Community succession and decomposition of microbial biomass during the composting of pot ale liquor

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ABSTRACT

Composting is normally viewed as a solid waste treatment process. In a new approach we have used composting to treat a liquid distillery waste, pot ale, comprised of predominantly dead yeast cells. The process is operated in continuous, batch-fed mode and degradation efficiency is dependent on maintaining a stable microbial community. The degradation of dead microbial biomass in the pot ale liquor was highly efficient resulting in a reduction of the five day Biological Oxygen Demand (BOD_5) by 99%. The composting of tree bark and pot ale liquor was studied in microcosms to determine the succession of microbial communities during the self-heating phase. Microbial community analyses (biomarkers and sole-carbon source tests) were used to characterize the successional changes which correlated with both physical and chemical changes in the substrate.

Introduction

Conventionally, composting is viewed as a low technology method to convert and stabilize low value organic wastes into a substrate useful for growing plants or mushrooms. However, the need for new sustainable waste treatment processes means composting is being considered for the bioremediation of contaminated solid wastes [15] and soil [1] as well as biologically active filters for remediating polluted waste waters [20] and contaminated air [17]. In addition, traditional agricultural and horticultural composts are being recognized increasingly for their biological quality, e.g. by the presence of growth promoting organisms [21] and organisms antagonistic to plant pathogens [13]. New applications and a new appreciation of the value of the composting process require greater understanding of which factors regulate microbial diversity during composting.

Microbial succession during composting is a classic ecological example of how the growth and activity of one group of organisms creates the conditions necessary for the growth of others. Temperature is the main driving force of succession but it also interacts with other environmental regulators such as pH, redox potential and gaseous exchange as well as the availability of C and energy sources. The turnover of biomass is an essential feature of community succession and total biomass is often seen to decline with time after the initial rapid growth [11,14]. Direct evidence of organisms living off necromass is difficult to obtain, although yeasts, usually reported as zymogenous organisms, have been reported living inside the disrupted cleistothecia of thermophilic fungi [5].

Our view of succession during composting is largely based on conventional plating and isolation procedures. More recently molecular biology techniques [16], phospholipid fatty acid analysis (PLFA) [12], and community level physiological profiles (CLPPs) using sole

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C source tests (Biolog) [14] have been used. The aim of this paper is to discuss the utility of some current methods of microbial community analysis for investigating the ecology of composting. A novel composting process used to treat pot ale liquor, a waste byproduct of the manufacture of whisky was studied. The efficiency of this process depends on maintaining a stable thermophilic community. The process called TAPP (Thermogenic, aerobic, plug-flow, percolation; UK Patent Application No GB 2322623A) was devised to treat pot ale liquor by percolating the liquor through compost stacks made from bark and wood wafers. Pot ale liquor cannot be discharged directly to waters because of its high biological oxygen demand (BOD), low pH and high Cu content. The solids content of pot ale comprise dead yeast cells and the efficiency of the process depends on developing a microbial population capable of degrading this necromass. The process produces a leachate with a BOD_5 reduced from 20000 to <100 ppm; the pH increased from 3.0 to between 5.0 and 6.5 and a Cu content reduced from 10 ppm to 16 ppb. The Cu is adsorped mainly to polyphenolic compounds in the bark but it was noted in early trials that bark on its own did not reduce the BOD as well as a combination of bark and wood wafers. Consequently, the microbial communities on the two different woody substrates, bark and wood wafers, were examined to test the hypothesis that they supported different microbial communities.

Materials and Methods

Experimental design

Pulverized fresh conifer bark and wood wafers were weighed separately into 1.5 L Kilner jars after being amended with either pot ale liquor or water (10% by volume). Thereafter pot ale liquor or water was added (2% v/v) every two days. The jars were closed with cotton wool bungs to allow free gas exchange and incubated at a predetermined temperature program starting at 30°C for one day and increasing by 10°C d^{-1} up to 50°C where it was maintained for a further 12 days. Samples were taken at 0, 7 and 14 d to compare the temporal development of the microbial communities.

Microbiological analysis

Total viable counts (TVC) were made on fresh samples extracted and diluted using de-ionized water and by spread plating onto Tryptone Soy Agar (TSA, 0.1 strength) and also malt extract agar (MEA). Duplicate sets of plates were incubated at 25 and 50 °C and counts made after 7 d. PLFA analysis was carried out on 10 g of freeze-dried sample using the Bligh & Dyer [3] method to extract and purify lipids that were analyzed by GC using a polar capillary column [8]. CLPPs were determined by direct incubation of compost extracts in microtitre plates containing different C sources in individual wells to determine changes in relative and absolute rates of utilization of individual substrates [4,10]. We tested 125 C sources using BIOLOG GN plates and customized BIOLOG MT plates in which the wells contained 30 additional, ecologically relevant C sources [4]. The 10^{-2} dilution was used to inoculate the Biolog plates and duplicate sets of C source profiles were inoculated and incubated at either 25 or 50°C to determine the profile of mesophilic and thermophilic organisms respectively. Plates were read twice daily for 5 d and ANOVA of the average well colour development over time was used to select comparable time points to avoid confounding effects of inoculum density differences between treatments [9]. Respiration (CO_2 evolution) was measured on samples of 10 g in 100 cm^3 soil jars, by

measuring headspace CO_2 accumulated over 6 h, at 25°C and 50°C, using a gas chromatograph.

Statistical analysis

Both the PLFA and CLPP multivariate data sets were analyzed by canonical variate analysis (CVA), after first reducing the dimensionality of the variates to less than the number of samples by principal components analysis (PCA). The mean Mahalanbois distance between treatment groups was compared to simulated data to calculate significant effects [4]. The CLPP data was also transformed by dividing by the AWCD before analysis. Treatment means and least significant differences at 5% ($LSD_{0.05}$) were calculated using a two way (treatment x sampling date) ANOVA. All computations were performed using Genstat 5.3 (NAG Ltd., Oxford, UK).

Results

The wood wafers had a higher initial starting C:N ratio than the bark and also a higher pH that was maintained over the 14 d (Table 1). The moisture content increased significantly over the 14 d until it reached an equilibrium level of 70%. The addition of pot ale with a C:N ratio of 4.8 significantly increased the N content of both bark and wood wafers but more so in the bark (Table 1).

Table 1. Changes in moisture content (%mc), pH , %C and %N, total PLFA and respiration in bark and wood wafers amended with pot ale liquor and incubated at 50°C .

	Day	% mc	pH	%C	%N	$PLFA_{tot}$ ηmol/g	Respiration µg CO_2-C/g/h	
							25°C	50°C
Bark	0	62.6	4.1	43.7	0.42	81	18.8	50.2
	7	64.3	4.2	47.5	0.39	89	9.9	31.3
	14	71.2	4.2	47.3	0.36	83	5.2	17.2
Bark + pot ale	0	63.2	4.5	47.3	0.71	98	64.5	148.6
	7	65.2	4.7	46.8	0.70	145	32.2	79.2
	14	72.9	4.9	46.3	0.68	150	15.5	43.9
Wood wafers	0	59.3	7.2	53.2	0.19	74	12.3	49.7
	7	55.0	6.6	52.6	0.20	66	8.0	32.2
	14	68.4	6.7	52.6	0.19	49	4.4	14.9
Wood wafers + pot ale	0	60.3	7.1	52.9	0.39	82	48.5	151.7
	7	59.8	7.1	52.7	0.44	183	21.8	62.3
	14	67.2	6.9	51.7	0.36	105	9.3	26.9
$LSD_{0.05}$		1.48	0.05	2.09	0.04	23	4.3	5.1

Respiration and biomass

Respiration (CO_2-C evolution) rates were increased almost 3 fold after the addition of the initial 10% of pot ale liquor and declined thereafter over the 14 d (Table 1). Respiration was significantly higher in the bark with pot ale than in the wood with pot ale and was approximately 2-4 times faster at 50°C than at 25°C. Biomass ($PLFA_{tot}$) was stable (in bark) or declined (in wood) without pot ale addition and was increased by 50% in the bark

with pot ale (Table 1). In the wood wafers with pot ale $PLFA_{tot}$ doubled after 7 d and then declined significantly ($P < 0.001$) by day 14.

Total Viable Counts (TVC)

TVC at 25 and 50°C, were increased by pot ale addition and increased over time in a similar manner for both bark and wood. TVC of thermophiles growing at 50°C increased from 10^4 to 2.10^7 after 14 d with mesophiles growing at 25°C present in equal or greater numbers ($3.10^6 – 2.10^8$). The wood wafers had higher fungal counts at both 25 and 50°C. The pot ale treated substrates (especially the wood wafers) had visibly profuse growth of hyphae within 7 d but this had disappeared by 14 d. Several bacterial isolates, found in large numbers (>10^5) on the MEA, were found to form long filaments. When sub-cultured and grown at different temperatures their cell length varied from short rods at 37°C to long rods at 50°C that after prolonged incubation grew into long filaments.

Community analysis

In all, 21 PLFAs were identified and quantified (not shown). Several other minor PLFA peaks were detected but were not quantified or identified. Multivariate analysis of the PLFA data of the 50°C-incubated composts showed distinct temporal patterns and some treatment differences (Figure 1). The main differences were between wood and bark irrespective of treatment or time on canonical variate 1 (explaining 79 % of the variation). After 7 d the PLFA patterns of microbial community on bark with addition of pot ale was

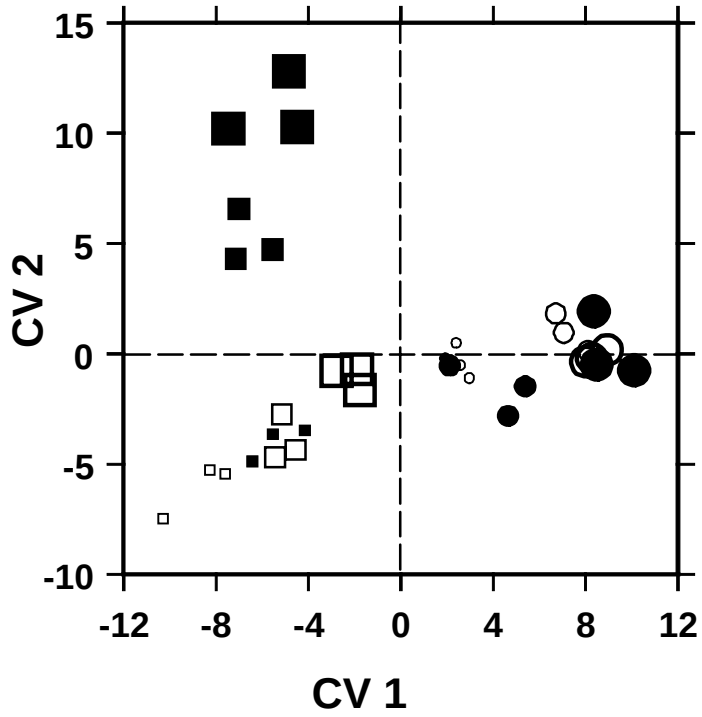

Fig. 1. Ordination plot of canonical variates 1 and 2 for PLFA data showing differences between bark (circles) and wood wafers (squares) incubated for 14 days at 50°C, with (solid symbols) and without pot ale (open symbols) on different sampling days (small symbols = day 0, medium symbols = day 7 and large symbols = day 14).

not significantly different from that of bark only (Figure 1). In contrast, however, the PLFA profiles on the wood wafers after 7 and 14 d with pot ale addition were significantly different from those on wood wafers only and from those on bark (Figure 1).

Some problems were encountered in applying the Biolog method to determine CLPPs. In a few replicates from samples taken on day 0 and 7, incubated at 50 ^{o}C, the indicator dye was spontaneously transformed in all wells including the control. Secondly, small discrete spots of intense colour were observed in a few wells so that colour development was not always uniform. Also fungal colonies grew profusely in some wells, especially at 50^{o}C and in some cases visible hyphal growth did not result in colour development.

For the thermophilic populations (incubated at 50^{o}C) there was no significant effect of pot ale addition on either substrate after 14 d but there was clear evidence of different populations on the bark and wood wafers (Figure 2a). For the mesophilic populations (incubated at 25^{o}C) the main difference in CLPP was also between the bark and wood substrates but there was also a significant difference between the bark with and without pot ale addition on canonical variate 2 (Figure 2b).

Discussion

Both the CLPP and PLFA data showed that the microbial communities on bark and wood wafers were different. Bark supported a larger and more active biomass, possibly due to its higher surface area. The wood wafers had a significantly higher pH and it appears that the two substrates are chemically and physically complementary for this type of application.

The CLPP did not demonstrate significant differences due to pot ale addition when incubated at 50^{o}C, whereas the PLFA technique showed not only that wood wafers resulted in a different community but also that wood amended with pot ale was different. The use of PLFA analysis has the advantage that biomarkers for a wide range of microbial groups can be identified and direct extraction means the problems associated with culturing techniques are avoided. The PLFAs with the highest loadings responsible for the discrimination of the treatments were i15:0, 16:1 ω9, i17:0,18:1 and 20:0. The PLFAs i15:0, 16:1 ω9 were increased by pot ale addition in both bark and wood and i17:0 was increased by pot ale addition but much more so in wood than in bark. PLFAs 18:1 and 20:0 were much lower in wood than in wood+pot ale. The PLFAs i15:0 and 17:0 are representative of Gram positive Bacillus species and may therefore suggest that the wood with pot ale additions favored the succession of these thermophilic species.

However, PLFAs do not necessarily distinguish between organisms that may be active at different temperatures and so may not necessarily distinguish the active groups at a particular time. It is clear that even at thermophilic temperatures maintained for 14 d that large numbers of mesophiles are viable but not necessarily active. The distinction of mesophiles and thermophiles is operationally defined and many organisms are active over wide temperature ranges e.g. the filament forming bacteria found. The presence of thermosensitive filament forming bacteria in composts seems to have received little attention in the literature and yet in this study they were present in large numbers ($> 10^5$). We have also isolated similar bacteria in other studies with temperature ranges from 25-50°C. Filamentation can also be induced by nutrient limitation [19] and it is probable that there is an ecological advantage to form filaments e.g. inhibition of predation [18] and enhanced gene transfer [7]. Not least this phenomenon raises questions about how to relate cell numbers to biomass when cell division but not growth is inhibited.

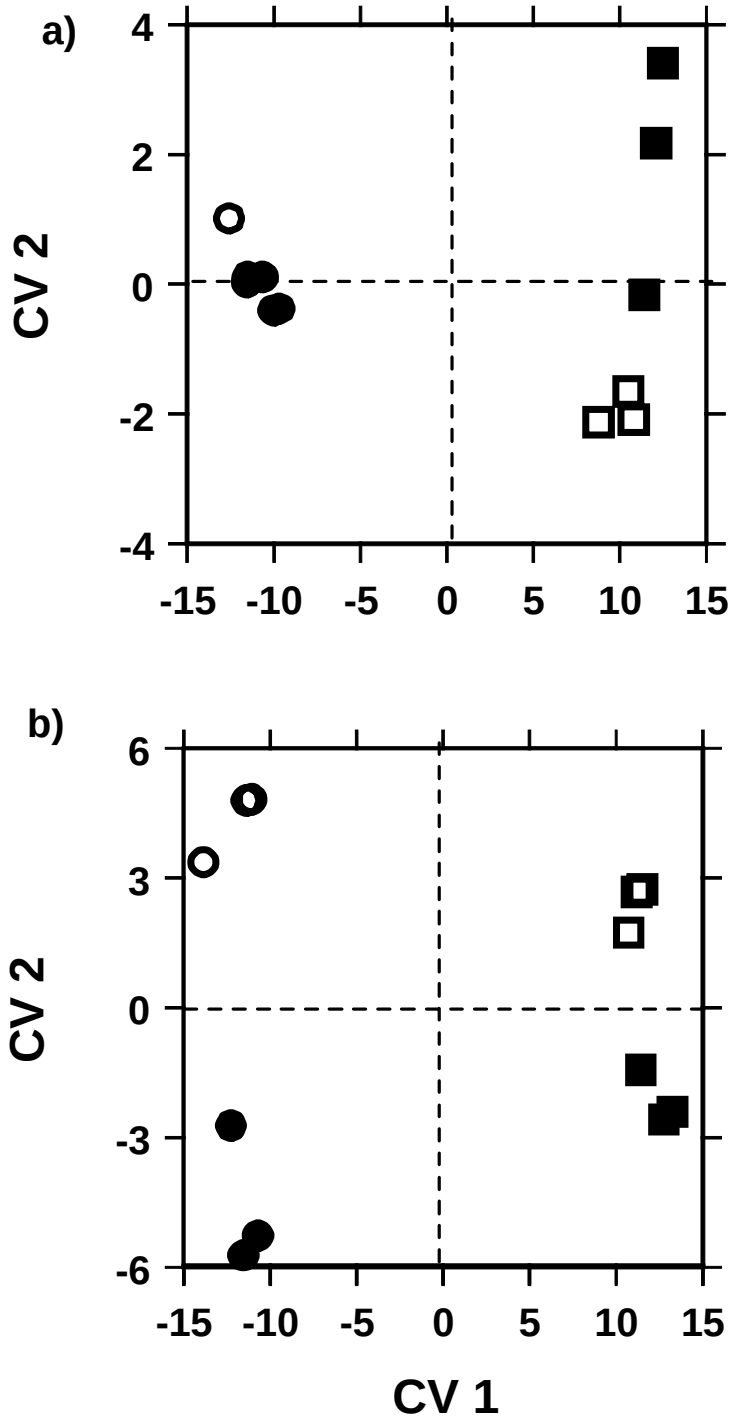

Fig. 2. Ordination plot of canonical variates 1 and 2 for CLPP sole carbon source data showing differences between bark (circles) and wood wafers (squares) either with (solid symbols) or without pot ale (open symbols) after 14 days incubation at a) 50°C and b) 25°C.

The use of CLPP was found to have several methodological problems but may still be useful if different sensing technology and approaches are developed [10]. The instantaneous colour formation and false positives may have been due to either carry over of C; the presence of reducing substrates or extracellular polysaccharide. The latter is often associated with *Bacillus* spp., which cause similar false positives and instantaneous reactions when Biolog plates are used to identify pure cultures [2]. The addition of a 1% thioglycollate solution to the inoculum can overcome such problems with pure cultures [2] and may perhaps also be valid for CLPP. Alternatively CLPPs might be obtained by the use of multiple-substrate induced respiration methods using whole compost samples that allow poorly soluble substrates to be evaluated [6]. This would allow a wider range of substrates to be tested and allow the inclusion of more polymeric C sources representative of organic matter and necromass.

The short-term experiments used here have demonstrated that there are differences in the microbial communities associated with bark and wood wafers used for the TAPP process. Longer-term studies, however, will be needed to determine if the microbial community reaches an equilibrium over the many months of waste treatment. CLPP and PLFA methods have showed broadly similar trends but also some differences such that both are potentially useful for following the successive changes in the microbial communities during composting.

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References

1. Balba MT**,** Al Daher R**,** Al Awadhi N**,** Chino H**,** Tsuji H (1998) Bioremediation of oil-contaminated desert soil: The Kuwaiti experience. Env Internat 24:163-173.
2. Biolog (1993) Manual for identification of Gram negative bacteria, Biolog Inc., Hayward, California, USA.
3. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physio 37:911-917.
4. Campbell CD**,** Grayston SJ**,** Hirst DJ (1997) Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. J Microb Meth 30:33-41.
5. Campbell CD, McHardy WJ (1994) Scanning electron microscopy of the microbial colonization of composted tree bark. Micron 25:253-255.
6. Chapman SJ**,** Campbell CD**,** Norrie A**,** Davidson MS (1998) Community substrate induced respiration, p.122 Abstracts Eight International Symposium on Microbial Ecology Halifax, Canada 9-14 August 1998
7. Debbia EA (1992) Filamentation promotes f'lac loss in *Escherichia coli* k12. J Gen Micro 138:2083-2091.
8. Frostegard A**,** Tunlid A **,** Baath E (1996) Changes in microbial community structure during long-term incubation in 2 soils experimentally contaminated with metals. Soil Biol Biochem 28:55-63.

9. Garland JL (1996) Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol Biochem 28:213-221.
10. Garland JL (1997) Analysis and interpretation of community-level physiological profiles in microbial ecology. Microb Ecol 24:289-300.
11. Hellmann B, Zelles L, Palojarvi A, Bai QY (1997) Emission of climate-relevant trace gases and succession of microbial communities during open-windrow composting. Appl Environ Microbiol 63:1011-1018.
12. Herrmann RF, Shann JF (1997) Microbial community changes during the composting of municipal solid waste. Microb Ecol 33:78-85.
13. Hoitink HJ, Stone AG, Han DY (1997) Suppression of plant diseases by composts. Hortscience 32:184-187.
14. Insam H, Amor K, Renner M, Crepaz C (1996) Changes in functional abilities of the microbial community during composting of manure. Microb Ecol 31:77-87.
15. Laine MM, Haario H, Jorgensen KS (1997) Microbial functional activity during composting of chlorophenol-contaminated sawmill soil. J Microb Meth 30:21-32.
16. Malik M, Kain J, Pettigrew C, Ogram A (1994) Purification and molecular analysis of microbial DNA from compost. J Microb Meth 20:183-196.
17. Matteau Y, Ramsay B (1997) Active compost biofiltration of toluene. Biodegradation 8:135-141.
18. Pernthaler J, Posch T , Simek K, Vrba J, Amann R, Psenner R (1997) Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. Appl Environ Microbiol 63:596-601.
19. Seeger M, Jerez CA (1993) Phosphate-starvation induced changes in *Thiobacillus-ferrooxidans*. Microb Lett 108:35-41.
20. Tomati U, Galli E, Pasetti L, Volterra E (1995) Bioremediation of olive-mill wastewaters by composting. Waste Managem Res 13:509-518.
21. Wiegant WM, Wery J, Buitenhuis ET, Debont JM (1992) Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom *Agaricus bisporus*. Appl Environ Microbiol 58:2654-2659.