Bioresource Technology 158 (2014) 374–377

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Biodegradation of polyester polyurethane during commercial composting and analysis of associated fungal communities

Urooj Zafar [a], Petrus Nzeram [b], Adrian Langarica-Fuentes [a], Ashley Houlden [a], Alan Heyworth [c], Alberto Saiani [d], Geoff D. Robson [a,*]

[a] Faculty of Life Sciences, Michael Smith Building, University of Manchester, Manchester M13 9PT, UK
[b] School of Chemical Engineering and Analytical Science, Faculty of Engineering and Physical Sciences, University of Manchester, Manchester M60 1QD, UK
[c] The TEG Group PLC, Westmarch House, 42 Eaton Avenue, Chorley PR7 7NA, UK
[d] School of Materials, Faculty of Engineering and Physical Sciences, University of Manchester, Manchester M13 9PL, UK

HIGHLIGHTS

- Considerable degradation of polyester PU occurs during composting process.
- Enrichment of fungi occurred on the surface of PU during biodegradation.
- Community on the surface of PU was different from compost.
- *Thermomyces lanuginosus* was found as PU degrader.

ARTICLE INFO

Article history:
Received 7 November 2013
Received in revised form 20 February 2014
Accepted 22 February 2014
Available online 4 March 2014

Keywords:
Polyurethane
Plastics
Biodegradation
Compost
Thermophilic fungi

ABSTRACT

In this study the biodegradation of polyurethane (PU) during the maturation stage of a commercial composting process was investigated. PU coupons were buried in the centre and at the surface of a 10 m high compost pile. Fungal communities colonising polyester PU coupons were compared with the native compost communities using culture based and molecular techniques. Putative polyester PU degrading fungi were ubiquitous in compost and rapidly colonised the surface of polyester PU coupons with significant deterioration. As the temperature decreased, fungal diversity in the compost and on the surface of the polyester PU coupons increased and selection of fungal community on the polyester PU coupons occurs that is different from the surrounding compost.

1. Introduction

In 2011/12 in the UK, 6.7 Million tonnes of waste was collected by local authorities and 43% of household waste was recycled (Plastic Europe, 2012) including waste directed to commercial composting facilities. Composting is a natural self-heating process with an increase in temperature occurring due to microbial growth and respiration as organic substrates are utilised. Commercial composting has become an effective strategy for diverting green and food waste from landfill sites as it is cost effective, benign and has little negative impact on the environment (Finstein and Morris, 1970).

Polyurethanes (PU) are a group of heteropolymer plastics have a wide range of applications (Krasowska et al., 2012). Polyester PUs are known to be vulnerable to microbial attack as they contain ester linkages within the backbone of the polymer that are naturally vulnerable to esterases as first reported by Darby and Kaplan (1968). Previously, it has been reported that fungi are the predominant microbes responsible for the biodegradation of polyester PU *in situ* (Barratt et al., 2003) and fungal polyester PU biodegraders have been isolated from soil (Cosgrove et al., 2007).

Recently, it has been reported that a number of fungal isolates are able to degrade impranil (liquid dispersion of PU) including thermotolerant and thermophilic fungi (Zafar et al., 2013). In this study, the potential of a commercial composting process to degrade PU has been investigated. This study reports that there were substantial physical changes to PU during this process and monitored the diversity and development of the fungal community over

* Corresponding author. Tel.: +44 (0) 161 275 5048.
E-mail address: Geoff.robson@manchester.ac.uk (G.D. Robson).

http://dx.doi.org/10.1016/j.biortech.2014.02.077
0960-8524/

the surface of the buried polyester PU coupons using both culture-based and molecular analysis (TRFLP and 454 pyrosequencing).

2. Methods

2.1. Burial of PU coupons in compost

In situ biodegradation of PU coupons was carried out at a commercial composting site (The TEG group, Todmorden, UK). Input material consisted largely of domestic green and food waste with material added daily to the top of silo cages (temperature ca. 60–75 °C) and after 14 days mechanically removed from the base of the silos periodically. Material removed from the silos is used to form large (ca. 8–10 m tall) compost piles and allowed to mature further without turning for ca. 28 days during which the temperature gradually declines (Langarica-Fuentes et al., 2014).

Polyester and polyether PU coupons were fabricated and buried at approx. 0.3 m (surface samples) and 4 m (centre samples) from the top of a 10 m tall compost pile, as it was formed. After 2, 14 and 28 days, temperature at the centre and surface of the compost pile was determined and three replicate PU coupons were recovered. Control coupons were placed in sealed sterile Petri plates and incubated at 45 °C and 55 °C for up to 6 months.

2.2. Measurement of physical deterioration of PU

Physical deterioration of PU coupons was measured by the loss in tensile strength and percentage elongation at break. Compost particles were removed from the surface of the coupons with a soft brush, briefly rinsed with water and wiped with 70% (v/v) ethanol. Dumb-bells (total length 5 cm, width at the end 1.9 cm with 19 cm gauge length) were cut from the coupons using a moulder cutter (Wallace Test equipment, Birmingham, UK) and tensile strength and percentage elongation at break was measured according to Zafar et al. (2013). Differential Scanning Calorimetry (DSC) was used to monitor changes in the physical structure of buried polyester PU coupons by determining the glass transition temperature (T_g). Samples (10 mg) were excised, hermetically sealed and analysed over a temperature range of −90 to −220 °C at a rate of 20 °C/min (DSC Q100, TA instruments, Delaware, USA).

2.3. Identification of putative PU degrading fungi

For cultivation and molecular analysis, biomass from the surface of polyester PU coupons was recovered according to Cosgrove et al. (2007) after 2, 14 and 28 days after burial. Triplicate samples were pooled, agitated for 5 min and 1 ml of the microbial biomass suspension used for serial dilution. The remaining suspension was centrifuged at 3000×*g* for 30 min at 4 °C, the supernatant discarded and the pellet used for DNA extraction.

The biomass suspension was serially diluted in PBS and plated out on Polyurethane agar (PUA, Crabbe et al., 1994) incubated at 37, 45, 50 and 55 °C for the isolation of polyurethane degrading microorganisms with Impranil (Bayer, Newbury, UK), as a sole carbon source. All colonies produced a zone of clearing suggesting that all were capable of degrading PU.

2.4. Extraction, amplification and purification of genomic DNA from impranil degrading fungal isolates

Genomic DNA was extracted from the mycelium of fungal colonies isolated from the surface of PU coupons according to Feng et al. (2010). Isolates were identified using the 28s region of rDNA (Supplement section 1).

2.5. Analysis of the fungal community by TRFLP and 454 pyrosequencing

Community genomic DNA was extracted from biomass obtained from the surface of polyester PU coupons and from compost using Powersoil DNA isolation kit (MO BIO Laboratories, Carlsbad, USA). For fungal community analysis by TRFLP, fungal 18s rDNA region was amplified, digested and analysed according to Zafar et al. (2013).

For the study of pyrosequencing, fusion primers (HPLC purified) and High Fidelity PCR system were used and DNA was amplified according to Zafar et al. (2013). PCR products were verified and excised after running on a 1% (w/v) agarose gel (ca. 575–700 bp expected size range) using a sterile rectangular blade and DNA extracted using a gel extraction kit (Qiagen, UK). Products were further purified through column purification (Qiagen, Manchester, UK) and amplicons were pooled in equal concentration to give a final concentration of 10 ng/μl. Pooled samples were sent for 454 Titanium platform pyrosequencing to the Centre for Genomic Research, University of Liverpool, UK. For processing the sequences and statistical analysis of data, QIIME routine was followed according to Zafar et al. (2013).

3. Results and discussion

3.1. Effect of composting on the percentage elongation and tensile strength of PU

Polyester PU recovered from the surface of the compost pile after 14 days, showed a significant reduction ($P < 0.05$) in both percentage elongation (ca. 50%) and tensile strength (>70%) but no further significant reduction was observed after 28 days ($P > 0.05$). PU coupons recovered from the centre of the compost pile showed a similar reduction in tensile strength but no significant change ($P > 0.05$) was found in percentage elongation (Fig. 1B and C). While, 60% and 95% loss have been reported after 1.5 months burial in lab based soil microcosms (Barratt et al., 2003) and 5 months burial in neutral and acidic soil in the environment (Cosgrove et al., 2007), respectively. Composting has found as the most efficient method among soil burial, bench scale aerobic degradation and exposure to axenic cultures and estrolytic enzymes (Chiellini et al., 1996). It offers enhanced opportunity for co-metabolism (Williams et al., 1992), a rich organic matrix (Kästner and Mahro, 1996) and elevated temperature which aid in biodegradation (Tuomela et al., 2000).

On the other hand, polyether PU has been reported extensively as a recalcitrant polymer (Darby and Kaplan, 1968; Krasowska et al., 2012). Polyether PU dumb-bells from centre and surface of compost pile after 28 days showed no significant change ($P > 0.05$) in either tensile strength or percentage elongation (50.1 ± 4 to 43.3 ± 3 MPa and 1277 ± 65 to 1192 ± 41 respectively).

3.2. Effect of composting on the glass transition and melting temperatures of polyester PU

There was a significant ($P < 0.05$) decrease observed in the T_g value for the soft segment of the polyester PU coupons recovered from centre of the compost pile after 14 days but did not fall further after 28 days but an increase in the T_g of hard segment (Table 1). The substantial reduction in the T_g of the amorphous region would suggest an increase in amorphous state in contrast to the T_g of the hard segment which increased in the polyester PU coupons from the centre. The combined effect of the changes in the soft and hard segments may explain the rubbery but fragile nature of the coupons buried in the centre, since its flexibility

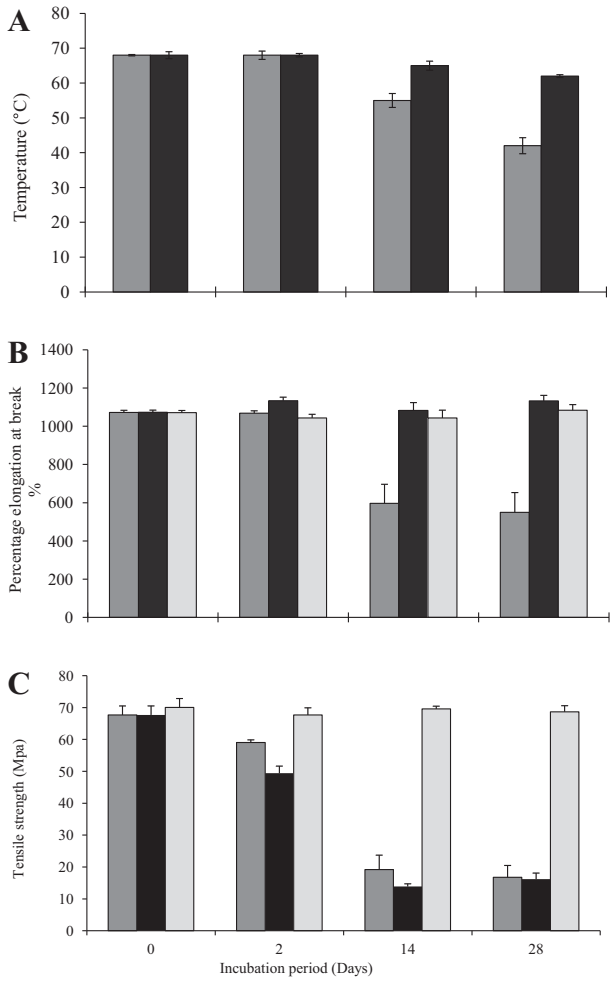

Fig. 1. Changes in the temperature of the compost pile and in the tensile strength of buried PU coupons over a 28 days period buried at 0.4 m depth from the surface (▒) and at the centre (■) of a compost pile at a commercial composting site. (A) Change in temperature. (B) Loss in percentage elongation of PU coupons. (C) Loss in tensile strength of PU coupons unburied control coupons were incubated at 55 °C (▒). Results are the means of 9 independent replicates ±SEM.

associated to the amorphous soft segment increased, whereas, physical crosslinks of the crystalline hard segment decreased. On the other hand, the reduction of the T_g and T_m of the hard segment and the more or less stable T_g of the of the soft segment of polyester PU coupons from surface would suggest a reduction in the crystalline phase of the hard segment, the crosslinks preventing plastic deformation, with a corresponding increase in its amorphous phase (Prisacariu, 2011). These differences between the samples at the centre and surface of the compost pile reflect that due to the temperature difference, bacteria and actinomycetes will be actively growing at the centre while at the surface where temperatures decline, fungi will be more active. The results were further justified by macro and micro images of PU coupons (data not shown).

3.3. Identification of isolates recovered from compost and surface of polyester PU coupons

Diversity in the fungal community recovered from polyester PU coupons buried at the surface of compost pile was dependent on the incubation temperature. At 37 °C, *Acremonium flavum* (JQ966574) and *Candida rugosa* (JQ966580), were consistant mesophilic species with dominant *Arthrographis kalrae* (JQ966577) on day 28. At 45 °C on day 2, the biomass obtained from the surface of buried polyester PU coupons were dominated by *Aspergillus* spp. and on day 28, a mixed community of *Lichtheimia* sp. (JQ966582), *Aspergillus fumigatus* (JQ966581) with occasional isolates of *Malbranchea cinnamomea* (JQ966583) and *Emericella nidulans* (JQ966573) were recovered. *A. fumigatus* and *E. nidulans* have previously been isolated as potential polyester PU degraders (Barratt et al., 2003). *M. cinnamomea* and *A. fumigatus* was also recovered from 50 °C. The major population at 50 and 55 °C was *Thermomyces lanuginosus* at all time points which is well characterized and is known to produce a variety of thermostable proteases, amylases, xylanases, ureases and lipases (Maheshwari et al., 2000) with some of these participating in polyester PU degradation (Howard et al., 1999). Polyester PU coupons recovered from the centre of the compost pile were mainly dominated by *A. flavum* and *C. rugosa* when plates were incubated at 37 °C, *A. fumigatus* at 45 °C and *T. lanuginosus* at 50 and 55 °C on day 28.

3.4. Comparison of the compost community with the community on the surface of polyester PU via TRFLP and pyrosequencing

TRFLP and pyrosequencing was used to study temporal changes in the fungal community in the compost pile and on the surface of buried polyester PU coupons. The number of TRFs recovered from compost (8, 8, 12) and from the surface of polyester PU coupons (10,13) taken from the centre of the compost pile remained relatively constant over 2, 14 and 28 days. In contrast, the number of TRFs detected from the surface of the compost pile (8,42,32) and polyester PU coupons (85,63) buried at surface of the compost pile increased over 2, 14 and 28 days. Interestingly, after day 28 the number of TRFs detected from the polyester PU coupons (63) buried at the surface of the compost pile was twofold higher than that detected in the surrounding compost (32). A significant increase in the Shannon index was observed in the compost samples from the surface of the pile from day 2 (1.8) to day 28 (2.78) corresponding to an increase in the diversity of the community and correlated with a decrease in the temperature of the compost. The assemblage of community (evenness) and diversity over the surface of the coupon remained fairly stable (0.59–0.61). An increase in the Evenness value (E) was observed from day 2 (0.65) to day 28 (0.75) in surface compost samples indicating that different members of

Table 1
Differential scanning calorimetry data of PU coupons recovered from surface and centre of the compost pile.

PU coupons recovered from compost pile	T_g[a] SS[c] (°C)		T_g HS[d]		T_m[b]HS (°C)	
	Surface	Centre	Surface	Centre	Surface	Centre
Unburied PU	−22.5 ± 0.5		68.5 ± 6		151.4 ± 5	
Day 14	−24.5 ± 0.9	−36.1 ± 2	55.3 ± 2.5	97.2 ± 1	113.7 ± 7	150.9 ± 3.5
Day 28	−24.4 ± 1.5	−36.0 ± 3	48.9 ± 5	97.2 ± 0.5	118.7 ± 4	150.9 ± 8

[a] T_g – glass transition temperature.
[b] T_m – melting temperatures (T_m).
[c] SS – soft segment.
[d] HS – hard segment, results are the means of 3 independent replicates ±sem.

the community are stabilising and getting proportional over time. TRFLP pattern generated accounted for 55% of total variance on PCA. The fungal community profiles clearly differed from the polyester PU samples buried at the surface of the compost pile after 14 and 28 days, there was less separation amongst the other samples (Supplement Fig. 1).

Following pyrosequencing analysis, total 55,430 sequences were obtained after denoising the samples, which were divided into 49 different OTUs that were shared between compost on day 0 and 28 and on the surface of polyester PU. Rarefaction curves levelled off and showed saturation of the libraries (data not shown). In agreement with the results of TRFLP, PCA of metagenomic profiles clearly separated the initial compost community (day 0) and the compost community after day 28 and the community on the polyester PU surface (data not shown). Good's estimate for all the samples were 100% with number of observed OTUs for compost day 0, day 28 and surface of PU coupons was 8.2, 15.6 and 22.0, respectively. The Shannon index of metagenomic profiles after 28 days on the surface of polyester PU (1.2) was less than compost on day 28 (2.1) but more than compost on day 0 (0.6). The ChaoI analysis suggested highest richness in community after 28 days on the surface of polyester PU (28.7), then compost on day 28 (17.2) with least in compost on day 0 (9.8). Sequences were associated with three phyla; *Ascomycota*, *Basidiomycota* and *Zygomycota* with the *Ascomycota* the most dominant phylum (Supplement Table 1).

T. lanuginosus, *A. fumigatus*, *A. kalrae* and *E. nidulans* that were cultured from the surface of polyester PU were also detected by pyrosequencing but at a low abundance. 454 pyrosequencing data suggested that *Thermomyces*, *Emericella*, *Lichtheimia* and *Aspergillus* are present but as a minor proportion of the total community, while the dominant fungus was identified as *A. kalrae* (Supplement Table 1). *A. kalrae* is a common pathogen ubiquitously found in soil and compost and has reported as having urease activity (Liu, 2011). The diversity demonstrated that distinct communities are present on polyester PU coupons, which are not dominant in compost at the same time point and shows selection for polyester PU degraders. Previously, Cosgrove et al. (2007) compared the community of PU coupons with soil and reported that PU community is the subset of the soil community because of the enrichment of the specie that colonises and/or degrades PU.

4. Conclusions

This is the first study to compare the dynamics of community on the basis of culture dependant and independent techniques in compost and on the surface of polyester PU coupons. This study suggests that the rate of degradation is enhanced under thermophilic and early maturation stage of commercial composting and that thermophilic and thermotolerant fungi have the capacity to cause significant polyester PU degradation. Thus, existing commercial composting systems designed to treat green and food waste have a potential to be modified to allow the input of PU as a possible alternative waste disposal strategy.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.02.077.

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