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Enzymatic hydrolysis of organic waste materials in a solid–liquid system

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Abstract

In the current climate of increasing emphasis on environmental protection and efficient waste management, regional management bodies and environmental agencies are striving to achieve an economical and environmentally acceptable system for the recycling of biodegradable organic wastes. Composting would appear to be a cost effective solution to this problem, but in its entirety, composting is an inherently lengthy and variable process and is restrictive in terms of the demand on resources and space in composting plants. The aim of this study was to compare a biological composting process of solid residues with an enzymatic hydrolysis process of residues. The length of time required to naturally compost three organic materials, spent mushroom compost (SMC), farmyard manure (FYM) and dairy wastewater sludge (DWS) under optimal conditions was 42 days, 98 days and 84 days, respectively. In an attempt to accelerate this process, commercial enzymes were added to the waste products in a heterogeneous solid–liquid system. The enzymes utilised included a range of proteases, cellulases, ligninases, lipases and pectinases, which are responsible for the hydrolysis of protein, cellulose, lignin, lipids and carbohydrates, respectively. Preliminary results indicate that all of the organic materials were stabilised within 9 h and that the enzymes used would, therefore, improve the efficiency of a waste management plant, if such a system were employed. Spent mushroom compost has a mean N/P/K ratio of 20:10:10 recorded for composted SMC, while a similar ratio of 20:10:20 was obtained for hydrolysed SMC. In contrast, composted farmyard manure has a N/P/K ratio of 30:0:30 and a ratio of 10:1:10 for hydrolysed FYM. Finally, composted DWS has a N/P/K ratio of 20:1:30 while DWS hydrolysate has a N/P/K ratio of 40:1:20, with the decrease in nitrogen in the composted DWS attributed to the addition of wood chippings and sawdust as a bulking agent. While all three materials have a considerable supply of plant nutrients, the variability in nutrients could be overlooked when employed as a soil amendment.
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1. Introduction

Waste management has become increasingly important in recent years as European law (99/31/EC) has stipulated that the quantity of organic waste discarded in landfills must not exceed 35% by weight with reference to 1995 levels, not later than 2016 (EC, 1999). Consequently, a greater emphasis has been placed on recycling and the reutilization of organic waste materials. Composting is considered to be a cost-effective solution to the problem of effective waste disposal, and this is witnessed in the fact that thirteen compost production units are currently operating in Ireland.

The objectives of this study were to investigate the addition of commercial extracellular enzymes to waste materials in an attempt to accelerate the degradation of the associated organic matter and to compare and contrast naturally composted and enzyme-hydrolysed compost with respect to selected physical and chemical parameters. The organic waste materials; spent mushroom compost (SMC), farmyard manure (FYM) and dairy wastewater sludge (DWS) were utilised in this study, all of which are produced in large quantities in Ireland each year.

2. Materials and methods

2.1. Organic waste materials

SMC was obtained from a mushroom production farm in Co. Cavan, Ireland; the farm had produced three flushes of mushrooms from the compost and was no longer

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capable of sustaining economic levels of mushroom production. The SMC was composed of wheaten straw, horse manure, poultry litter and gypsum. FYM was collected from a dairy farm in Co. Limerick, Ireland, with hay being used as the only constituent of bedding. Finally, DW sludge was obtained from a dairy industrial plant in Michelstown, Co. Cork, Ireland. The dairy waste sludge was composed mainly of cheese whey, together with washings from all of the yards and instruments in the plant, which were treated using a biological membrane reactor resulting in the degradation of COD, NH_4N and other organic compounds (Anon, 2006). The resultant sludge was separated from the treated effluent using an ultrafiltration membrane, and the remaining water was drained off into the local water system in conjunction with the requirements stipulated by the EPA (Anon, 2006).

2.2. Composting

The composting of each waste was carried out in individual small-scale (5 gallon) drums. To provide adequate aeration, holes (0.5 cm^2) were drilled in each drum and the drums were tumbled every 3 days. A moisture content of 50% was maintained for SMC and FYM throughout the composting process. Due to the initial high moisture content of the DWS sludge, a mixture of wood chippings and sawdust were subsequently added in a ratio of 4:1, which successfully reduced the moisture content to the recommended level of 50% (Lohr et al., 1984).

Waste material pH (2:1 ratio, Jenway 3010 pH meter), moisture content, CO_2, NH_3 (Solvita® maturity index test) and temperature (Tiny Tag® data logger) were measured throughout the composting process. The process was deemed complete when the CO_2 and NH_3 concentrations were reduced to maturity index 7 or 8 (Solvita®), which represented a matured compost. Also, pH stabilisation and temperature decrease were noted during the maturation stage, indicating that all readily degradable organic matter was decomposed (Bardos and Lopez-Real, 1988). The initial waste materials and subsequent composts were analysed to evaluate their merit as fertiliser.

2.3. Enzymatic hydrolysis

In order to hydrolyse the three organic materials, initial analyses were carried out to determine the quantities of the various constituents present in each. Accordingly, the moisture content and the carbohydrate, mineral, protein, fat and lignin concentrations were determined. The mineral content was determined by the quantity of residue left after ashing 2 g of each waste overnight at 500 °C, in a Gallenkamp muffle furnace. The amount of protein present was determined using the Kjeldahl method for plant material (Rowell, 1994), whereas the concentration of fat was established using the soxhlet method, which is a semi-continuous solvent extraction (Min and Steenson, 1998). The lignolytic material was calculated using a special plant analysis procedure, which involved refluxing and ashing of the composts, as outlined by Byrne (1979). Finally, the amount of carbohydrate present was determined by subtracting the combined weights of crude protein, total fat and minerals from the weight of the waste sample, the results of which are outlined in Table 1.

2.4. Enzymes

From these results, the amount of each enzyme responsible for the hydrolysis was then calculated. Each enzyme was added in excess of 2% volume, to ensure that if any variability occurred, the particular enzyme would not be limiting in the reaction. Five commercially prepared enzymes were used in total, all of which were supplied by Novo Industries, Bagsvaerd, Denmark. The amount of each enzyme used during the hydrolysis process for each material is given in Table 2.

Alcalase 2.4 L FG is a proteolytic food grade enzyme that is generated by the submerged fermentation of a strain of *Bacillus licheniformis*. Pectinex Ultra SP-L is a pectolytic enzyme produced from a strain of *Aspergillus aculeatus* and although it is successful in the breakdown of hemicellulolytic materials, celluclast 1.5 L was also used in this study, to aid in the degradation of cellulosic substances. Celluclast® is generated from the submerged fermentation of a selected strain of the fungus *Trichoderma reesei*. NS 44064 was also utilised to aid the breakdown of lignolytic materials. NS 44064 is a laccase (phenol oxidase) and is produced by the submerged fermentation of *Aspergillus oryzae*, which has been genetically modified. Finally, Lipozyme® TL 100 L is a lipolytic food grade enzyme prepared from *Thermomyces lanuginosus* by the submerged fermentation of the genetically modified *Aspergillus oryzae* (Anon, 2003).

Table 1
Summary of percentage composition of the study materials

	Moisture (%)	Carbohydrate (%)	Minerals (%)	Fat (%)	Protein (%)	Lignin (%)
SMC	50.6	65.6	20.3	3.37	8.20	2.49
FYM	86.7	74.3	9.52	3.98	6.84	5.33
DWS	92.6	17.6	27.5	20.0	34.7	0.25

SMC, Spent mushroom compost; FYM, farmyard manure; DWS, dairy wastewater sludge.

Table 2
Quantity of each enzyme added (μl) to the study materials

	Lipozyme® (μl)	Pectinex® (μl)	Celluclast® (μl)	Alcalase® (μl)	Laccase® (μl)
SMC	84.1	1641	1641	205	62.1
FYM	99.5	1858	1858	171	133
DWS	500	439	439	867	6.13

SMC, Spent mushroom compost; FYM, Farmyard manure; DWS, Dairy wastewater sludge.
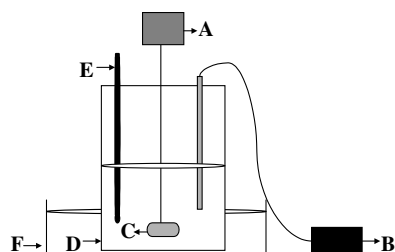
Fig. 1. Schematic diagram of reaction vessel for the hydrolysis of the organic wastes. A, Motor/speed regulator; B, pH data logger; C, Mechanical stirrer; D, Reaction vessel; E, Thermometer; and F, Water bath.

2.5. Analytical procedure

Hydrolysis experiments were carried out in 500 ml reaction vessels (Fig. 1); 50 g of each dried material (2 mm) was hydrated with approximately 150 ml of distilled water and was allowed to equilibrate at 50 °C for 1 h. The mixture was agitated throughout this time and during the extent of the hydrolysis, with a mechanical over-head stirrer (Heidolph Instruments, Schwabach, Germany). The pH was measured and adjusted to between 9 and 10 using NaOH, before the addition of the enzyme lipozyme®. This pH and temperature provide optimum conditions for lipozyme® to function efficiently (Anon, 2003).

Lipozyme®, the enzyme responsible for the hydrolysis of fat, was added and the pH was continuously monitored at 2.5-s intervals, using a Pasco pH data logger, until the pH remained constant for at least 1 h. The pH was adjusted to 6 using HCl, which is optimal for the remaining enzymes (pectinex®, celluclast®, alcalase® and laccase®) to function. The reaction was allowed to proceed until the pH remained unchanged for 1 h, indicating that most of the components had been hydrolysed. Hydrolysate samples were then heated at 90 °C, for 10 min to denature the enzyme activities. Samples were then cooled and stored at 4 °C until required for analysis.

Control runs were also carried out where no enzymes were added. Lignin, cellulose, hemicellulose and organic matter content were determined on these control samples to establish whether chemical breakdown may have transpired at high pH.

2.6. Analyses

All three organic materials were analysed to establish variations, if any, between fresh, composted and hydrolysed composts and also to evaluate their stability and the suitability of each for possible usage in horticulture or agriculture. Twenty-four parameters, both physical and chemical, were measured in total.

2.7. Physical analyses

The organic matter content was determined following overnight ashing at 500 °C, in a Gallenkamp muffle furnace, while acid detergent lignin (ADL), acid detergent fibre (ADF) and hemicellulose constituents were determined using an adapted procedure outlined by Goering and Van Soest (1970). Particle size distribution (PSD) for each compost was analysed on samples <0.53 μm using laser diffraction on the Malvern Mastersizer 2000® (Houghton et al., 2002). The value reported for this parameter, is the $d(4,3)$ value, which is the volume mean diameter of particles, which takes into account the number and volume of particles in a particular medium. Finally, the percentage germination index values were established using cress seeds (Hogg et al., 2002) and is an eminent test in determining the phytotoxicity of compost.

2.8. Chemical analyses

Total nitrogen was determined using the Kjeldahl method (Rowell, 1994); total phosphorus and potassium were also analysed as per Rowell (1994), with the phosphorus filtrate analysed colorimetrically by UV spectrophotometry at 712 nm (UV Spect 2000) and potassium levels determined by atomic absorption spectrophotometry (AAS) (Varian Spectra AA 1989) using an air–acetylene flame.

The remaining elements, copper, zinc, iron, manganese, cadmium, chromium, nickel and lead, along with the cations calcium, sodium and magnesium, were also determined on the atomic absorption spectrophotometer, using a procedure developed by Soon and Abboud (1993). The appropriate standard curves were achieved, using commercially available AA standards. The standards were prepared within the range for optimum working conditions, as outlined in the operator's manual (Varian Spectra AA 1989).

The electrical conductivity of the materials was determined using a procedure developed by Bower and Wilcox (1965) using a Jenway 4200 portable conductivity meter, while pH values were obtained using a Jenway 3010 pH meter, according to Hendershot et al. (1993).

The C/N ratio, which is an important parameter in determining the stability of compost, was calculated from the total nitrogen and organic matter values on the assumption that organic matter contains 58% carbon (Haug, 1993). Finally, the degree of hydrolysis (DH) values were quantified using the trinitrobenzenesulfonic (TNBS) method (Adler-Nissen, 1979), which measures the degree to which the protein has been hydrolysed by the enzyme alcalase®.

2.9. Statistical analysis

The data obtained were analysed by independent-sample T-tests using the ANOVA programme of SPSS, version 11.0 (SPSS, 2002).

3. Results and discussion

3.1. Composting

The duration of the composting process for each of the three organic materials is outlined in Fig. 2. SMC aged in
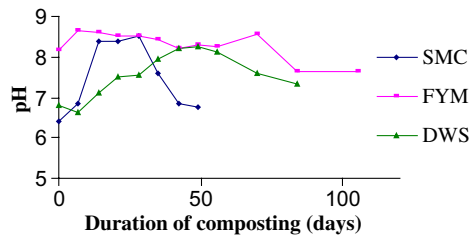
Fig. 2. Changes in pH with time in aerobically composted SMC, FYM and DWS

just over 6 weeks, at which stage a consistent pH of 6.6 was recorded for 2 weeks. This was in agreement with Lohr et al. (1984), employing a similar composting method. The pH increased significantly from day 7 to day 30 ($P < 0.05$) as is evident in Fig. 2, which also coincided with an increase in temperature to 39 °C within the first 10 days (Fig. 3). This was in accordance with the findings of Lohr et al. (1984) and Maher et al. (1993). The volume of compost in the composting drum deceased greatly due to the breakdown of organic matter by microorganisms and the consequent loss in structure (Maher et al., 1993). The resulting compost was dark brown/black in colour, was peat-like in texture and odourless. This relatively short composting process was anticipated, as SMC is already partly decomposed at the end of the mushroom cropping cycle, as shown in Fig. 2 by the absence of stable pH values.

Farmyard manure was deemed to be successfully composted after 14 weeks, as the compost had no odour, was dark in colour, and the pH had stabilised at 7.62 (Fig. 2). The temperature had also dropped below ambient levels. As is evident in Fig. 2, the pH increased from 8.18 to 8.65 with the initial days of composting, whereas it decreased from 8.54 to 8.34 in the study undertaken by Wang et al. (2004). The duration of the composting process may have been attributable to the initial higher organic matter content of the FYM and the particle size of the hay present, resulting in a subsequent reduction in surface area for microbial attack (Crawford, 1983).

Following the addition of the bulking agent, the length of time required to stabilise DW sludge was 12 weeks (Fig. 2). Das et al. (2003) suggested that the addition of bulking agents such as bark/wood chippings would help establish optimal moisture levels, maintain air-filled porosity, and generally improve the overall structure of the compost. This resulted in the amplification of microbial activity within the compost and the concomitant reduction in odour concentration (Das et al., 2003), all of which should facilitate the rapid composting of the organic material, which was noted in this study (Fig. 3).

3.2. Enzymatic hydrolysis

Interpretation of Fig. 4 shows clearly that the time taken for the pH to stabilise following hydrolysis was about 8 h. Following the initial addition of lipozyme®, the pH increased swiftly within the first 2 h, and then levelled off, illustrating that the hydrolysis of lipids within the samples was complete within 4 h, which coincidentally was within the recommended reaction time specified on the lipozyme® product sheet (Anon, 2003). On addition of the remaining enzymes, the pH remained around 6 for the first 30 min and then dropped significantly for all three materials. The pH subsequently remained constant with the notable exception of SMC, where the pH increased slightly before stabilising.

The SMC hydrolysate was dark brown in colour, and was quite viscous, with minute solid particles remaining. The final pH of the SMC was 6.1, but when the compost suspension was brought back to room temperature the pH recorded was 6.48, which is only slightly lower than the findings of Lohr et al. (1984), who recorded a pH of approximately 6.7 for SMC aerobically composted over 60 days.

The FYM hydrolysate was somewhat similar to that of SMC, as it was quite viscous, with some solids remaining, and was slightly darker in colour. However, the pH of the hydrolysed FYM was 4.61, which is extremely low for mature compost (Crawford, 1983). The DWS hydrolysate was also darker in colour; however, a slight odour persevered indicating that perhaps the DWS was not fully hydrolysed. The addition of sawdust prior to hydrolysis
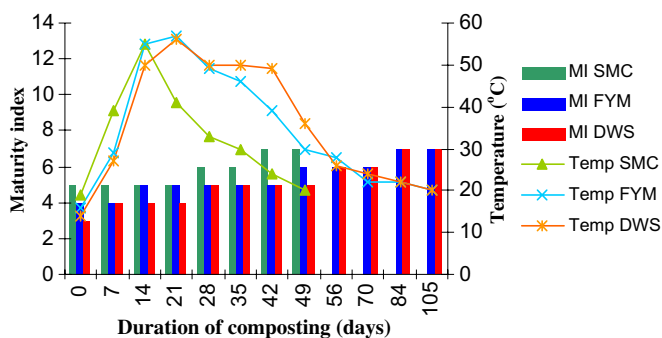

Fig. 3. Changes in temperature and maturity index with time in aerobically composted SMC, FYM and DWS. MI: Maturity index (Solvita®); Temp: Temperature (°C).
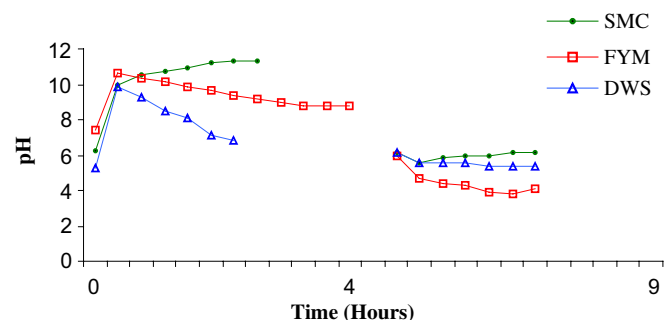

Fig. 4. pH changes during the hydrolysis of three organic waste materials

may prove successful in this case, as it may reduce the odour significantly in advance and/or the inclusion of enzymes more specific to the various components present in DWS.

3.3. Physical and chemical analysis

The variation of properties for all fresh organic materials, and the changes they undergo following hydrolysis and natural composting, are outlined in Tables 3–5. Evidently, there is great variation between all three materials, particularly among the cations and the fertiliser elements N, P and K, while all heavy metals are consistently low, and for the most part are well below the recommended standards (Hogg et al., 2002).

The organic matter content of the three organic materials decreased during hydrolysis and composting. Changes in the organic matter content during composting is most likely to be due to the microbial utilisation of readily decomposable substances within the organic matter, such as sugars, starches, lipids, pectin, amino acids, and nucleic acids (Bardos and Lopez-Real, 1988).

The range of pH values determined for fresh and composted FYM and hydrolysed SMC suggest that these materials would successfully raise the pH of soil, if employed as a soil conditioner or amendment (Wang et al., 1984). By comparison, the pH of the hydrolysed FYM and DWS are quite low; nevertheless, they are within the recommended pH range (4–7) for compost (Verdonck, 1988).

In comparison, the EC value, which is a measure of the total dissolved salts in the residue, increased slightly for the SMC and FYM composted materials and it is hypothesised

that this increase is due to the release of salts during the decomposition of organic matter (Lohr et al., 1984) and is also attributed to the mass of the compost as it becomes denser during composting. In relation to the EC values measured for the hydrolysed composts, there was a vast increase in the magnitude of EC, due primarily to the addition of sodium hydroxide, required for pH change. This increase resulted in imbalances amongst nutrients, so as a preventative measure, further research is required into enzymes that work at a more neutral pH and will thus eliminate the use of any chemicals for pH adjustment. Elevated EC levels will almost certainly have a negative effect on the growth of salt sensitive plants, and perhaps on the growth of young seedlings. In a standard commercial growing medium, the EC levels are usually between 1.2 and 1.5 mS cm^{-1} (Maher et al., 2000); however, almost all of the organic materials analysed in this study were above this threshold value, with the exception of DWS compost, while EC values differed significantly ($P < 0.01$) for all composts.

Each compost has more than adequate amounts of the fertiliser elements nitrogen, potassium and phosphorus present and with the exception of nitrogen, their values varied significantly during the composting and hydrolysing processes (Tables 3–5). A decrease was noted in phosphorus levels for all composted and hydrolysed materials, which is of particular interest given recent emphasis on nutrient management in agriculture (Kurz et al., 2005). Of all of the macronutrients and heavy metals analysed, little variation was noted between composts, with sodium being the solitary exception. Levels of sodium were amplified for all hydrolysed samples, owing to the NaOH additions during hydrolysis. These amplified values may be a

Table 3
Physical and chemical properties of fresh, composted and hydrolysed SMC on a dry matter basis

Parameter	Fresh	Fresh/composted	Composted	Fresh/hydrolysed	Hydrolysed	Composted/hydrolysed
OM (%)	68.3	***	36.1	**	65.5	***
pH	6.42	***	7.12	***	6.48	*
EC (mS cm^{-1})	5.32	**	7.05	***	13.4	***
Total N (g kg^{-1})	20.7	***	23.2	***	19.1	***
Total P (g kg^{-1})	8.21	***	5.13	***	7.20	*
Total K (g kg^{-1})	20.1	***	12.9	***	21.6	***
C/N ratio	19.2	***	8.99	***	20.8	***
Total Ca (g kg^{-1})	27.4	***	27.3	***	30.5	***
Total Mg (g kg^{-1})	7.29	***	9.31	***	6.77	***
Total Na (g kg^{-1})	2.65	*	2.55	***	120.4	***
Total Cu (g kg^{-1})	0.022	*	0.041	***	0.046	**
Total Zn (g kg^{-1})	0.020	***	0.013	***	0.015	
Total Fe (g kg^{-1})	0.029	*	0.061	***	0.077	**
Total Mn (g kg^{-1})	0.128	***	0.074	***	0.059	**
Total Cd (g kg^{-1})	0.058	NS	0.077	NS	0.076	NS
Total Cr (g kg^{-1})	0.013	NS	0.023	NS	0.030	NS
Total Ni (g kg^{-1})	0.018	NS	0.010	NS	0.018	NS
Total Pb (g kg^{-1})	0.024	NS	0.007	NS	0.000	NS
Lignin (%)	27.0	***	21.8	***	21.0	NS
Cellulose (%)	52.4	NS	52.4	***	44.9	***
Hemicellulose (%)	45.7	***	39.4	***	35.4	***
d(4,3)	20.6	***	22.0	***	21.1	*
Germination index (%)	18.0	***	53.1	***	0.00	***

Composts significantly different at $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*) levels. NS, not significant.

Table 4
Physical and chemical properties of fresh, composted and hydrolysed FYM on a dry matter basis

Parameter	Fresh	Fresh/composted	Composted	Fresh/hydrolysed	Hydrolysed	Composted/hydrolysed
OM (%)	91.9	***	75.9	***	52.2	***
pH	7.39	***	7.95	***	4.66	***
EC (mS cm^{-1})	2.38	***	4.84	***	20.8	***
Total N (g kg^{-1})	14.4	***	25.3	***	7.61	***
Total P (g kg^{-1})	2.02	***	0.028	***	1.11	***
Total K (g kg^{-1})	44.6	***	27.3	***	14.1	***
C/N ratio	37.1	***	17.4	***	39.9	***
Total Ca (g kg^{-1})	27.2	***	27.9	***	21.1	***
Total Mg (g kg^{-1})	9.31	***	9.77	***	7.30	***
Total Na (g kg^{-1})	5.27	***	9.21	***	346.4	***
Total Cu (g kg^{-1})	0.321	*	0.223	***	0.527	***
Total Zn (g kg^{-1})	1.980	***	1.073	***	0.425	***
Total Fe (g kg^{-1})	0.528	***	0.375	***	1.26	***
Total Mn (g kg^{-1})	6.10	***	1.35	***	2.44	***
Total Cd (g kg^{-1})	0.095	NS	0.077	NS	0.097	NS
Total Cr (g kg^{-1})	0.031	NS	0.028	NS	0.055	NS
Total Ni (g kg^{-1})	0.006	NS	0.023	NS	0.027	NS
Total Pb (g kg^{-1})	0.000	NS	0.000	NS	0.000	NS
Lignin (%)	5.42	***	21.1	***	2.17	***
Cellulose (%)	62.1	***	54.8	***	18.0	***
Hemicellulose (%)	51.1	***	41.8	***	12.5	***
$d(4,3)$	24.5	***	28.4	***	56.5	***
Germination index (%)	91.8	***	47.9	***	8.25	***

Composts significantly different at $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*) levels. NS, not significant.

Table 5
Physical and chemical properties of fresh, composted and hydrolysed DWS on a dry matter basis

Parameter	Fresh	Fresh/composted	Composted	Fresh/hydrolysed	Hydrolysed	Composted/hydrolysed
OM (%)	71.9	***	92.5	***	70.0	***
pH	6.46	**	6.62	***	4.64	***
EC (mS cm^{-1})	6.21	***	1.75	***	13.4	***
Total N (g kg^{-1})	60.4	***	20.2	***	43.9	***
Total P (g kg^{-1})	0.13	***	1.33	***	1.32	
Total K (g kg^{-1})	8.91	***	26.3	***	17.2	***
C/N ratio	6.91	***	26.6	***	9.26	***
Total Ca (g kg^{-1})	22.6	***	7.85	***	39.7	***
Total Mg (g kg^{-1})	7.78	***	0.769	***	9.11	***
Total Na (g kg^{-1})	6.19	***	2.48	***	235.5	***
Total Cu (g kg^{-1})	0.098	**	0.028	***	0.048	***
Total Zn (g kg^{-1})	0.753	***	0.525	**	0.933	***
Total Fe (g kg^{-1})	2.65	***	2.76	***	2.61	**
Total Mn (g kg^{-1})	0.679	***	0.190	NS	0.706	***
Total Cd (g kg^{-1})	0.059	NS	0.108	NS	0.127	NS
Total Cr (g kg^{-1})	0.019	NS	0.000	NS	0.052	NS
Total Ni (g kg^{-1})	0.008	NS	0.000	NS	0.028	NS
Total Pb (g kg^{-1})	0.000	NS	0.000	NS	0.000	NS
Lignin (%)	16.6	***	10.40	***	14.3	***
Cellulose (%)	13.47	***	65.5	***	55.5	***
Hemicellulose (%)	12.32	***	26.5	***	16.5	***
$d(4,3)$	33.3	***	31.1	***	50.2	***
Germination index (%)	0.00	***	99.5	***	0.000	***

Composts significantly different at $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*) levels. NS, not significant.

factor in the poor germination index values obtained over-all for hydrolysed compost. A germination index (GI) of 90% is recommended for mature compost (Hogg et al., 2002), but this only occurred in two of the samples, namely fresh FYM and composted DWS. However, as hydrolysed samples are in solution, they will not be solely used as a growing medium, but merely as a soil conditioner.

Lignocellulose is composed of lignin, cellulose and hemi-cellulose, and forms the foremost part of biomass in nature, and subsequently its degradation is fundamental

within the carbon cycle (Tuomela et al., 2000). Levels of lignocellulytic material were relatively high in each compost analysed, with straw, hay, wood chippings and sawdust being primary providers of such components. Lignin, cellulose and hemicellulose values appeared to increase during both the composting process and hydrolysis. This is probably due to the fact that the more readily degradable carbon sources have been depleted, and most of the residual compost is lignocellulytic in nature. Lignocellulytic components are not easily degradable and can only be naturally hydrolysed through the secretion of extracellular enzymes that are produced by only a small number of compost microorganisms. Overall, lignin appears to be the most resistant component to microbial attack (Crawford, 1983). There were significant differences evident in these components between most of the compost samples, in particular between all composted and hydrolysed materials ($P < 0.001$), with the exception of lignin values for SMC. Overall, the lignolytic components of the hydrolysates are considerably lower in amount than those of their corresponding composted samples, which demonstrates the success of the laccase® and pectinex® over naturally produced enzymes excreted by compost microbes. The possibility that the high pH of the hydrolysates may chemically break down the cellulytic material was not applicable, as the cellulytic values of the control samples were significantly higher than the hydrolysates (Table 6).

3.4. Particle size distribution

Particle size distribution is an essential feature of the composting process, as microbial activity is greatly improved when particle sizes are minute, resulting from an increased surface area. However, if the particle size is too minute, void spaces are then reduced, resulting in a reduced rate of gaseous diffusion (Crawford, 1983). The particle size distributions carried out in this study were carried out on samples >0.53 µm, as particles greater than this size may abrade the cell lens. The $d(4,3)$ values for fresh, composted and hydrolysed SMC are outlined in Fig. 5, and significant variations ($P < 0.05$) in particle size was observed for all treated substrates. An increase in particle size occurred for composted and hydrolysed SMC, which was probably due to the release of humic acids during hydrolysis and the composting process (Grabbe, 1988), resulting in the particles binding together, to form a more stable complex.
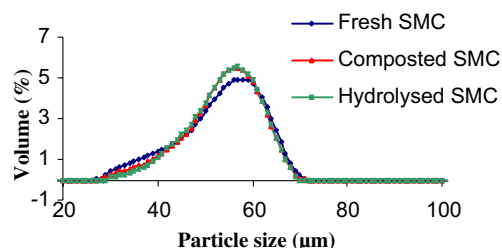

Fig. 5. Particle size distribution ($d(4,3)$) for fresh, composted and hydrolysed SMC.

Similar to the particle size distributions in SMC, the composted FYM showed a greater increase in particle volume and size, when compared to both fresh and hydrolysed materials (Fig. 6). There was a significant difference ($P < 0.01$) noted between all treated substrates and again these could be due to the differences in biodegradability of each material (Houghton et al., 2002).

Finally, for the DW sludge, the fresh DWS proved to have the largest volume of particles present (Fig. 7), and differed significantly ($P < 0.01$) from composted and hydrolysed DWS. This indicates that the breakdown in organic matter during hydrolysis and composting influenced the particle size of the materials, but not the percent volume <0.53 µm.

3.5. TNBS analysis

The degree of hydrolysis (DH) for SMC was 86.3%, while, for FYM and DWS, the DH values measured were 3.5% and 38.14%, respectively. This indicates that the type

Table 6
Organic matter properties of control samples without enzyme addition

	SMC	FYM	DWS
Organic matter (%)	67.7	84.0	67.4
Lignin (%)	21.5	5.73	38.3
Cellulose (%)	15.8	42.6	13.6
Hemicellulose (%)	11.3	44.4	34.4

SMC, Spent mushroom compost; FYM, Farmyard manure; DWS, Dairy wastewater sludge.
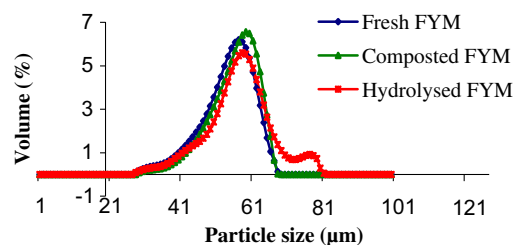

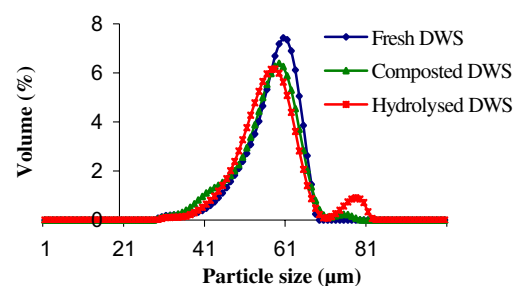Fig. 6. Particle size distribution ($d(4,3)$) for fresh, composted and hydrolysed FYM.

Fig. 7. Particle size distribution ($d(4,3)$) for fresh, composted and hydrolysed DWS.

of substrate in SMC appeared to be more suited to the alcalase® enzyme, while DWS and FYM were not as suitable even under optimal conditions as a reduced number of peptide bonds were cleaved during proteolysis.

4. Summary

The addition of commercial enzymes in a heterogeneous solid–liquid system proved successful in aiding the breakdown of organic materials. However, some adjustments may be necessary in order for this process to work more efficiently and cost effectively. These include the possibility of acquiring enzymes that function at a more neutral pH and yield a high degree of hydrolysis, which would eliminate the use of chemicals for pH adjustment. Furthermore, if this system was employed at a pilot scale, the recycling of the commercially available enzymes could prove a successful cost cutting measure. As it stands, the hydrolysates obtained in this study could also be used in the bioenergy industry, where fermentation of the hydrolysates could successfully lead to the generation of ethanol and other useful by-products.

The possibility of nutrient removal, in a similar way to that employed in wastewater treatment plants, is also an option as the organic waste hydrolysates are in solution. It would also require a critical observation to the C/N rate and NH_4 concentrations. Furthermore, the fact that the hydrolysed compost is in solution may also be beneficial to farmers who wish to use this compost as a soil amendment or conditioner since conventional injection or band spreaders are widely available on farms nowadays.

The economic feasibility of employing the hydrolysis methodology on a pilot scale has not yet been explored, yet it is anticipated that aerated system similar to that functioning in water treatment facilities may be suitable. Moreover, stringent temperature controls and monitoring will be required. Initial estimates of cost for enzymes use to treat 1 t of SMC in this liquid–solid system is €803.90, which excludes any mechanical or operational expenses. This cost effectiveness of this system would improve, particularly when the system works more efficiently and through the recycling of these enzymes.

5. Conclusions

The length of time required to naturally compost three organic materials, spent mushroom compost (SMC), farmyard manure (FYM) and dairy wastewater sludge (DWS) under optimal conditions was 42 days, 98 days and 84 days, respectively. DWS has great potential for the production of high-quality compost as it yielded a germination index of 99.9% following the addition of wood chippings.

Enzymatic hydrolysis is an effective methodology for the treatment of organic waste components, as stable hydrolysates were obtained within 9 h. The hydrolysates obtained have high fertiliser merit, which were comparable to those of their corresponding composts. Spent mushroom com-

post hydrolysate had a mean N/P/K ratio of 20:10:20, farmyard manure hydrolysate had a N/P/K ratio 10:1:10, while DWS hydrolysate had a ratio of 40:1:20.

The hydrolysis of organic manures in general has the potential to generate vast quantities of reducing sugars, which could be further fermented into ethanol (Wen et al., 2004), and this area of research is relatively unexplored.

References

Adler-Nissen, J., 1979. Determination of the degree of hydrolysis of food protein hydrolysates by the trinitrobenzenesulfonic acid method. Novo Enzyme Products Ltd., Technical Information, TN/004.

Anon, 2003. Novo Enzyme Products Ltd., Technical Information.

Anon, 2006. MBR at Dairygold – effective treatment of dairy processing waste for over five years. Water and Waste Water International, <http://www.water-int.com/categories/treatment-plants/mbr-at-dairygold-effective-treatment-of-dairy-processing-waste-for-over-five-years.asp> (accessed February 2006).

Bardos, R.P., Lopez-Real, J.M., 1988. The composting process: susceptible feedstocks, temperature, microbiology, sanitisation and decomposition. In: Bidlingmaier, W., L'Hermite, P. (Eds.), Proceedings – Compost Processes in Waste Management, Monastery of Neresheim (FRG) 13–15 September 1988. CEC, Brussels, pp. 179–190.

Bower, C.A., Wilcox, L.V., 1965. Soluble salt. In: Black, C.A. (Ed.), Methods of Soil Analysis. Part II, Chemical Methods. American Society of Agronomy, Madison, Wis, pp. 933–951.

Byrne, E., 1979. Chemical analysis of agricultural materials. An Foras Taluntais.

Crawford, J.H., 1983. Composting of agricultural wastes – a review. Process Biochemistry, 14–18.

Das, K.C., Tollner, E.W., Eiteman, M.A., 2003. Comparison of synthetic and natural bulking agents in food waste composting. Compost Science and Utilization 11 (1), 27–35.

EC, 1999. Council directive on the landfill of waste. (99/31/EC) Official Journal L182, Document 399L0031, pp. 1–19.

Goering, H.K., Van Soest, P.J., 1970. Forage fibre analysis. In: Agricultural Handbook, vol. 379. USDA, Washington DC, USA, pp. 1–20.

Grabbe, K., 1988. Definition of compost-quality – a need of environmental protection. In: Bidlingmaier, W., L'Hermite, P. (Eds.), Proceedings – Compost Processes in Waste Management, Monastery of Neresheim (FRG) 13–15 September 1988. CEC, Brussels, pp. 179–190.

Haug, R.T., 1993. The Practical Handbook of Compost Engineering. Lewis Publishers, p. 698.

Hendershot, W.H., Lalande, H., Duquette, M., 1993. Soil reaction and exchangeable acidity. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, pp. 141–145.

Hogg, D., Barth, J., Favoino, E., Centremoero, M., Caimi, V., Amlinfer, F., Devliegher, W, Brinton, W. and Antler, S. 2002. Comparison of compost standards within the EU, North America and Australasia. Review of Compost Standards in Ireland. WRAP, Banbury, Oxon.

Houghton, J.I., Burgess, J.E., Stephenson, T., 2002. Off-line particle size analysis of digested sludge. Water Research 36, 4643–4647.

Kurz, I., Coxon, C., Tunney, H., Ryan, D., 2005. Effects of grassland management practices and environmental conditions on nutrient

concentrations in overland flow. Journal of Hydrology 304 (1–4), 35–50.

Lohr, V.I., Wang, S.H., Wolt, J.D., 1984. Physical and chemical characteristics of fresh and aged spent mushroom compost. Hortscience 19 (5), 681–683.

Maher, M.J., Smyth, S., Dodd, V.A., McCabe, T., Magette, W.L., Duggan, J., Hennerty, M.J., 2000. Managing Spent Mushroom Compost. Teagasc, Dublin, p. 1–41.

Maher, M.J., Lenehan, J.J., Staunton, W.P., 1993. Spent *Mushroom Compost – Options for Use*. Teagasc, Dublin, p. 1–44.

Min, D.B., Steenson, D.F., 1998. Crude fat analysis. In: Nielson, S. (Ed.), Food Analysis. Aspen Publishers, US, pp. 201–235.

Rowell, D.L., 1994. Soil Science: Methods and Applications. Longman Group Ltd., UK.

Soon, Y.K., Abboud, S., 1993. Cadmium, chromium, lead and nickel. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, pp. 101–107.

SPSS, 2002. SPSS for Windows (Version 11). Chicago.

Tuomela, M., Vikman, M., Hatakka, A., Itävaara, M., 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technology 72, 169–173.

Verdonck, O., 1988. Composts from organic waste materials as substitutes for the normal used horticultural substrates. In: Bidlingmaier, W., L'Hermite, P. (Eds.), Proceedings – Compost Processes in Waste Management, Monastery of Neresheim (FRG) 13–15 September 1988. CEC, Brussels, pp. 83–90.

Wang, P., Changa, C.M., Watson, M.E., Dick, W.A., Chen, Y., Hoitink, H.A.J., 2004. Maturity indices for composted dairy and pig manures. Soil Biology and Biochemistry 36 (5), 767–776.

Wang, S.H.L., Lohr, V.I., Coffey, D.L., 1984. Growth response of selected vegetable crops to spent mushroom compost application in a controlled environment. Plant and Soil 82, 31–40.

Wen, Z., Liao, W., Chen, S., 2004. Hydrolysis of animal manure lignocellulosics for reducing sugar production. Bioresource Technology 91 (1), 31–39.