Gaseous emissions during the solid state fermentation of different wastes for enzyme production at pilot scale

Caterina Maulini-Duran, Juliana Abraham, Sheila Rodríguez-Pérez, Alejandra Cerda, Pedro Jiménez-Peñalver, Teresa Gea, Raquel Barrena, Adriana Artola*, Xavier Font, Antoni Sánchez.

Composting Research Group
Department of Chemical Engineering
Universitat Autònoma de Barcelona
Bellaterra, 08193-Barcelona (Spain).

*Corresponding author: Adriana Artola
Universitat Autònoma de Barcelona, Departament d'Enginyeria Química, Escola d'Enginyeria, Edifici Q, Campus de Bellaterra, 08193- Cerdanyola del Vallès, Spain.
Tel.: +34 935814480
Fax: +34 935812013
Email address: adriana.artola@uab.cat

Abstract

The emissions of Volatile Organic Compounds (VOC), CH_4, N_2O and NH_3 during the Solid State Fermentation process of some selected wastes to obtain different enzymes have been determined at pilot scale. Orange peel + compost (OP), hair wastes + raw sludge (HW) and winterization residue + raw sludge (WR) have been processed in duplicate in 50L reactors to provide emission factors and to identify the different VOC families present in exhaust gaseous emissions. Ammonia emission from HW fermentation (3.2 ± 0.5 kg Mg^{-1} dry matter) and VOC emission during OP processes (18 ± 6 kg Mg^{-1} dry matter) should be considered in an industrial application of these processes. Terpenes have been the most emitted VOC family during all the processes although the emission of sulphide molecules during HW SSF is notable. The most emitted compound was dimethyl disulfide in HW and WR processes, and limonene in the SSF of OP.

Keywords: Solid State Fermentation, emission factors, VOC characterization, ammonia, methane.

1. Introduction

In biotechnology, solid-state fermentation (SSF) represents an interesting alternative to submerged fermentation (SmF) processes for the production of some industrially useful compounds (Singhania et al., 2009). Indeed, SSF presents some advantages compared to SmF such as higher concentrations of gaseous oxygen with higher overall mass transfer capacity, volume reduction of the fermentation vessel owing to a lower water volume and the possibility of carrying out the process using non-sterilized substrates (Subramaniyam and Vimala, 2012). Moreover, the use of organic wastes suppose a clear advantage towards a sustainable alternative process.

Enzymes are one of the valuable products that can be successfully produced through SSF. Lipases, proteases and cellulases have been obtained processing selected organic wastes such as residual hair from the tanning industry, soybean, pomace waste, orange peels or oil cakes (Abraham et al., 2014). These enzymes have an important role in biotechnological, pharmaceutical and chemical industries. They can also be used in food applications or wastewater treatment. There is a general interest in obtaining new, more specific and stable enzymes which can be produced through SSF processes (Thomas et al., 2013).

Many works in literature refer the use of specific microorganisms in SSF processes. Another alternative is to mix a common waste or amendment that will act as inoculum with a specific waste that will act as the substrate to produce the enzyme (Santís-Navarro et al., 2011). Compost or sludge from a wastewater treatment plant are materials that can be used as inoculum and also to adjust moisture or pH levels. The specific waste will depend on the final added-value product desired e.g.: oil cake (as a source of lipids to produce lipases), hair waste (as a source of proteins to produce proteases) or orange peel (as a source of cellulose to produce cellulases). It is technically and economically more unfavorable to use as inoculum a pure culture of a known microorganism that can degrade a specific waste (Dhillon et al., 2012).

Once the useful enzymes have been obtained, the biodegradation process should continue to produce a stabilized product that can be used as an organic amendment (Thomasa et al., 2013). Thus, when wastes are being used as substrates, SSF can be considered a waste valorization process where a value added product and a final organic amendment are obtained from the biological degradation of the waste under controlled conditions, closing the cycle for the organic matter.

The environmental impacts of the SSF process must be taken into account when considering SSF benefits. However, to our knowledge, this point has not been studied in the scientific literature. Gaseous emissions play an important role in the environmental impact of processes used to manage solid organic substrates (Colón et al., 2012). Greenhouse gases (GHG), ammonia and volatile organic compounds (VOC) are the main gases emitted during SSF processes.

Regarding GHG, biogenic CO_2 is the main compound emitted although it is not normally accounted as GHG (Colón et al., 2012). Also CH_4 and N_2O are often released, and present a global warming potential of 34 and 298 kg CO_2 eq., respectively (IPPC, 2013). On the other hand, the emission of VOC and ammonia has been associated to the generation of unpleasant odors and other environmental impacts such as acidification, eutrophication or the formation of photochemical oxidants. Terpenes, alcohols, ketones, sulfur-containing compounds and amines have been reported among the VOC emitted from waste handling and treatment processes described in literature (Komilis et al., 2004).

The objective of this work is to study the emissions of VOC, CH_4, N_2O and NH_3 during the production of different enzymes through SSF using specific wastes. Emission factors (amount of compound emitted per amount of waste treated) have been determined for the studied compounds, providing data to compare the different processes. Special attention has been addressed to VOC emissions identifying and quantifying the main VOC present.

2. Materials and Methods

2.1 Materials

Raw sludge (RS) from a wastewater treatment plant located in Manresa (Barcelona, Spain) and final compost from a mechanical-biological treatment plant located in Montcada i Reixac (Barcelona, Spain) were used as inoculum. The wastes used were orange peel from Valencia (Spain), winterization residue provided by an oil refining facility in Montcada i Reixac and hair wastes from a local tanning industry in Igualada (Barcelona, Spain). Orange peel was mixed with compost (1:0.1, w:w), winterization residue was mixed with raw sludge (0.1:1, w:w) and hair wastes were mixed also with raw sludge (1:2, w:w). The ratios of each specific waste to inoculum were different for each waste since the composition of the raw materials is different (Table S1). These ratios were adjusted in previous experiments at smaller scale (Abraham et al., 2014; Santís-Navarro et al., 2011). All these mixtures were finally mixed (1:1, v:v) with wood chips to adjust moisture content and porosity to a value within the range recommended for the adequate development of the biodegradation process under full aerobic conditions (Ruggieri et al., 2009). A homogeneous sample of each mixture and raw material was separated and stored at -18 ºC to be used for waste characterization prior to introducing the mixture in the reactors. It is reported that freezing did not change the biological activity of the waste during this period.

The main characteristics of the initial mixture and the final products obtained from each experiment are presented in Table S1 (Supplementary information). Dry and organic matter and pH were determined in triplicate following the standard procedures (US Department of Agriculture and US Composting Council, 2001).

2.2 Pilot plant

The results presented in this study were obtained, in duplicate, in 50 L pilot plant closed reactors (batch size 25 kg). A schematic diagram of the pilot reactor and a detailed description can be found in Puyuelo et al. (2010). Temperature, exhaust gas oxygen concentration and inlet airflow were monitored during the trials. The data acquisition and control system is described in detail in Maulini-Duran et al. (2014). The experiments were performed using a control strategy based on the Oxygen Uptake Rate (OUR), as described by Puyuelo et al. (2010). The main objective of the controller was to obtain an automatic airflow regulation to maximize the biological activity in the reactor measured as OUR. OUR control permits the optimization of energy consumption during the process while achieving a high degree of stability in the final product. This control reduces the variability of gaseous emissions typically found when simple control strategies are used (Maulini-Duran et al., 2014, 2013a; Puyuelo et al., 2010).

2.3. Sampling

Gas samples were collected in 1-L Tedlar® bags for VOC, N_2O and CH_4 determination. Also a 250-mL glass gas collector was used for VOC composition determination and identification. In all cases, one sample was taken per day and per reactor.

Reactors were opened twice a week to monitor the degradation process. In these cases, the material was homogenized and a 0.5 kg sample was taken to analyze moisture, pH, organic matter and enzymatic activity. A total of 2.5 kg were withdrawn from each reactor. Emissions potentially released during sampling were quantified prior sampling by stripping all the gas in the reactor with airflow of 10 L/min for 5 minutes and collecting this gas in a 50 L Tedlar sampling bag.

2.4. Enzymatic activity

The procedure followed to determine lipase activity is detailed in Santis-Navarro et al. (2011). The determination of enzymatic activity from proteases is described in Abraham et al. (2014). Extract for cellulase activity determination was obtained by mixing 10 g of a wet sample, from the 0.5 kg taken from the reactor, with 150 mL of citrate buffer 4.8 (1:10 w/v ratio). The enzyme extraction was carried out by incubating the samples in a wrist action shaker for 30 min at 100 rpm. After incubation, the samples were centrifuged at 10000 g and 4°C for 15 min and the supernatant was analyzed for cellulase activity (Dhillon et al., 2012). To measure total cellulase activity (TC), the modified filter paper assay, was used (Ghose, 1987). Instead of using filter paper strips as substrate, microcrystalline and insoluble Sigmacell cellulose type 20 μm was used (high purity cellulose, Sigma-Aldrich). Modifications were mainly made due to several authors report filter paper assay as very laborious and time consuming method, requiring many manual manipulations that lead to non-reproducible results (Coward et al., 2003). Carboxymethylcellulase (CMCase) and β-glucosidase activity were also measured according to Ghose (1987). Released reducing sugars were measured by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). For TC and CMCase, one activity unit (UA) was defined as the amount of enzyme that produces 1 μmol of reducing sugar (glucose equivalent) per minute. For β-glucosidase one activity unit (UA) was defined as the amount of enzyme which produces 1 μmol of glucose per minute.

2.5. Stability degree

The dynamic respiration index (DRI) of initial mixtures and final products was measured in triplicate using a dynamic respirometer (Ponsá et al., 2010) on the basis of the methodology proposed by Adani et al. (2006) to assess the degree of biological stability.

2.6. Determination of gaseous emissions

VOC, CH_4 and N_2O analysis was performed by means of gas chromatography (Agilent Technologies 6890N Network GC system, Madrid, Spain) as explained in Colón et al. (2012). All samples were analyzed in triplicate for each compound. The deviation found per each triplicate was lower than 10% for all the compounds.

Ammonia concentration was measured in situ at the gas outlet of the composting reactor by means of an ammonia sensor (Industrial Scientific sensor iTX-T82, Oakdale, PA, USA) with a measurement range of 0 to 1200 ppmv. The sensor was placed inside a hermetic recipient with inlet and outlet holes that allowed gas circulation.

2.7. GC-MS detection

VOC characterization was performed using gas samples analyzed by SPME (Solid Phase Micro Extraction)/GC-MS. Detailed method for VOC identification and quantification has been described in detail in Maulini-Duran et al. (2013b).

3. Results and Discussion

3.1 Process evolution

Temperature, airflow, oxygen concentration and OUR were monitored on line, while enzymatic activity was determined twice a week, in the six SSF trials. Figure S1 (Supplementary information) shows the profiles for all these parameters obtained in one of the two process replicates undertaken for each of the three mixtures studied: orange peel + compost (OP), winterization residue + raw sludge (WR) and hair wastes + raw sludge (HW). Similar OUR, oxygen, temperature and airflow profiles were observed in the duplicates for the same waste.

The process evolution for the SSF of orange peel with compost (Figure S1a) was as follows: thermophilic conditions were achieved in the third day of process, although maximum temperature was not achieved until the twelfth process day (OP-I: 67.43ºC; OP-II: 61.21ºC). The delay in temperature peaking may be due to different factors: initial acidic pH of the mixture (5.88, Table S1) rose during the process reaching a final value of 8.45; the lack of homogeneity of the initial mixture was overcome through successive mixing when sampling, contributing to mass transfer between the different materials inside the reactor; finally, a potential inhibition by the presence of limonene is further discussed in this paper. As expected, the lowest oxygen values (OP-I: 11.8 % OP-II: 9.6 %) and the highest OUR values (OP-I: 10.3 g $O_2 \cdot h^{-1}$; OP-II: 13.2 g $O_2 \cdot h^{-1}$) matched with the maximum temperature achieved (highest microbial activity). The highest airflow (OP-I: 3 ml min^{-1}; OP-II: 3.5 ml min^{-1}) was set by the controller in the third day of process, a fact related to the achievement of thermophilic phase and the initial increase in OUR. The maximum cellulase activity was detected on the thirteen processing day for both trials (OP-I: 52.9 UA g^{-1} DM; OP-II: 48.7 UA g^{-1} DM) in coincidence with the highest temperature.

Regarding the SSF of winterization residue with raw sludge (Figure S1b), thermophilic conditions were achieved during the first day while maximum temperature was reached during the third day (WR-I: 64.26ºC; WR-II: 58.8ºC). In this case, the degradation started earlier than in the OP process because of the higher biodegradable organic matter content of the mixtures and no impairment due to pH. Maximum OUR values (WR-I: 14.5 g $O_2 \cdot h^{-1}$; WR-II: 18 g $O_2 \cdot h^{-1}$) were found around the third day of process, matching with the thermophilic peak and the minimum oxygen concentration (WR-I: 10.3% and WR-II: 9.8%). The maximum airflow was observed in the sixth day in both reactors (WR-I: 2.6 ml min^{-1}; WR-II: 3.8 ml min^{-1}), coinciding with the maximum lipase activity detected (WR-I: 4.5 UA g^{-1} DM; WR-II: 10.8 UA g^{-1} DM).

On the process of hair wastes with raw sludge (Figure S1c), thermophilic conditions were achieved in the first hours of the experiment and the maximum temperature was reached at the beginning of the second day (HW-I: 72.31 ºC; HW-II: 69.87 ºC). This peak matches with maximum OUR (HW-I: 24.8 g $O_2 \cdot h^{-1}$; HW-II: 19.3 g $O_2 \cdot h^{-1}$), also with lowest oxygen content (7.6 %) and highest airflow value in HW-I (1.7 ml min^{-1}). In the HW-II experiment, minimum oxygen value (5.23 %) and maximum airflow supplied (4.7 ml min^{-1}) were recorded slightly later, on the third and fourth day respectively. However, on both replicates the maximum protease activity was found on the fourth day (HW-I: 27135 UA g^{-1} DM; HW-II: 22400 UA g^{-1} DM).

According to the temperature profile and the values of DRI of the final material (Table S1), the process evolution was correct, and the final product was stabilized (Adani et al., 2006) in all the experiments. Maximum temperatures, maximum OUR values and enzymatic activity are consequence of biological activity and, therefore are clearly related occurring, in almost all the experiments, at the same time. The highest OUR values were recorded in the HW process.

3.2 Gaseous emissions

Figure 1 presents the evolution of the emissions of the four gaseous pollutants studied through the degradation process. Table 1 summarizes the emission factors obtained, and includes emission factors for raw sludge of same origin previously published for comparison (Maulini-Duran et al., 2013b). The values for raw sludge were obtained in the same reactors under the same conditions.

CH_4 emissions

Figure 1a presents the evolution of methane emission for the three treated mixtures. Methane emissions are related to the presence of anaerobic zones, due to excessive moisture, insufficient porosity or an inappropriate aeration strategy (Puyuelo et al., 2010). During HW and WR processes the highest emissions were detected during the high-rate stage (days 0 to 12 and 1 to 8 for HW and WR respectively), as has been previously described in similar processes degrading other wastes (Maulini et al., 2013b; Ahn et al., 2011). OP presented a different profile, showing high methane emissions from day 13 to day 23, also coinciding with highest OUR values (Figure S1a). However a peak was also observed on the fifth day due to the low airflow supplied at that moment and the oxygen decrease (Figure S1a).

N_2O emissions

Figure 1b presents the evolution of N_2O emissions. The main N_2O emissions released during the six processes were found during the first week, as has been previously reported for the aerobic degradation processes of other wastes (El Kader et al., 2007). However, other authors related the inhibition of N_2O production with thermophilic temperatures (Fukumoto et al., 2003). In Figure S1 it can be observed that thermophilic temperatures were achieved during the first week of process (for HW and WR) pointing that N_2O was emitted even at thermophilic temperatures. There were no considerable differences between the emission factors obtained for N_2O in the different processes studied (Table 1). N_2O emission factors present the lowest values among the analyzed contaminants, as found in similar biological processes (Maulini-Duran et al., 2014, 2013b). In spite of that, from an environmental impact point of view the contribution of N_2O to global warming is significantly higher than CO_2 or CH_4. Additionally, it should be highlighted that N_2O presents the highest deviation obtained between emission factors calculated for the two replicates, specifically in the case of WR.

NH_3 emissions

The evolution of NH_3 emissions is presented in Figure 1c. HW trials emitted an important quantity of ammonia during the first week, matching with the thermophilic stage of the processes. This fact agrees with the results reported by Pagans et al. (2006a) where NH_3 emissions from the composting process were strongly related to the process temperature. Keratin is the essential component of hair, a protein formed by the combination of 18 amino acids. It contains large quantities of cysteine that has a very stable structure that makes it difficult to degrade by most proteolytic enzymes (Coward-Kelly et al., 2006). The degradation of these proteins can provoke the differences among the ammonia emissions from the different treated wastes. From the values presented in Table 1 for raw sludge and HW, it can be deduced that ammonia was emitted mainly from hair wastes in HW trials although raw sludge also contributes to these emissions. A similar trend was also observed during WR processes, in this case ammonia emissions were clearly related to the presence of raw sludge, as the winterization residue did not contribute to the nitrogen content of the mixture. Values in Table S1 show considerable higher nitrogen content in HW compared with the other mixtures. Also, nitrogen contain in WR is higher than in OP (Table S1), where no ammonia emissions were detected (Table 1). However, WR emissions were lower than RS emissions due to the higher bioavailable C/N ratio (Puyuelo et al., 2011).

To enhance the properties of the final product to be used as an organic amendment, it would be beneficial to reduce ammonia emissions retaining nitrogen in the solid phase. Further investigation on the strategies to reduce these significant nitrogen losses is necessary to assume the feasibility of a complete valorization process of the hair wastes, as it has been highlighted in other studies (Puyuelo et al., 2011).

VOC emissions

Figure 1d presents the evolution of VOC emissions. It should be noted than different scales on y axis have been used for OP than for WR and HW due to the large amount of VOC emitted during OP processes. Once again, the evolution of VOC emission is related to temperature. The VOC emission peak (Figure 1d, right axis) matches with OUR and temperature peak (Figure S1b and S1c) in WR and HW processes. This was not observed in the OP processes where VOC were mainly emitted from day 2 to day 6 (37-50ºC) but higher thermophilic temperatures were reached on days 11 to 16. On the other hand, VOC emission in first OP processing day was more than 10-fold the emission from HW or WR even if these residues were under thermophilic conditions. Differences in temperature, OUR and VOC emissions during OP processes, show that VOC emission was not only a consequence of the organic matter degradation, as has been previously reported (Pagans et al., 2006b). OP processes emitted important quantities of VOC from the beginning of the process, as a consequence of the stripping occurred when the airflow passes through the solid matrix. VOC emissions increased when temperature and biological activity (OUR) started to rise up. Moreover, VOC emissions in OP were one order of magnitude larger than in HW and WR processes until day 15. The difference in VOC emission could be also observed in Table 1, being the OP emission factor 100-fold the emission factor of the other processes.

To summarize, the emissions of the studied compounds were mainly produced when maximum biological activity (maximum temperature and maximum OUR values) was achieved. An appropriate treatment to avoid the release of important amounts of VOC and NH_3 to the atmosphere must be designed if these SSF process are implemented at industrial scale, as it has been previously reported in studies on related topics such as composting (Pagans et al., 2006b). The emission factors calculated in this study could be useful for this design.

3.3 VOC characterization by SPME/GC-MS

A large number of different volatile organic compounds was identified in the emissions from the six processes. These compounds were classified into the following chemical families: alcohols, esters, furans, ketones, aliphatic hydrocarbons, aromatic hydrocarbons, aldehydes, halogenated compounds, nitrogen-containing compounds, sulfur-containing compounds, volatile fatty acids and terpenes. Table 2 summarizes the percentage of each VOC family for each process. A quantitative analysis was also carried out for fifteen specific and typical VOC emitted during similar biodegradation processes (Scaglia et al., 2011). Eucalyptol and decane were not found and the 13 characterized compounds and their amounts are summarized in Table 3.

Broadly, similar distribution percentages were found for furans, esters, volatile fatty acids, aliphatic hydrocarbons, aldehydes and nitride molecules among the different processed wastes, being the less abundant families in the gaseous emissions. The differences between the maximum and the minimum percentage found in the emission of these compounds in the six SSF processes were below 2.9 points. Alcohols, ketones and aromatic hydrocarbons presented some differences depending on the treated waste, with differences between the maximum and the minimum percentage over 12.9 points. The emission of sulphide molecules presented a higher dispersion, with a difference of 28.2 points between the maximum and the minimum percentage. Terpenes were the most emitted family although showing a substantial dispersion in the percentages for the different processes, specifically 51.9 points.

The less abundant families were furans, volatile fatty acids and esters. The percentage of furans, mainly represented by 2-pentylfuran, was higher in WR processes. This molecule was related to grassy odors (Suffet et al., 2009). Volatile fatty acids and esters have been only found in OP processes. The acid pH of the mixture contributed to the release of some volatile fatty acids. The presence of alcohols in OP processes together with the above mentioned volatile fatty acids could indicate the presence of some esterification reactions, thus obtaining esters.

Aliphatic hydrocarbons were present in low percentages in all the trials. Aldehydes also presented low percentages in OP and HW processes and were not detected in WR. As can be seen in Table 3, decanal and nonanal were the aldehydes found in HW processes and decanal and hexanal the ones found in OP processes. Probably, in the case of HW, these compounds come from hair wastes, because no aldehydes were found either in RS or in WR.

Ketones were found in higher percentages in the processes using raw sludge. 2-pentanone was not present in OP processes, while it was found in all the other trials. Similarly happens for nitrogen-containing compounds. This family is represented by pyridine, a product related to a putrid odor (Suffet et al., 2009). Pyridine was found and quantified mainly in process containing raw sludge. Pyridine was emitted in HW in one order of magnitude higher than in all the other processes. This fact could be also a consequence of the high nitrogen content of hair wastes (Table S1).

Aromatic hydrocarbons were found in all the experiments. However, toluene, styrene and xylene were not found in HW trials (Table 3) where phenol, benzene and naphthalene were the main aromatic hydrocarbons emitted.

Terpenes are the family presenting higher emissions in all the experiments (Table 2). This dominance was also observed in other studies where organic wastes were biodegraded in similar conditions (Maulini-Duran et al., 2014, 2013a, 2013b). Terpenes have been described as the main compounds responsible for odorous pollution in similar biodegradation processes dealing with organic wastes such as composting (Staley et al., 2006). Terpenes could be produced as an intermediate in the aerobic metabolism. During OP trials, the percentage of terpenes in gaseous emissions was above 80%. Terpenes dominance in OP processes and the higher percentage compared to the other processes is mainly due to limonene. Limonene is related to the fruity or citrusy sensorial experience (Suffet et al., 2009). Limonene is a harmless compound widely used in fragrances, but some studies revealed that reacting in ambient with ozone could form dangerous secondary pollutants, including formaldehyde (Walser et al., 2007). Table 3 shows that limonene emission factor is, at least, 10-fold higher in OP than in all the other processes studied. In fact, there are some studies of biological processes involving orange peels that report the need to remove limonene as a pretreatment because it is considered an anti-microbial agent (Martin et al., 2010). In the light of these studies, the lag phase observed at the beginning of the OP process can also be attributed to the high concentration of limonene in the material. The increase in airflow observed during the first days of process, which coincides with an increment of total VOC emissions (Figure S1a and Figure 1d), enhanced the release of limonene by stripping, as can be observed in Figure S2, which shows the daily evolution of limonene emission factor during OP processes. The lower limonene concentration inside the reactor could favor the microbial proliferation from day 6th on. This fact could explain the late appearance of the thermophilic peak and its mismatch with the total VOC emission peak, both discussed before in this study.

Alpha and beta pinene have been specifically related to emissions coming from woody bulking agents (Maulini-Duran et al., 2014). In the present study the amount of bulking agent was the same in all the processes and, as can be observed in Table 3, the emission factor for these two compounds is quite similar among the different SSF trials. This confirms the study by Büyüksönmez and Evans (2007), working with green waste composting, who reported that terpenes emission is closely related to the material used as bulking agent.

Percentages of sulfur-containing compounds during HW processes were higher than those from OP, WR and RS and also higher than those observed in previous works (Maulini-Duran et al., 2014, 2013a, 2013b). It can be seen in Table 2 that the percentage of sulfur-containing compounds was similar in RS and WR but much higher for HW. The same raw sludge was used in RS, WR and HW trials. In consequence, the differences on sulfur-containing compounds between HW and RS or WR can be attributed to hair wastes. As discussed before, the hair structure contains large quantities of cysteine, a sulfur-containing compound. The degradation of cysteine provokes a higher emission of volatile sulfur compounds (López del Castillo-Lozano et al., 2008). In fact, as can be observed in Table S1 sulfur content in HW is higher than WR. Dimethyl disulfide was detected as the main sulfur-containing compound and it was quantified (Table 3). Dimethyl disulphide is a strong odorant and moderately toxic volatile compound that was detected in most of the samples analyzed during the different experiments, often over its odor threshold (EPA, 2010), 0.007 mg m^{-3}. According to the percentage of sulfur-containing compounds the process with the highest emission factor of dimethyl disulfide was HW.

Most of the VOC emitted in the studied processes are harmless, but some of them are or could be transformed into hazardous compounds for the environment and for human health as it has been particularly explained for limonene (Walser et al., 2007).

Summarizing, terpenes is the most abundant emitted family for all the treated wastes, but the emission of sulfur-containing compounds during HW processes is also remarkable. Therefore, the most emitted compounds were dimethyl disulfide in HW and WR processes and limonene in OP SSF.

4. Conclusions

A systematic study on SSF gaseous emissions has been presented using pilot easily scalable reactors. Emission factors are strongly dependent on the waste processed under SSF. For instance, for most of the wastes, emission factors for CH_4 and N_2O are of similar order of magnitude whereas for VOC and NH_3 differences among emission factors values can differ in several orders of magnitude.

Hair waste SSF process is characterized by high emission of ammonia. Winterization and hair waste SSF processes were also characterized by the presence of sulfur-containing compounds; while in orange peel SSF process limonene was emitted in higher amount.

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Table 1 . Emission Factors for VOC, CH_4, N_2O and NH_3 (kg of compound emitted Mg^{-1} of dry matter). RS data are from Maulini-Duran et al. (2013b) for reference. For each waste, values for each replication are presented jointly with the average of the two duplicated reactors with the corresponding deviation.

Trial	VOC	CH_4	N_2O	NH_3
OP-I	21.6	0.0120	0.00238	n.d.
OP-II	13.4	0.0164	0.000657	n.d.
OP-mean	18 ± 6	0.014 ± 0.003	0.002 ± 0.001	n.d.
WR-I	0.227	0.00668	0.00118	0.405
WR-I	0.341	0.00896	0.00997	0.194
WR-mean	0.28 ± 0.08	0.008 ± 0.002	0.006 ± 0.006	0.3 ± 0.1
HW-I	0.213	0.00387	0.00152	2.89
HW-II	0.186	0.00328	0.00267	3.61
HW-mean	0.2 ± 0.02	0.0036 ± 0.0004	0.0021 ± 0.0008	3.2 ± 0.5
RS-I	0.361	0.0238	0.0112	1.53
RS-I	0.240	0.0210	0.00470	0.480
RS-mean	0.3 ± 0.1	0.022 ± 0.002	0.008 ± 0.005	1.0 ± 0.7

n.d. = not detected

Table 2. Percentages of different VOC families emitted during the processes studied. RS data from Maulini-Duran et al. (2013b).

Trial	Furans	Esters	Volatile fatty acids	Alcohols	Aliphatic hydrocarbons	Aldehydes	Ketones	Nitrogen containing compounds	Aromatic hydrocarbons	Terpenes	Sulphur containing compounds
OP-I	0.1	0.9	0.3	4.0	0.2	0.1	1.2	0.0	0.4	92.8	0.0
OP-II	0.2	2.9	1.2	6.1	1.2	0.6	2.0	0.1	2.4	83.3	0.0
WR-I	1.3	0.0	0.0	3.0	0.2	0.0	10.9	1.2	12.9	61.0	9.5
WR-I	0.6	0.0	0.0	2.2	0.7	0.0	13.4	0.8	9.8	61.4	11.1
HW-I	0.6	0.0	0.0	3.9	0.0	0.6	7.4	0.4	8.4	50.6	28.2
HW-II	0.6	0.0	0.0	10.7	0.4	0.7	14.0	1.4	6.9	40.9	24.4
RS-I	0.1	0.0	0.0	8.9	0.4	0.0	4.2	0.1	3.3	72.0	11.1
RS-I	0.1	0.0	0.0	15.1	0.1	0.0	5.1	0.0	3.8	64.3	11.4

Table 3. Total emission of selected individual VOC (kg of compound emitted Mg^{-1} of dry matter). RS data from Maulini-Duran et al. (2013b).

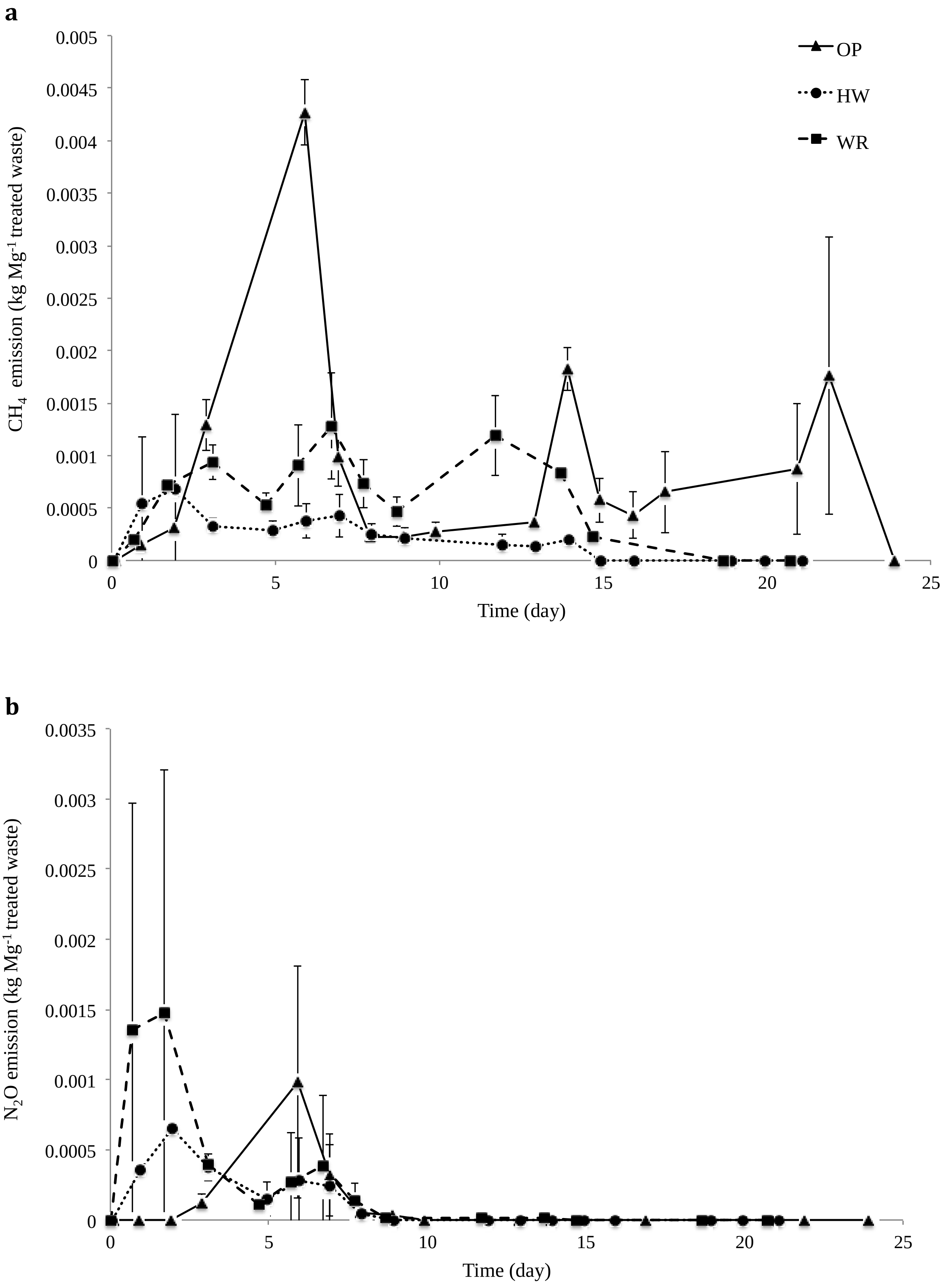
Trial	alpha-Pinene	beta-Pinene	Limonene	Dimethyl disulfide	Nonanal	Pyridine	Decanal	2-pentanone	Toluene	Styrene	Xylene	Hexanal	Cyclo hexanone
OP-I	0.0396	0.0697	1.04	0.00128	n.d.	0.000265	0.000189	0.0164	0.00302	0.000212	n.d.	0.000493	n.d.
OP-II	0.0352	0.0684	0.82	0.000803	n.d.	n.d.	0.0000989	0.0292	0.000380	0.0000442	n.d.	0.000441	n.d.
WR-I	0.0053	0.0021	0.00220	0.0348	n.d.	0.000142	n.d.	0.00205	0.0000730	0.0000600	0.00000604	n.d.	n.d.
WR-I	0.0120	0.0044	0.00445	0.0704	n.d.	0.000276	n.d.	0.00563	0.000298	0.0000973	0.0000204	n.d.	n.d.
HW-I	0.0485	0.0172	0.0123	0.671	0.000702	0.00496	0.0000240	0.00207	n.d.	n.d.	n.d.	n.d.	n.d.
HW-II	0.0165	0.00561	0.00435	0.139	0.000358	0.00267	0.00459	0.00291	n.d.	n.d.	n.d.	n.d.	n.d.
RS-I	0.0137	0.00940	0.00153	0.222	n.d.	0.000126	n.d.	n.d.	0.0000151	0.0000798	0.000316	n.d.	0.000375
RS-I	0.0188	0.00189	0.00164	0.202	n.d.	0.000341	n.d.	0.00115	n.d.	0.0000806	0.0000521	n.d.	0.000494

n.d. = not detected

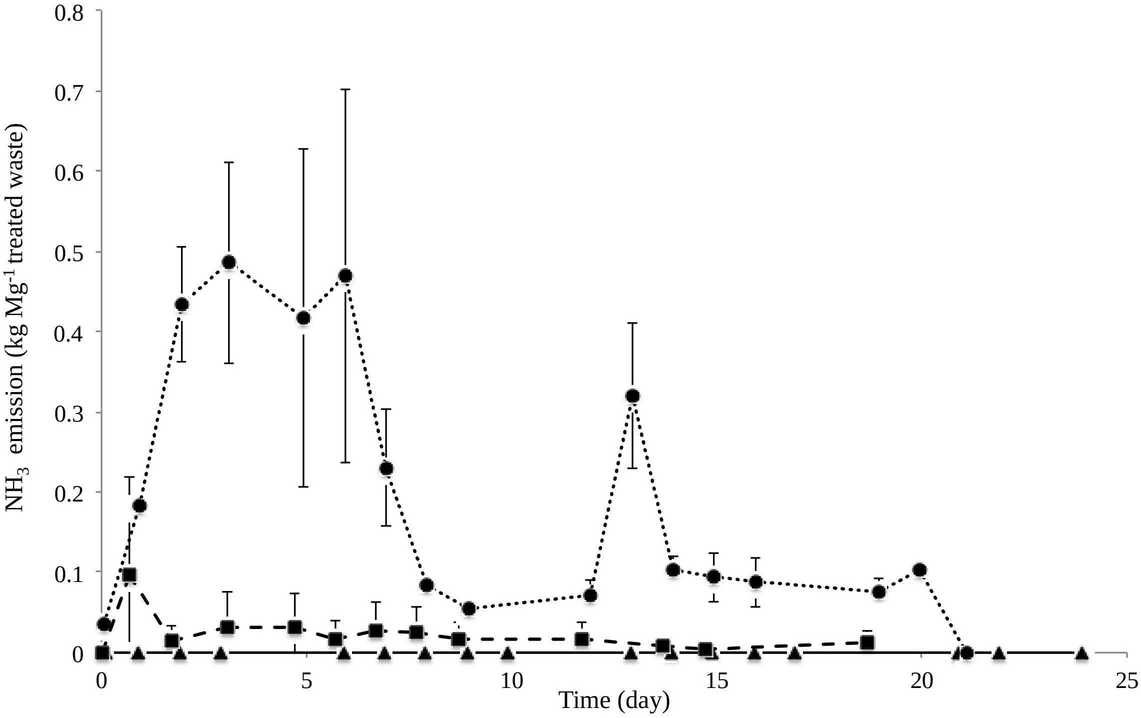
Figure Legends

Figure 1. Daily emission factors (kg of compound Mg^{-1} treated waste) evolution for (a) CH_4, (b) N_2O, (c) NH_3 and (d) VOC, for the six trials carried out. Values presented are an average of the two duplicated reactors for each waste with the corresponding deviation. Legend in Figure 1a is the same for Figure 1b, 1c and 1d. VOC (Figure 1d) from WR and HW processes have to be read on the right axis.

Figure 1

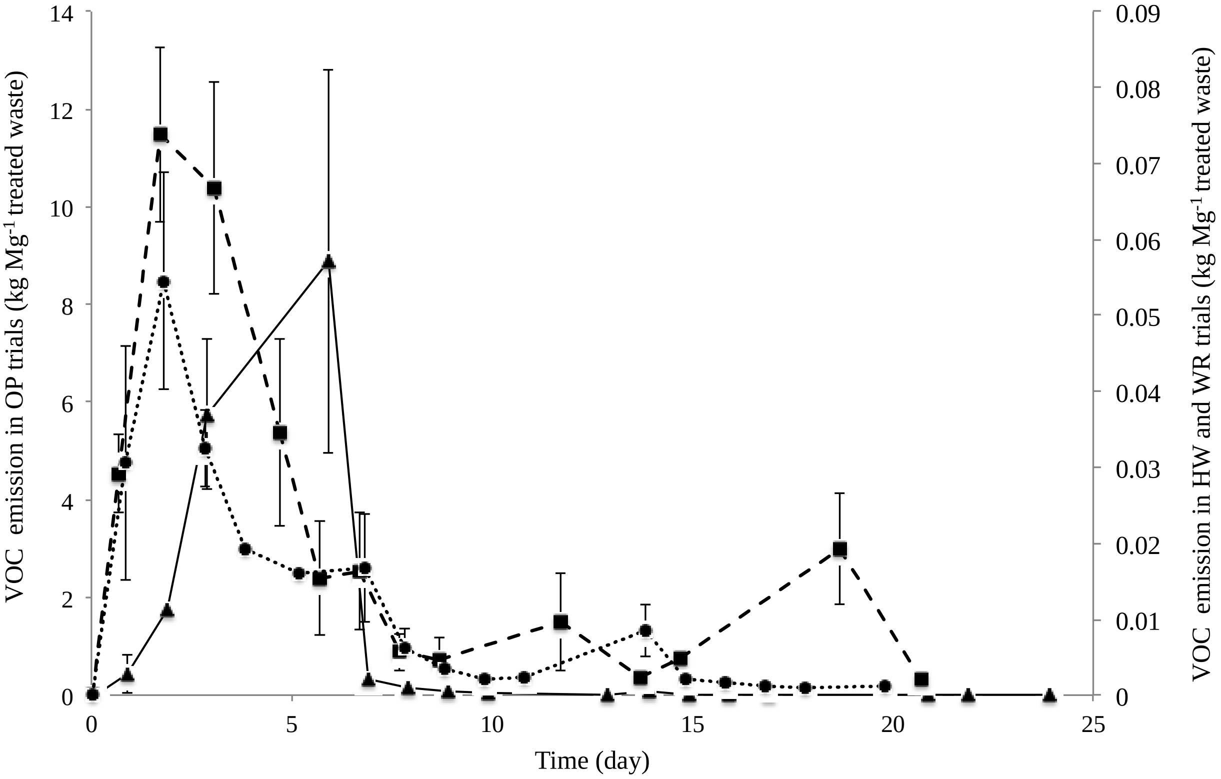

a
0.005
0.0045
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
CH_4 emission (kg Mg^{-1} treated waste)
0
5
10
15
20
25
Time (day)
OP
HW
WR
b
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
N_2O emission (kg Mg^{-1} treated waste)
0
5
10
15
20
25
Time (day)

c



NH3 emission (kg Mg-1 treated waste)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Time (day)

d



VOC emission in OP trials (kg Mg-1 treated waste)
14
12
10
8
6
4
2
0
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
VOC emission in HW and WR trials (kg Mg-1 treated waste)
0
5
10
15
20
25
Time (day)

Supplementary Information

Gaseous emissions during the solid state fermentation of different wastes for enzyme production at pilot scale

Caterina Maulini-Duran, Juliana Abraham, Sheila Rodríguez-Pérez, Alejandra Cerda, Pedro Jiménez-Peñalver, Teresa Gea, Raquel Barrena, Adriana Artola*, Xavier Font, Antoni Sánchez.

Composting Research Group
Department of Chemical Engineering
Universitat Autònoma de Barcelona
Bellaterra, 08193-Barcelona (Spain).

*Corresponding author: Adriana Artola

Universitat Autònoma de Barcelona, Departament d'Enginyeria Química, Escola d'Enginyeria, Edifici Q, Campus de Bellaterra, 08193- Cerdanyola del Vallès, Spain.

Tel.: +34 935814480

Fax: +34 935812013

Email address: adriana.artola@uab.cat

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Number of figures: 2

Number of tables: 1

Table S1. Characteristics of the initial mixtures (waste and by-products with bulking agent) and materials obtained at the end of the process.

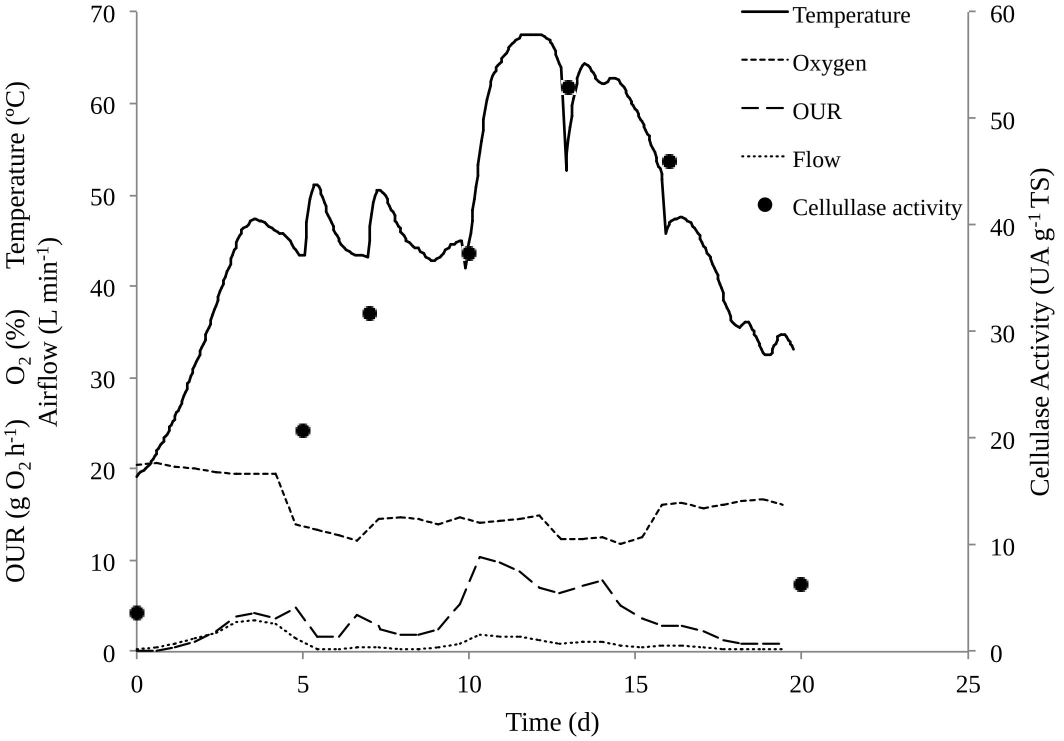
(HW: Hair waste, WR: Winterization waste (oil cake), OP: Orange peel)

Material	**DRI*** **(mg O_2 g^{-1} OM h^{-1})**	**Dry Matter (%, wb*)**	**Organic Matter (%, db*)**	**Air filled porosity (%)**	**pH**	**N (%,db*)**	**S (%,db*)**	**C (%,db*)**
HW (initial mixture)	1.66 ± 0.05	36 ± 1	90 ± 3	71	7.40	7.4 ± 0.2	1.0 ± 0.1	43.5 ± 0.3
HW-I (final product)	0.83 ± 0.04	28.9 ± 0.3	86 ± 3	69	8.84	6.3 ± 0.4	1.1 ± 0.2	43.7 ± 0.7
HW-II (final product)	0.32 ± 0.01	31 ± 1	85 ± 2	68	8.76	7.3 ± 0.1	1.4 ± 0.2	40.4 ± 0.5
WR (initial mixture)	2.6 ± 0.1	40 ± 2	76 ± 1	60	6.21	2.8 ± 0.1	0.26 ± 0.03	40.8 ± 0.2
WR-I (final product)	0.54 ± 0.04	37.9 ± 0.9	72 ± 1	56	8.80	3.13 ± 0.05	0.31 ± 0.04	39.4 ± 0.6
WR-II (final product)	0.64 ± 0.04	39 ± 2	75 ± 2	55	8.47	3.32 ± 0.03	0.32 ± 0.01	38.8 ± 0.5
OP (initial mixture)	1.5 ± 0.2	41.9 ± 0.8	89.9 ± 0.2	74	5.88	1.37 ± 0.04	0.13 ± 0.04	32.5 ± 1.0
OP-I (final product)	0.44 ± 0.04	32 ± 2	81 ± 1	68	8.45	2.7 ± 0.3	0.28 ± 0.03	30.3 ± 1.2
OP-II (final product)	0.56 ± 0.05	33 ± 3	80.3 ± 0.8	67	8.45	2.3 ± 0.3	0.14 ± 0.04	34.0 ± 2.7

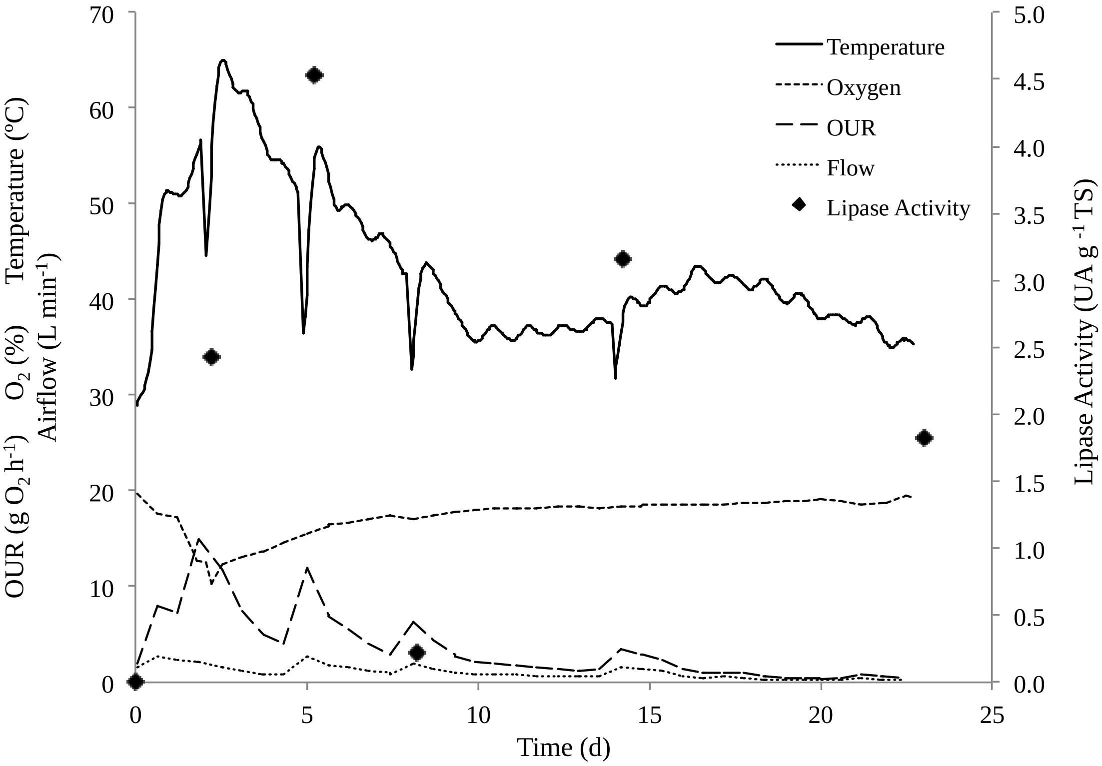
***DRI:** Dynamic respiration index, **wb**: wet basis; **dw**:dry basis

Figure S1

a


Temperature
Oxygen
OUR
Flow
Cellullase activity
OUR (g O$_2$ h^{-1}) O$_2$ (%) Temperature (°C)
Airflow (L min^{-1})
Cellulase Activity (UA g^{-1} TS)
Time (d)
0
10
20
30
40
50
60
70
0
5
10
15
20
25
0
10
20
30
40
50
60

b


Temperature
Oxygen
OUR
Flow
Lipase Activity
OUR (g O$_2$ h^{-1}) O$_2$ (%) Temperature (°C)
Airflow (L min^{-1})
Lipase Activity (UA g^{-1} TS)
Time (d)
0
10
20
30
40
50
60
70
0
5
10
15
20
25
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

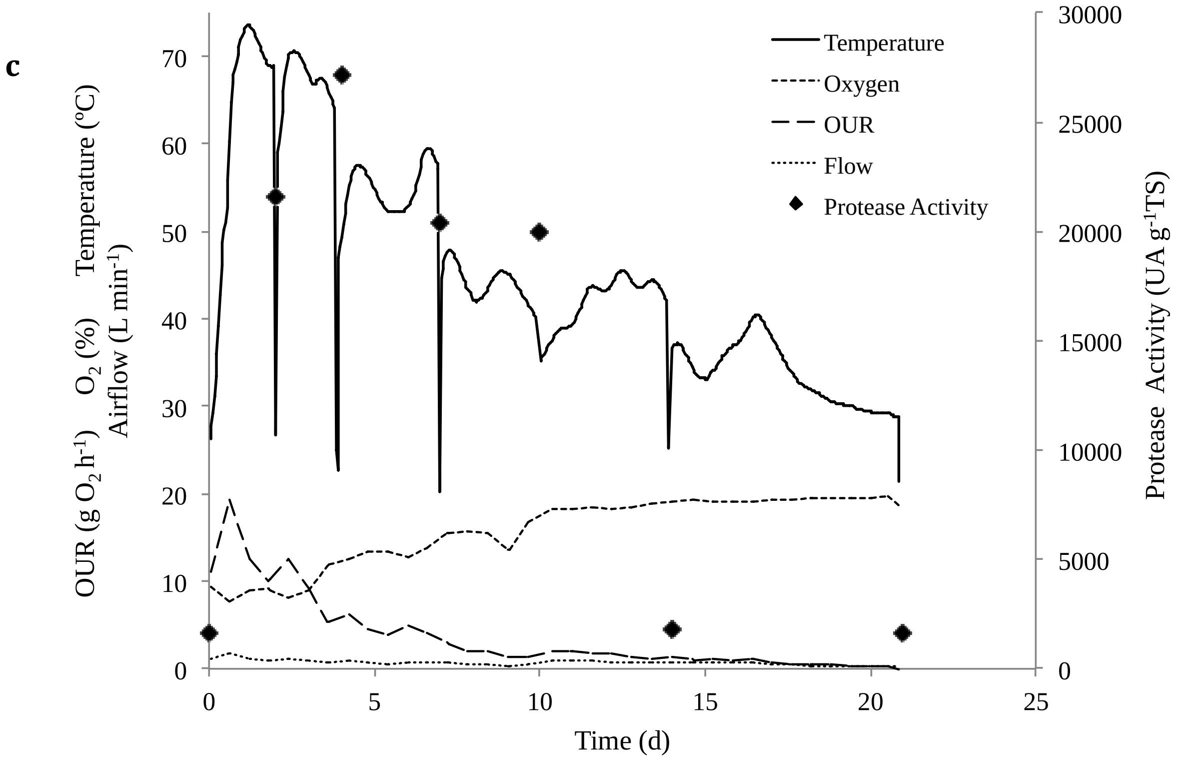


Figure S1. Evolution of temperature, airflow, OUR, oxygen content and enzymatic activity during each process: (a) OP, (b) RW and (c) HW. Due to the similarity of profiles among duplicates, only one graph is shown for each waste. Note the different axis and scale for the enzymatic activity.

Figure S2:

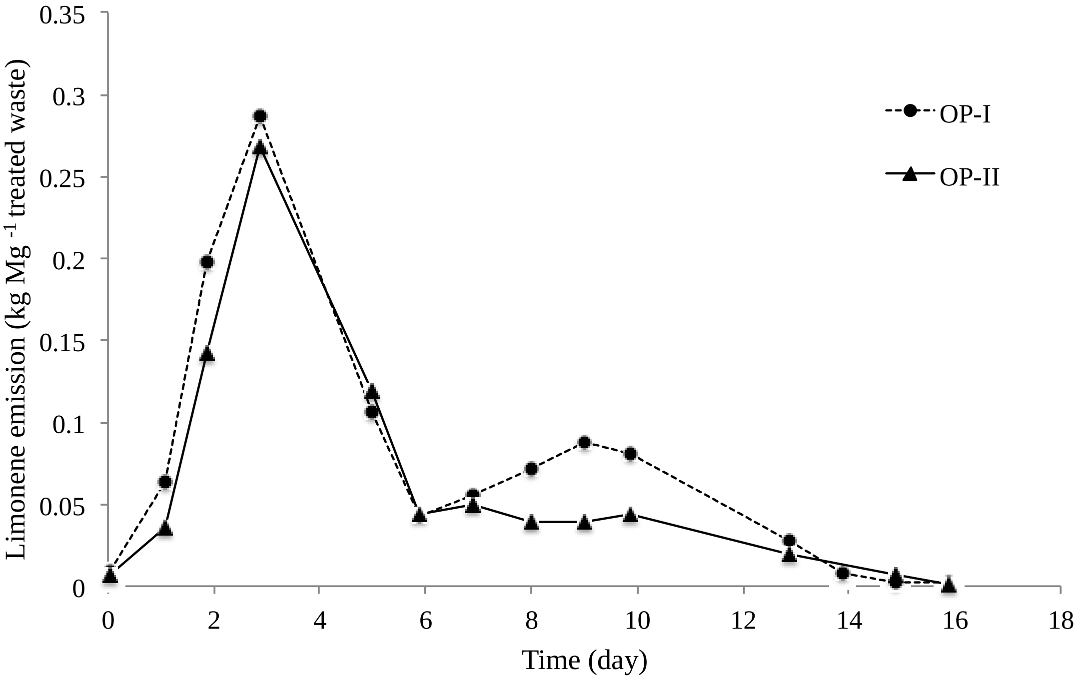


Figure S2. Limonene daily emission factors (kg of compound Mg^{-1} treated waste) evolution during OP processes.