bt inoculation + 6 days of R1' allowed for Bt acclimation + 30 days of RA and RB operation) was monitored by

temperature, sOUR, and Bt viable cell and spore counts. The results are summarised in Figure 3. . The process evolution is shown for reactors RA (Fig. 3a) and RB (Fig. 3b). The temperature and cell-count profiles until day 18 are common to both RA and RB, as shown in Figure 3, and correspond to reactor R1 (OFMSW, until day 12) and R1′ (OFMSW + Bt, until day 18)The temperature in R1 evolved as in previous experiments, with a maximum of 65ºC attained at day 5 of the process. Thermophilic temperatures were maintained for 7 days. A similar profile was obtained in R2 (not shown), ensuring sanitation of the OFMSW during SSF. In day 18, the content of R2 was mixed with the content of R1' giving RA and RB which temperature and OUR profiles are presented separately (Fig 3a and 3b respectively). Table 2 presents the mass balance and process parameter values.

As proven by the initial and final values of Bt viable cells and spores in Table 2, Strategy 3 using the solid matter of a reactor initially inoculated with Bt (produced by submerged fermentation) to inoculate a second reactor should be considered a valid option for reactor operation. In fact, the final Bt viable-cell and spore concentrations were higher in reactors RA ($1.7 \cdot 10^7$ and $1.3 \cdot 10^7$ CFU g^{-1} DM for viable cells and spores, respectively) and RB ($2.2 \cdot 10^7$ and $2.1 \cdot 10^7$ CFU g^{-1} DM, respectively) than the initial concentration in R1′ ($6 \cdot 10^6$ and $5 \cdot 10^6$ CFU g^{-1} DM, also viable cells and spores, respectively) from which RA and RB originated. In addition to the increment in Bt concentration, the total amount of final Bt-containing material (4,544 g, RA + RB final weight in Table 2) was 1.5-fold the initial quantity in R1′ (3,000 g, organic matter degradation through SSF should be considered), reinforcing the positive balance in Bt production. The stability of the final product reflected in the final sOUR values in Figure 3 should also be highlighted. Relevant characteristics of the end SSF material

such as pH, organic matter and moisture content can be found in Table 2 as final values for reactors RA and RB. Although a stable product was obtained, further curing is required to be used as soil amendment. In other tests undertaken in domestic composting bins comprising a maturation stage, final materials with similar characteristics were obtained from Bt inoculated and non-inoculated bins (pH=8.15-8.17, MC= 33-39%, OM= 58%; C/N= 12.9-13.2; N= 3-3.1% in dry basis). In that case, values for respirometric and germination indices indicated that final materials were mature and stable (Dynamic Respiration Index < 1 gO_2 kg^{-1} OM h^{-1}; Germination Index > 100%, (Komilis and Tziouvaras, 2009)), with no significant differences between Bt enriched and non-enriched materials. Those materials presented also similar microbial biodiversity except for the presence of Bt in the inoculated bin while pathogenic microorganisms were not detected in any of them.

Thus, Strategy 3 permitted the set-up of a semi-continuous SSF process for Bt-enriched compost-like amendment production. The possibility of doing SSF without a previous SmF process to produce the inoculum is of great interest, especially if scale up is considered. The cost of commercial culture media for Bt growth is one of the key points in process economics (Zou et al., 2016), thus also important if inoculum has to be prepared by SmF.

Comparison of Bt concentrations obtained in the present work to literature values has not been possible due to the lack of works studying SSF of non-sterile wastes. Concentrations of Bt spores in the 10^9–10^{10} CFU g^{-1} range (clearly higher than those reached in the present study) have been obtained using sterile wastes (Balasubramanian and Tyagi, 2017). However, the current process strategy avoids energy consumption

and operational difficulties associated with solid waste sterilisation. A complete energy balance and economics study will be required to complete the evaluation of the process's viability. After a maturation period of the SSF product, Bt-enriched compost application study will also be necessary to determine the usage, dosage, and effects of this novel product.

4. Conclusions

Non-sterile OFMSW was used as substrate for Bt growth in an SSF process at the 10 L scale, and an operational strategy that permits the set up of a semi-continuous process to produce a compost-like material enriched with Bt suitable for use as an organic amendment was developed. This strategy allows Bt inoculation to OFMSW using the material obtained from a previous SSF process, thus avoiding inoculum production through submerged fermentation. The results obtained at the 10 L scale are promising although further pilot-scale studies are recommended.

Acknowledgments

The authors thank the Spanish Ministry of Economía y Competitividad (CTM2015-69513-R) with FEDER funding for financial support, the Consorci del Bages per a la Gestió de Residus for the waste supply, and Dr. Calvo Torras from the Department of Microbiology and Health Services, Veterinary School, UAB, for advice on Bt identification. Cindy Ballardo thanks the Peruvian Government for the President of the Republic Scholarship (PRONABEC). Raquel Barrena is grateful to TECNIOspring fellowship programme (TECSPR15-1-0051) co-financed by the European Union through the Marie Curie Actions and ACCIÓ (Generalitat de Catalunya).

References

1. Abraham, J., Gea, T. Sánchez, A., 2014. Substitution of chemical dehairing by proteases from solid-state fermentation of hair wastes. J Clean Prod. 7, 191-198.
2. Balasubramanian, S., Tyagi, R.D., 2017. Biopesticide Production From Solid Wastes. Current Developments in Biotechnology and Bioengineering. Solid Waste Manage. 43-58.
3. Ballardo, C., Abraham, J., Barrena, R., Artola, A., Gea, T., Sánchez, A., 2016. Valorization of soy waste through SSF for the production of compost enriched with *B thuringiensis* with biopesticide properties. J Environ Manage. 169, 126-131.
4. Bansal, N., Tewari, R., Soni, R., Soni, S.K., 2012. Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manage. 32, 1341–1346.
5. Barrena, R., Gea, T., Ponsá, S., Ruggieri, L., Artola, A., Font, X., Sánchez, A. 2011. Categorizing raw organic material biodegradability via respiration activity measurement: A review. Compost Sci and Ut. 19, 105-113.
6. Brock, T.D., Smith, D.W., Madigan, M.T., 1984. Biology of Microorganisms, 4th edition. Prentice-Hall, Inc., Englewood Cliffs, NJ.
7. Chandler, D., Bailey, A.S., Tatchel, G.M., Davidson, G., Greaves, J., Grant, W.P., 2011. The development, regulation and use of biopesticides for integrated pest management. Philos T Roy Soc B. 366, 1987-1998.
8. Devi, P.S.V., Ravinder, T., Jaidev, C., 2005. Cost-effective production of *Bacillus thuringiensis* by solid-state fermentation. J Invertebr Pathol. 88, 163-168.
9. European Commission, 2015. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Closing the loop - An EU action plan for the

Circular Economy, COM/2015/0614. Brussels, 2/12/2015. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52015DC0614. Last accessed: January 2017.

10. Forster-Carneiro, T., Bernib, M.D., Dorileoc, I.L., Rostagnoa, M.A., 2013. Biorefinery study of availability of agriculture residues and wastes for integrated biorefineries in Brazil. Resour Conserv Recy. 77, 78-88.
11. González, I., Robledo-Mahon, T., Silva-Castro, G.A., Rodríguez-Calvo, A., Gutiérrez, M.C., Martín; M.A., Chica, A.F., Calvo, C., 2016. Evolution of the composting process with semi-permeable film technology at industrial scale. J. Clean Prod. 115, 245-254.
12. Komilis, D. P., Tziouvaras, I. S., 2009. A statistical analysis to assess the maturity and stability of six composts. Waste Manage, 29, 1504-513.
13. Murthy, P.N., Naidu, M.M., 2012. Sustainable management of coffee industry by-products and value addition-A review. Resour Conserv Recy. 66, 45-58.
14. Ozcan, O., Icgen, B., Ozcengiz, G., 2010. Pretreatment of poultry litter improves *Bacillus thuringiensis*-based biopesticides production. Biores Technol. 101, 2401–2404.
15. Pham, T.T.H., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., 2010. Optimization of Fenton oxidation pre-treatment for *B. thuringiensis* – Based production of value added products from wastewater sludge. J Environ Manage. 91, 1657-1664.
16. Prabakaran, G., Balaraman, K., 2006. Development of a cost-effective medium for the large scale production of *Bacillus thuringiensis* var *israelensis*. Biol Control. 36, 288-292.
17. Puyuelo, B., Gea, T., Sánchez, A., 2010. A new control strategy for composting process based on the oxygen uptake rate. Chem Eng J. 165, 161-169.

18. Sánchez, A., Artola, A., Gea, T., Barrena, R., Font, X., 2015. A new paradigm for waste management of organic materials. Waste Manag. 42, 1–2.

19. Singhania, R.R., Patel, A.K., Soccol, C.R., Pandey, A., 2009. Recent advances in solid-state fermentation. Biochem Eng J. 44, 13-18.

20. Smitha, R.B., Jisha, V.N., Pradeep, S., Josh, M.S., Benjamin S., 2013. Potato flour mediated solid-state fermentation for the enhanced production of *Bacillus thuringiensis*-toxin. J Biosci Bioeng. 116, 595-601.

21. Suarez-Estrella, F., Jurado, M.M., Vargas-García, M.C., López, M.J., Moreno, J., 2013. Isolation of bio-protective microbial agents from eco-composts. Biol Control. 67, 66-74.

22. Test Methods for the Examination of Composting and Compost, 2001. The US Department of Agriculture and The US Composting Council, Edaphos International, Houston, USA.

23. Thomas, L., Larroche, C., Pandey, A., 2013. Current developments in solid-state fermentation. Biochem Eng J. 81, 146-161.

24. Vidyarthi, A., Tyagi, R., Valero, J., Surampali, R., 2002. Studies on the production of *B. Thuringiensis* based biopesticides using wastewater sludge as a raw material. Water Res. 36, 4850-4860.

25. Yezza A., Tyagi R.D., Valéro J.R., Surampalli R.Y., 2006. Bioconversion of industrial wastewater and wastewater sludge into *Bacillus thuringiensis* based biopesticides in pilot fermentor. Biores Technol. 97, 1850–1857.

26. Zhang, W., Qiu, L., Gong, A., Cao, Y., Wang, B., 2013. Solid-state Fermentation of Kitchen Waste for Production of *Bacillus thuringiensis*-based Bio-pesticide. Bioresources. 8, 1124-1135.

27. Zhuang, L., Zhou, S., Wang, Y., Liu, Z., Xu, R., 2011. Cost-effective production of *Bacillus thuringiensis* biopesticides by solid-state fermentation using wastewater sludge: Effects of heavy metals. Biores Technol. 102, 4820-4826.
28. Zou, H., Ding, S., Zhang, W., Yao, J., Jang, L., Liang, J., 2016. Study on influence factors in *Bacillus thuringiensis* production by semi-solid state fermentation using food waste. Procedia Environmental Sciences, 31, 127-135.
29. Zulkeflee, Z., Shamsuddin, Z.H., Aris, A.Z., Yusoff, M.K., Komilis, D., Sánchez, A. (2016). Performance, yield and characteristics of bioflocculants (UPMBF13) produced by *Bacillus subtilis* UPMB13 during submerged and solid-state fermentation. Global Nest. 18, 3, 611-620.

Figure captions

Figure 1. Evolution of temperature, sOUR, and Bt viable-cell and spore concentrations during SSF in 10 L adiabatic reactor using non-sterile OFMSW under Strategy 1: Bt inoculated at day 0 (day 28: end of reactor operation, material was kept at room temperature).

Figure 2. Evolution of temperature, sOUR, and Bt viable-cell and spore concentrations during SSF in 10 L adiabatic reactor using non-sterile OFMSW under Strategy 2: Bt inoculated at day 15, after thermophilic period (day 30: end of reactor operation, material was kept at room temperature).

Figure 3. Strategy 3: Semi-continuous operation in 10 L reactors. Evolution of temperature, sOUR, and Bt viable-cell and spore concentrations during SSF in 10 L adiabatic reactors using non-sterile OFMSW. The first 18 days of the process are identical in a) and b) and correspond to day 0–day 12, R1 without Bt inoculum and day 12–day 18, R1′ Bt inoculated. Day 18, R1 was mixed with R2 (evolution similar to R1, data not shown) originating RA (a) and RB (b).

Table 1. Characteristics of the biowaste used in the study (values represent average and standard deviation of at least three samples collected during the study)

Parameter	OFMSW + bulking agent (1:1 v:v) as collected in waste treatment plant
Moisture (% wb)	59.3 ± 2.1
Organic matter (% db)	67.4 ± 1.9
pH	5.6 ± 0.1
C/N	16.9
C (% db)	42.3 ± 1.0
H (% db)	5.7 ± 1.4
N (% db)	2.5 ± 2.2
S (% db)	< 0.1
Conductivity (mS cm^{-1})	5.4 (26.4°C)

% wb: wet basis; % db: dry basis

Table 2. Process parameters and mass balances during non-sterile OFMSW SSF in 10 L reactors operating under Strategy 3.

	R1 (non-sterile OFMSW)	R1' (R1 + Bt)	R2 (non-sterile OFMSW)	RA 1/2 (R1'+R2)	RB 1/2 (R1'+R2)
B. thuringiensis inoculum	No	10 % (v/w)	No		
Process time (d)	12	6	11	28	28
Initial weight (g)	3800	3000	4000	3067	3067
Final weight (g)	3000	2107	3250	2232	2311
Initial MC (% wb)	67.4	65.5	63.1	66.6	66.3
Final MC (% wb)	64.2	62.1	60.5	62.6	62.8
Initial OM (% db)	72.9	78.4	76.6	70.5	70.3
Final OM (% db)	68.1	72.0	73.1	63.8	64.3
Initial pH	5.38	8.22	5.53	8.23	8.32
Final pH	8.16	8.36	8.23	8.83	8.45
Initial VC (CFU g^{-1} DM)	-	6E+06 ± 1E+06	-	7.8E+05 ± 8E+04	1.1E+06 ± 3E+05
Final VC (CFU g^{-1} DM)	-	3.8E+06 ± 8E+05	-	1.7E+07 ± 3E+06	2.2E+07 ± 6E+06
Initial spores (CFU g^{-1} DM)	-	5E+06 ± 3E+06	-	1.5E+06 ± 3E+05	1.42E+06 ± 4.5E+04
Final spores (CFU g^{-1} DM)	-	3.52E+06 ± 4.9E+04	-	1.3E+07 ± 2E+06	2.1E+07 ± 2E+06
Total initial (VC+S) (CFU g^{-1} DM)	-	1.1E+07 ± 1E+06	-	2.3E+06 ± 3E+05	2.5E+06 ± 3E+05
Total final (VC+S) (CFU g^{-1} DM)	-	7.4E+06 ± 8E+05	-	3.02E+07 ± 5E+06	4.3E+07 ± 8E+06
Total initial (VC+S) (CFU)	-	1,14E+10	-	2,36E+09	2,58E+09
Total final (VC+S) (CFU)	-	5,91E+09	-	2,52E+10	3,70E+10
sOUR max (g O_2 kg^{-1} M h^{-1})	4.9	0.8	6.1	2.5	2.2

Figure 1.

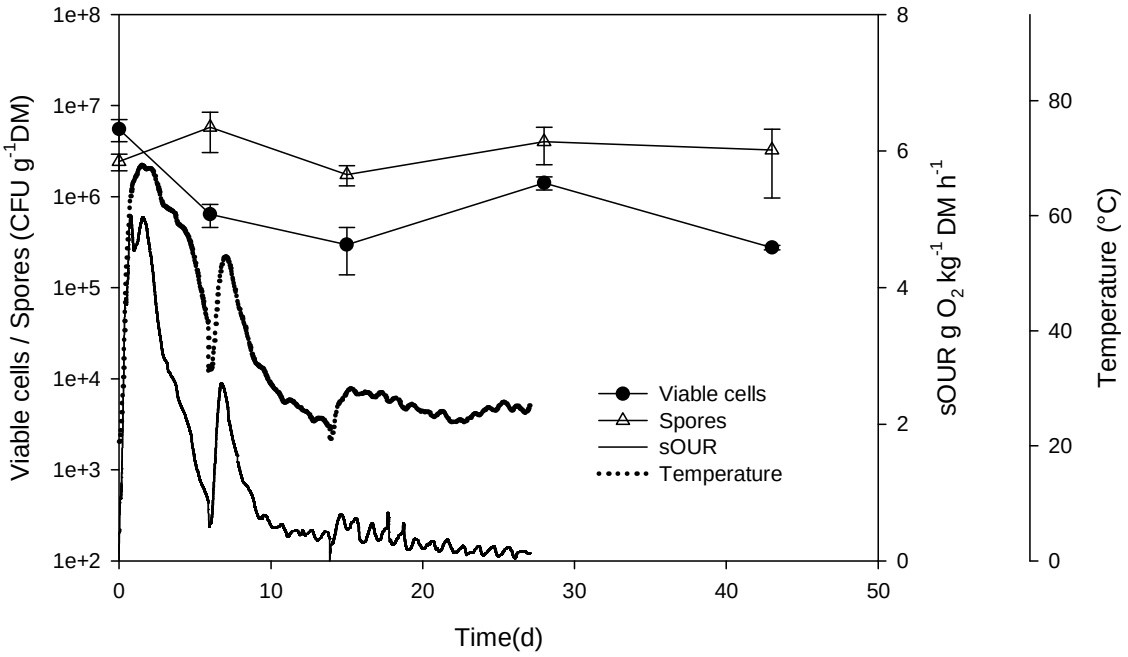

1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
Viable cells / Spores (CFU g-1DM)
0
10
20
30
40
50
Time(d)
8
6
4
2
0
sOUR g O2 kg-1 DM h-1
80
60
40
20
0
Temperature (°C)
Viable cells
Spores
sOUR
Temperature

Figure 2.

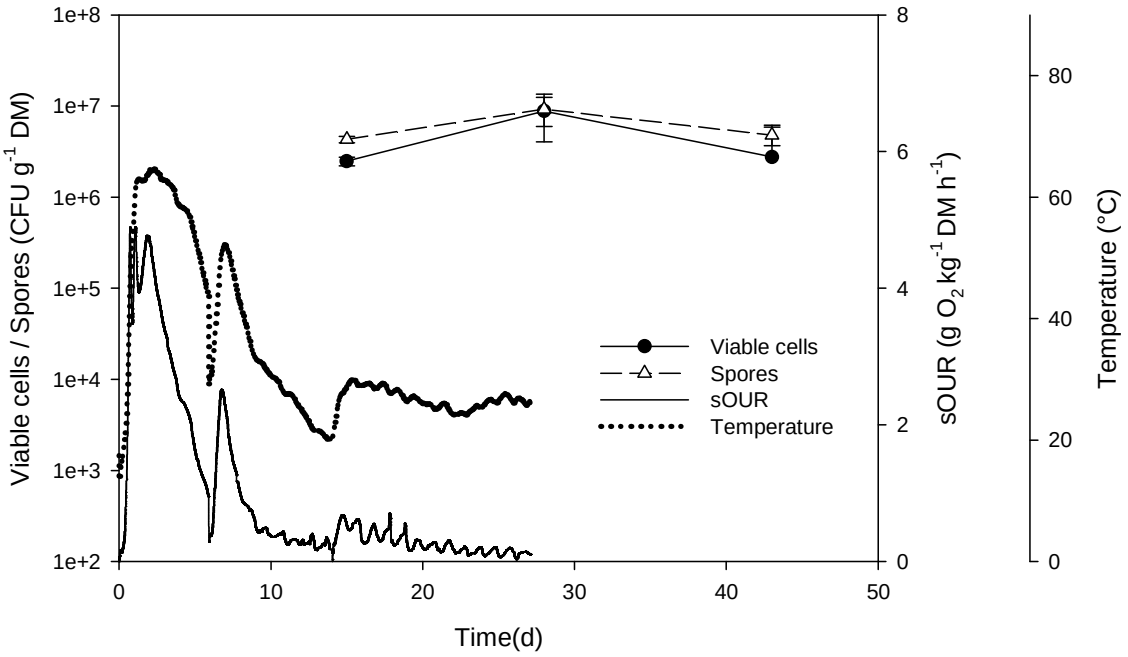
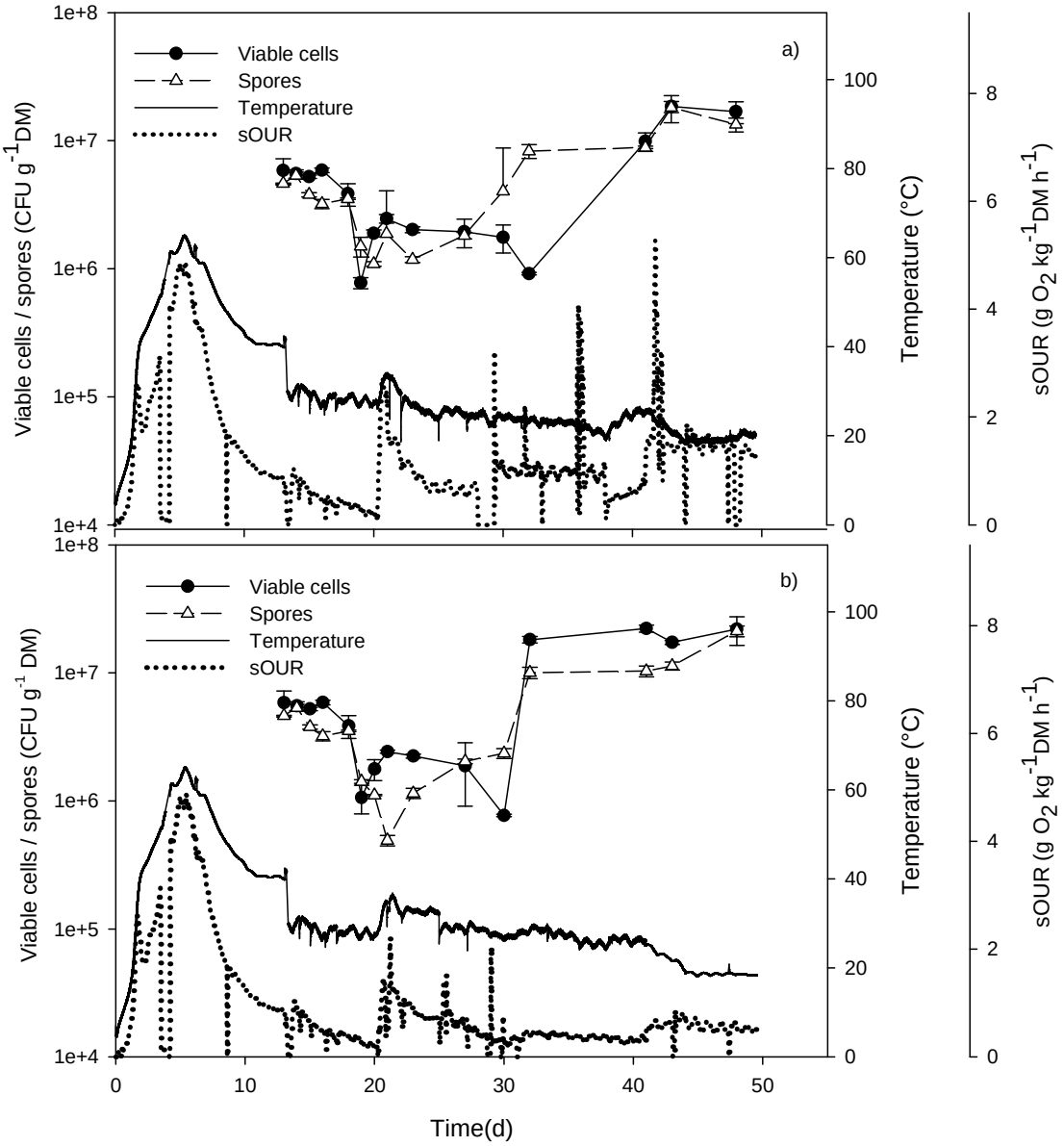

Viable cells / Spores (CFU g-1 DM)
sOUR (g O2 kg-1 DM h-1)
Temperature (°C)
Time(d)
Viable cells
Spores
sOUR
Temperature
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
8
6
4
2
0
80
60
40
20
0
0
10
20
30
40
50

Figure 3.


a)
b)
Viable cells
Spores
Temperature
sOUR
Viable cells / spores (CFU g-1DM)
Temperature (°C)
sOUR (g O2 kg-1DM h-1)
Time(d)
1e+8
1e+7
1e+6
1e+5
1e+4
0
10
20
30
40
50
100
80
60
40
20
8
6
4
2