Biodegradation of animal fats in a co-composting process with wastewater sludge

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Scientific relevance:

This paper includes a study about the biodegradation of fats in high proportion (40-50%) by means of a composting process where wastewater sludge is used as co-substrate. Several aspects of the composting process and their relation with biodegradation of fats are analysed in detail, including composting routine parameters (temperature, moisture content and porosity) and biochemical parameters related to fats biodegradation, such as respiration activity and lipolytic activity exhibited by microbial populations. The effects of turning the material are also reported. The results obtained can be used for the fats recycling to obtain a stabilised compost with agronomical value.

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Abstract

A composting process was proposed as an effective technology for the biodegradation of fats in a proportion of 40-50%. Anaerobically digested sludge was used as co-substrate for animal fats to balance the C/N ratio of the composting mixture and to provide additional biodegradable organic matter and active biomass. Two different strategies were studied: static pile and dynamic turned pile. Air-filled porosity was initially adjusted to 40% for both experiments. It was observed that non-turned strategy increases the formation of material agglomerates which derived in a non-homogeneous fat distribution, the development of filamentous fungi, and a considerable increase in the amount of leachate generated. Turning the composting material resulted in the best results for composting fat enriched wastes, preventing the formation of agglomerates. An effective biodegradation up to 92% of the fats was observed under these conditions. Besides, the addition of fats increased significantly the duration of the thermophilic period of the composting process.

Key words: Animal fats; Composting; Porosity; Turning; Wastewater sludge.

1. Introduction

Fats and oils are among the main components of organic matter in wastewater (Saatci et al., 2001) and solid wastes, especially those produced by the food industry (Galli et al., 1997; Mari et al., 2003). Fats and oils are essentially triglycerides consisting of linear fatty acids attached, as esters, to glycerol. The biodegradation of fats begins with the enzymatic hydrolysis of the ester bond carried out by lipases, followed by the consumption of glycerol and beta-oxidation of fatty acids (Lalman and Bagley, 2000). However, fat biodegradation can be limited by its insolubility in water (Lefebvre et al., 1998).

Among the available technologies to recycle organic solid wastes, composting is often presented as a low-technology and low-investment process to convert organic solid wastes to a soil amendment known as compost. Composting is a biotechnological process by which different microbial communities initially biodegrade organic matter into simpler nutrients and, in a second stage, form complex organic macromolecules such as humic acids (Hsu and Lo, 1999). The composting process is characterized by the rapid development of thermophilic temperatures due to the heat generation in the first degradation stage in which a significant pathogen content reduction is also achieved. Composting is an aerobic microbial process that requires optimal moisture and porosity (Haug, 1993). Temperature, oxygen and moisture content are often selected as control variables of the composting process along with other chemical, biochemical or microbiological properties such as enzymatic activity or respiration indices (Barrena et al., 2005; Saviozzi et al., 2004; Tiquia, 2005).

References can be found in literature regarding the composting of fat-enriched wastes. Special attention has been given to wastes derived from the olive oil industry (Albuquerque et al., 2006a; Cegarra et al., 2006). In general, routine composting parameters show that composting fat-enriched wastes is possible with low percentages of fats (5-15%) in the initial mixture (Filippi et al., 2002; García-Gómez et al., 2003; Lemus and Lau, 2002), which causes a longer thermophilic phase attributed to the high chemical energy content of fats (Nakano and Matsumura, 2001). On the other hand, reported values of lipid degradation are usually high, within 80-90% (Baeta-Hall et al., 2005; García-Gómez et al., 2003; Lemus and Lau, 2002) and up to 97% when long composting periods are considered (Baddi et al., 2004). However, references to the degradation of fats of animal origin or organic matrices with high fat content (over 15%) are scarce.

Composting of fats is inherently difficult due to their nutrients deficiency, with especially low nitrogen and phosphorous content relative to high carbon content (Sasaki et al., 2003). This fact usually implies the utilization of a co-substrate to compensate the C/N ratio of the initial mixture and to act as inoculum. Different types of sludge, due to their typical low C/N ratio, are considered suitable for being composted with fats or fat-enriched wastes (Wakelin and Forster, 1997).

Water and oxygen are necessary for the biological activity of microorganisms and their availability is directly related to the total porosity and the air-filled porosity (AFP) of the material. These physical properties considerably affect the biological activity of a composting matrix (Malinska and Richard 2006). Annan and White (1998) suggested an AFP range between 30-60% as optimal, depending on the nature of the material to be composted. The use of a bulking agent is required in sludge and fat composting due to the low AFP of both materials. A long list of waste materials have been proposed as bulking agents in several studies. The most widely used materials are wood chips and sawdust (Larsen and McCartney, 2000; Wong and Fang, 2000). Also the type and proportion of bulking agent has been highlighted as being responsible for the thermophilic phase duration and rate of oil biodegradation (Manios et al., 2006).

Our previous work (Gea et al., 2007a) showed that animal fat could be successfully composted with sludge at high ratios to obtain a stable and sanitized product. Composting of mixtures with a fat content of up to 50% was possible although a maximum content of 30% was recommended to obtain high fat degradation (85%) and to avoid excessively long composting periods. In addition, this work displayed the importance of bulking agent selection.

The main objective of the present work is to study the biodegradation of animal fats present in a high proportion in a composting process at laboratory scale with digested sewage sludge used as co-substrate and to determine the influence of the strategy used for composting (static or dynamic). The effects of initial adjustment of AFP are also studied. Chemical and physical properties of the materials were monitored during the process, also the lipolytic activity and the respiration index were used to measure the biological activity.

2. Materials and Methods

2.1. Composting materials

Animal fat (Trg Debo Fancy, KAO Corporation S.A., Spain) collected from a cow slaughterhouse was mixed with anaerobically digested sewage sludge from the wastewater treatment plant of Granollers (Barcelona, Spain). Long chain fatty acids (LCFA) profile of animal fat used was: 3% mystiric acid (C14:0), 30% palmitic acid (C16:0), 17% stearic acid (C18:0), 38% oleic acid (C18:1), 6% linoleic acid (C18:2), 6% other LCFA. Wood chips from a local carpentry were used as bulking agent. Initial characteristics of composted materials are summarized in Table 1.

2.2 Composting mixtures

Two composting strategies were tested: static system and dynamic turned system. In both experiments, a high animal fat content (40-50%) was used. The initial characteristics of the composted mixtures are summarized in Table 2.

Bulking agent particle size was in the range of 0-10 mm. A mixing ratio of bulking agent to sludge/fat mixture was calculated to adjust initial the AFP to 40% as porosity requirement (Eftoda and McCartney, 2004). The approximate volumetric ratio to achieve the porosity requirements was in the range of 2:1-1:1 bulking agent:sludge.

2.3 Composting experiments

The experiments were carried out using a 30 l insulated vessel conditioned for composting with an O_2 control system. The online parameters recorded were temperature and O_2 content. Gas content was measured with an O_2 sensor (Sensox 6C, Sensotran, Spain). One Pt-100 sensor (SR-NOH, Desin, Spain) inserted at the centre of the reactor was used for temperature monitoring. All sensors were connected to a self-made data acquisition system implemented in a personal computer. Oxygen control was performed by a feedback oxygen control that automatically supplied fresh air (room temperature) to the reactor by means of a flow meter (Sensotran mod. MR3A18SVVT) when oxygen concentration was under 10%. Water was manually added to the composting mixture when necessary to maintain moisture content in the optimal range for composting (40-60%) (Haug, 1993).

Two strategies were used in the composting experiments. Static composting was carried out without homogenization of the composting mass, whereas in dynamic composting a complete manual mixing and homogenization of the material was carried out at the moment of sampling. This procedure was used to simulate static (non-turned) and dynamic (turned) composting conditions.

2.4. Air-filled porosity

Air-filled porosity is expressed as the ratio of gas-filled pore volume of the sample to total sample volume. AFP was measured using a self made constant volume air pycnometer according to the description of Annan and White (1998) and Oppenheimer et al., (1997) with an effective sample chamber volume of 1.65 l and using an initial pressure of 6 bar. AFP determinations were carried out in triplicate. The average value of AFP was calculated.

2.5. Sampling procedure

For each analyzed sample, a representative sample (2 l) was carefully taken out from the core of the reactor to minimize the possible alterations of the composting mass for AFP determination. After analyzing AFP a mixed sub-sample of approximately 700 ml was used to determine the rest of the parameters studied during the experiments, whereas the remaining sample volume was returned to the reactor. It was assumed that these high sample volumes were representative of the whole composting mass in the reactor.

2.6. Analytical Methods

Fat content was determined by a standard Soxhlet method (U.S. Environmental Protection Agency, 1998) using n-heptane as organic solvent. Additional analytical methods to determine moisture content, volatile organic matter (OM), nitrogen Kjeldhal and pH were carried out according to the standard procedures (U.S. Department of Agriculture and U.S. Composting Council, 2001).

2.7. Static Respiration Index

Static Respiration Index (SRI) was determined in a static respirometer according to the original model described by Ianotti et al. (1993) and following the modifications and recommendations given by the U.S. Department of Agriculture and U.S. Composting Council (2001). Assays were run at process temperature at the moment of sampling. A complete description of the equipment and procedure can be found in Barrena et al. (2005). Three replicates were used in each case and the average value was recorded. SRI is expressed as mg O_2 g OM^{-1} h^{-1}.

2.8. Lipolytic Activity

Lipolytic activity was determined using a commercial kit (Roche/Hitachi Lip num 1821792) as described by Lopez et al. (2002). Lipases were extracted from 5 g of a representative sample of the composting material using 50 ml of 400 mM tris-HCl buffer with 10 mM $CaCl_2$ (pH 8). Triton X-100 (Panreac, Barcelona, Spain) at 5% (w/w) was added in order to assure a quantitative extraction of lipases. It was assumed that lipolytic activity is mainly non-water extractable, for that reason its presence in leachate was considered negligible (Gessesse et al., 2003). After 30 minutes of extraction using a magnetic stirrer, supernatant was centrifuged (25 min, 7000g) and filtered (0.45 μm) to remove biomass and suspended solids. This sample was used for lipolytic activity determination. Standard lipolytic activity assays were run at 30ºC. Lipolytic activity was expressed as activity units per gram of dry matter (AU g^{-1}). One activity unit was defined as the quantity of enzyme necessary to release 1 μmol of fatty acid per minute under the specified conditions.

2.9. Long chain fatty acids (LCFA)

50 ml of heptane (99% purity) were added to 5 g of sample and mixed in a magnetic stirrer for 30 minutes to extract LCFA. Afterwards, the suspension was centrifuged (30 min, 7000g) and the resulting supernatant filtered through a Millipore Millex FGS filter (0.2 μm). This extract was used for free LCFA determination by gas chromatography using a Perkin-Elmer AutoSystem XL Gas Chromatograph with a flame ionization detector (FID) and a HP Innowax 30 m x 0.25 x 0.25 μm column. The carrier gas was Helium and a split ratio of 13 was used. An initial temperature of 120ºC was kept for 1 min; then, it was increased up to 250ºC at 8ºC min^{-1}, and maintained at this temperature for 7 min. The system was calibrated with different LCFA standards (including lauric, mystiric, palmitic, stearic, oleic and linoleic acid from Sigma, Spain) of concentrations in the range of 0-100 mg l^{-1}.

3. Results

3.1. Initial porosity adjustment

For the reported composting experiments, air-filled porosity of initial samples was experimentally measured by air pycnometry and adjusted to 40% by adding bulking agent. This is a new procedure to adjust porosity in composting mixtures, since the typical recipes for composting are only based on volumetric ratios of bulking agent:substrate (Haug, 1993), which do not directly consider the available porosity. In low-porosity materials, such as wastewater sludge and fats, an experimental measure of porosity should be recommended for an enhanced biodegradation. However, despite the significance of AFP in the composting process, only few publications reflect its measurement and evolution along the composting process (Eftoda and McCartney, 2004; Su et al., 2006).

In relation to the optimal values of porosity, previous experiments (data not shown) with an initial AFP value of 30% did not follow the expected evolution and thermophilic range of temperatures was not reached, being the biodegradation of fats negligible. Thus, an initial AFP of 40% was selected in further experiments and could be considered as a minimum porosity requirement in the composting of mixtures of low-porosity materials.

3.2. Static composting

Figures 1a, 1b and 1c show the results of different physical, chemical and biological parameters monitored during the static composting experiment (without turning). The fat content in the initial organic matrix was 43.7% (Table 2).

Figure 1a shows the temperature and SRI profiles obtained. Thermophilic temperatures were reached at the second day of composting and maintained during 25 days. Afterwards, a cooling phase and a mesophilic maturation stage were observed. SRI registered the highest value on day 11 (6.36 mg O_2 g OM^{-1} h^{-1}), which indicates a high metabolic activity at that moment, coinciding with temperatures above 60ºC. On day 30 the temperature and the SRI decreased considerably, which indicates a reduction of the biological activity. SRI followed the same pattern as temperature and has proved to be a reliable measure of biological activity, biodegradability and stability of the material (Barrena et al., 2005). Similar maximum values of SRI have been obtained at the initial thermophilic stage in composting experiments with different types of wastes such as paper sludge, hair wastes and anaerobically digested sludge. Higher values (12 mg O_2 g OM^{-1} h^{-1}) have been observed for more readily biodegradable materials as the organic fraction of municipal solid waste and raw sludge from municipal wastewater treatment plant (Barrena et al., 2007; Gea et al., 2004).

Figure 1b shows moisture content and AFP profiles. Additional watering of the matrix was required in several occasions during the process to maintain moisture in the recommended range for composting (40-60%, Haug, 1993). When the reactor was opened for sampling, water was homogeneously added on the surface of the composting mass without additional mixing. However, due to the hydrophobic nature of the initial organic matrix, one hour after watering approximately the 50% of the added water was released as leachate. This fact highlights the difficulty of water content control in hydrophobic matrices under static conditions.

Different stages can be observed in AFP and moisture content evolution during the process (Fig 1b). Firstly, AFP increased as moisture decreased due to aeration and high temperatures. Secondly, after watering (day 4), AFP decreased, as the added water filled the free air pores. This inverse relationship was expected as total porosity (pores occupied by water plus AFP) remains constant assuming that no physical or structural changes occur. In the second stage (days 7-15), AFP decreased as moisture decreased, reflecting an overall decrease of total porosity. This could be the result of fat melting due to the high temperatures of the composting mass. During sampling, it was observed that fat, initially homogenously integrated with sludge, melted and flowed from it, filling the macro pores, resulting in the compaction of the organic matrix and a lower value of AFP (Figure 2a). From day 15 to 40, AFP and moisture content increased expectedly upon the biodegradation of organic matter, which was confirmed by the high values of final AFP and moisture content.

Agglomeration was observed when temperature decreased in the cooling phase prior to the maturation stage of composting (day 25). Figure 2b shows the agglomeration of organic material due to filamentous fungi. Rojas-Avelizapa et al. (2007) reported an increase in fungal biomass over bacterial biomass in the final stage of aerobic treatment of fat-enriched wastes. The formation of agglomerates caused larger intra particular pores (Ahn et al., 2007) that contributed to the increase in observed AFP values. Consequently, preferential paths for water circulation appeared at the inner section of the mass and an increase in leachate generation was observed due to the hydrophobic characteristics of the substrate. The lack of mixing favoured agglomeration. The need for turning fat-enriched matrices in a composting process has been highlighted as a measure to reduce compaction and to provide homogenization and re-inoculation (Albuquerque et al., 2006a, 2006b).

Figure 1c shows fat content, organic matter and lipolytic activity profiles. The most significant reduction was observed in the first days of process (31% fat content at day 11) and from that moment until the end of the process only a slight reduction occurred.

On the other hand, high temperatures and high SRI values (Figure 1a) were registered until day 25. This demonstrates that biological activity was not inhibited. Lipolytic activity (Figure 1c) was detected during the second week of process and increased progressively during the thermophilic phase to reach and maintain maximum levels in the mesophilic cooling and maturation stages. This profile is opposite to temperature and SRI profiles, which was probably due to an accumulation of lipolytic activity.

Table 3 shows the concentration of free LCFA in three different moments of the process. It can be observed how LCFA concentration increased considerably from day 14 to day 25. From this data, it can be deduced that in the initial stage of the composting process, fat hydrolysis to LCFA was not limited, and the LCFA consumption was the rate limiting step in biological activity, which was confirmed by the presence of lipolytic activity (Figure 1c).

Different mechanisms for LCFA and lipid hydrolysis inhibition have been reported in aerobic and anaerobic biodegradation processes (Alves et al., 2001; Angelidaki et al., 1999; Becker et al., 1999; Fernández et al., 2005; Hanaki et al., 1981; Lalman and Bagley 2001; Loperena et al., 2006). LCFA concentrations found in this experiment are much higher than those found as inhibitory in other references. It is thus possible that an inhibition of the degradation of triglycerides, due to LCFA accumulation, provoked a diminution of fat degradation.

3.3. Dynamic (turned) composting

Figures 3a, 3b and 3c show the results obtained for composting under dynamic (turned) conditions. Initial organic matrix used in this experiment presented a fat content of 47.5%. This value is slightly higher than that of static composting experiment. However, it should be noted that it is very difficult to adjust the fat content of the mixture. In any cases, both experiments (static and dynamic), fat content can be considered as very high since reported values are in the range of 5-15% (Filippi et al., 2002; García-Gómez et al., 2003; Lemus and Lau, 2002).

In the dynamic experiment, the composting mass was mixed at the moment of sampling to emulate the homogenization provided by a dynamic composting system. Agglomerates did also appear but were in fewer amount and smaller in size than those formed using a static strategy. Consequently, the development of fungal communities and agglomeration was limited as it was previously observed (Albuquerque et al., 2006b; Cegarra et al., 2006).

Figure 3a shows the temperature and SRI profiles obtained in the composting process, where thermophilic temperatures were reached on day 3 and maintained during 21 days. SRI followed the same profile than temperature and showed an activity decrease on day 21. In general SRI values in thermophilic phase were higher than those of static material indicating a slightly more active process (maximum value 6.95 mg O_2 g OM^{-1} h^{-1} at day 19).

Figure 3b shows moisture content and AFP profiles. Only two moisture adjustments were necessary during the process to maintain moisture content within the range recommended for the composting process (Haug, 1993). This important difference from static experiment was attributed to the fact that water was fully integrated in the mixture. As a result, the leachate volume generated was inferior to the volume generated in static conditions in a 38%. AFP increased during the process as organic matter biodegraded. On day 19, a large increase in AFP occurred, along with the end of the thermophilic phase. The high values of AFP (around 70%) registered until day 33 coincided with the highest rate of fat biodegradation during the process, from 31% to 7% (figure 3c). During the last stage of the process (days 33 to 50) AFP decreased to 55%. This can be attributed to the compaction phenomena observed in highly degraded organic wastes (Mohee and Mudho, 2005).

Figure 3c shows fat content, organic matter and lipolytic activity. Both fat content and organic matter decreased considerably during all the process. No LCFA accumulation was observed during the composting process, which confirms the hypothesis that periodical homogenization of the composting mass and the agglomerates destruction favoured the biological activity. Moreover, fat content decreased progressively during the composting process reaching a final value of 5.4%, which implies a global fat biodegradation of 92%.

Although an important fat content reduction was observed, no lipolytic activity was detected until the second week of process. As observed under static conditions, lipolytic activity presented an increasing trend through the process. However, under dynamic conditions the values of lipolytic activity were more erratic, which suggested a possible analytical problem, for instance, in the lipase extraction method used for solid samples. Further research is required in order to establish an effective method for enzyme extraction and enzymatic activity determination in composting environments, especially when quantitative determinations are necessary. A possible alternative for lipolytic activity analysis could be the determination on a solid sample finely dispersed without solid-liquid extraction. Although other enzyme activities such as dehydrogenase have been measured using this approach (Barrena et al., 2008), no results for lipase activity have been reported, which is probably due to the inherent interface catalytic mechanism of lipases involving mass transfer limitations (Verger, 1997). Anyway, a procedure of lipase extraction for composting samples could be the aim of future works.

4. Discussion

Table 4 shows the reduction of weight, dry mass, organic matter and fat content obtained for the experiments carried out. Composting under dynamic conditions showed the highest reduction of weight, dry mass, organic matter and fat content. Final fat content was 5% under these conditions. This indicates that there is a residual fraction of fats present in the anaerobically digested sludge, which is not biodegradable under composting process conditions. This fact has been reported previously (Réveillé *et al.*, 2003). However, it can be concluded that sewage sludge acts as an adequate cosubstrate in fats composting in terms of nitrogen source and providing additional biodegradable organic matter and active biomass.

From Figures 1a and 3a it can be deduced that a high fat content in the composting mass leads to a longer thermophilic stage compared to those typically reported for laboratory scale sewage sludge composting experiments (Gea et al, 2004), which confirms previous observations (Gea et al., 2007a). This fact could be an inconvenient for the management of composting plants when dealing with fat-enriched wastes (Manios et al., 2006). However, fats can be useful as co-substrate in the case of composting low energy content wastes to fulfil the international requirements on compost sanitation (temperature above 55ºC for a total period of 2 weeks, U.S. Environmental Protection Agency, 1995). Lipids contain twice the energy of other organic materials like sugars and starch (Fernandes et al., 1988; Viel et al., 1987). This high-energy content represents a clear advantage for processes where thermophilic temperatures are desirable.

On the other hand, it has been demonstrated that an initial AFP adjustment of 40% permits to reach the thermophilic range of temperatures. However, only considering the initial AFP value does not guarantee the desired development of the biodegradation process. Monitoring AFP along the composting time can ensure that this parameter does not fall under the recommended values for aerobic microbial activity, but AFP does not give information about porosity distribution. A visual observation of the composting matrix is additionally necessary when composting particular types of wastes to detect structural changes of the material as the agglomerates formation. Material turning should complement adequate AFP values in those cases to achieve a correct porosity distribution.

Finally, it should be highlighted that moisture requirements and leachate generation were lower under dynamic conditions because the formation of agglomerates was avoided. These are important facts in considering the cost and the environmental impacts of the waste management used for fats biodegradation (Wei et al., 2001). A mechanical turning system without forced ventilation has been suggested as optimal for fat-enriched wastes from olive oil mills (Cayuela et al., 2006, Cegarra et al., 2006).

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Figure Legends

Fig. 1. Composting results for static experiment: a) Temperature and Static Respiration Index; b) Moisture Content and Air filled Porosity (sampling and watering points are also indicated); c) Organic Matter, Fat Content and Lipolytic Activity.

Fig. 2. Composting material in static experiment.

Fig. 3. Composting results for turned experiment. a) Temperature and Static Respiration Index; b) Moisture Content and Air filled Porosity (sampling and watering points are also indicated); c) Organic Matter, Fat Content and Lipolytic Activity.

Table 1: Main initial characteristics of composted materials.

Parameter	Wastewater sludge	Wood Chips	Animal Fat
Moisture content (%)	72.7	11.8	<2
Organic matter (% dry basis)	61.5	95.3	> 99
Fat content (% dry basis)	8.0	2.9	> 99
N-Kjeldhal (% dry basis)	2.6	0.1	<0.02
C/N ratio	8	500	>4000
pH	7.6	-	-
Air-filled porosity (%)	30.0	87.6	3.0

Table 2: Initial characteristics of the mixtures in static and turned strategies.

Parameter	Static	Turned
Total mass (kg)	15.3	12.8
Moisture content (% wet basis)	52.0	44.3
Organic matter (% dry basis)	75.2	72.6
Fat content (% dry basis)	43.7	47.5
Air-filled porosity (%)	40	40
pH	7.2	7.0

Table 3: LCFA concentration (mg g^{-1}, dry basis) in different days of the composting process for static material.

Day of process	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Total LCFA
14 (thermophilic)	7.2	2.4	9.7	19.3
25 (cooling phase)	25.5	13.4	72.1	111
39 (maturation)	12.7	5.0	40.3	58

Table 4: Total mass, dry mass, organic matter and fat content reductions (in percentage) obtained in the composting processes.

Parameter	Static	Turned
Total mass	16.8	24.5
Dry mass	30.1 ± 9.9	34.9 ± 10.0
Organic matter	36.8 ± 10.4	43.5 ± 11.1
Fat content	56.5 ± 18.1	92.6 ± 19.2