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Morphological Classification of Bioaerosols from Composting using Scanning Electron Microscopy

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Abstract:

This research classifies the physical morphology (form and structure) of bioaerosols emitted from open windrow composting. Aggregation state, shape and size of the particles captured are reported alongside the implications for bioaerosol dispersal after release. Bioaerosol sampling took place at a composting facility using personal air filter samplers. Samples were analysed using scanning electron microscopy. Particles were released mainly as small (< 1 μm) single, spherical cells, followed by larger (>1 μm) single cells, with aggregates occurring in smaller proportions. Most aggregates consisted of clusters of 2-3 particles as opposed to chains, and were <10 μm in size. No cells were attached to soil debris or wood particles. These small single cells or small aggregates are more likely to disperse further downwind from source, and cell viability may be reduced due to increased exposure to environmental factors.

Keywords: Bioaerosols, dispersion, SEM, particle size, aggregation

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25

1. Introduction

Bioaerosols are airborne particles of biological origin (Cox and Wathes, 1995), ranging from 0.02 to 100 µm in size (Dowd and Maier, 2000; Ariya and Amyot, 2004), including living microorganisms such as bacteria, fungi, yeasts and protozoans, or fragments and constituents of microorganisms (ADAS/SWICEB, 2005). Bioaerosols are released as a consequence of compost agitation activities (shredding, turning and screening), but do also occur naturally in the environment and exposure to bioaerosols is not limited to composting facilities (Dutkiewicz, 1997; Lacey, 1997; Nielsen et al., 1997; Reponen et al., 1998; Sánchez-Monedero and Stentiford, 2003; Seedorf et al., 1998; Swan et al., 2003). Under prolonged or acute exposure conditions, bioaerosols have the potential to pose health risks to immune-compromised or vulnerable humans, particularly where high concentrations are emitted close to residences, schools, hospitals and other public facilities (Environment Agency, 2007).

The physical and morphological characteristics of bioaerosols are central to understanding emissions and downwind dispersal from composting facilities. The behaviour of bioaerosols after release is governed by physical factors, including gravitational forces and Brownian motion, as well as environmental factors, such as wind speed, relative humidity and temperature (Pillai and Ricke, 2002). Bioaerosol properties such as size, shape, aspect ratio, surface characteristics and their affinity for aggregation, also affect their behaviour and are important factors in predicting their dispersal (Levetin, 1995; Madelin and Johnson, 1992; McCartney, 1994; McCartney et al., 1997). For example, a larger particle or aggregate might be subject

50 to higher deposition velocities than a smaller one (Wheeler et al., 2001; Swan et al.,
51 2003), with implications for the distance and time particles remain airborne (Pillai and
52 Ricke, 2002).
53
54 Research examining bioaerosol size distribution and aggregation from composting
55 emissions is limited. Kanaani et al. (2008) found that deposition rates for bioaerosols
56 and non-biological particles were a function of particle size, not the nature of the
57 particle. Byeon et al. (2008) found that aerodynamic diameters of microorganisms
58 were larger than expected and attributed this to the possibility that they were
59 suspended as aggregates with other bioaerosols and/or with dust particles. Feng et
60 al. (2011) claim that size and shape of bioaerosols can be clarified in real-time
61 environmental monitoring by means of analysing the special distribution of scattered
62 light, although their research is still requires further development.
63
64 Our research attempts to improve understanding of bioaerosol transport from source
65 to sensitive receptor. In an attempt to improve characterisation of aggregation and
66 size distribution of compost bioaerosols, experiments were undertaken to:
67 a) determine the size distribution of particulates released from compost and
68 composting facilities, and
69 b) examine and characterise the nature of bioaerosol aggregates released from
70 compost and composting facilities.
71
72 Images of microorganisms and their aggregates have been published before using
73 Scanning Electron Microscopy (SEM) from either pure cultures or from substrates
74 other than composts (Heikkilä et al., 1988; Klich, 2002; Kormendy and Wayman,

1972; Karlsson and Malmberg, 1989; Prescott et al., 1999a, b; Wittmaack et al., 2005). SEM has also been previously used as a technique for characterising morphological properties of small particles and bioaerosols (Friedbacher and Grasserbauer, 1995; Hiranuma *et al.*, 2008; Pasanen *et al.,* 1989; Skujins *et al.*, 1971; Williams, 1970). SEM was therefore chosen as the method to study bioaerosols emitted from compost.

2. Materials and Methods

Bioaerosols were initially sampled under controlled experimental conditions, with samples being analysed using SEM and through traditional culture techniques. These results confirmed the suitability of SEM as an analysis method and the presence of bioaerosols typically sampled from composting facilities, notably *Aspergillus fumigatus* (for further details see Tamer Vestlund, 2009).

2.1. Site sampling techniques

Samples were collected from a composting facility from a windrow (static source) using a wind tunnel and from agitation activities as described below (Jiang and Kaye, 2001; Taha et al., 2005). Particles were sampled in triplicate at a height of 1.8 m for a period of 30 minutes at ten locations around the windrows and screening area (one upwind; three at 10, 50 and 100 m downwind of the windrows respectively; two by the screening area, and two at source). Calibrated (with an SKC Ltd. rotameter) personal SKC (Universal dust and vapour) air filter samplers were connected to IOM sampling heads by a 10 mm internal diameter Tygon tube (Taha et al., 2006; 2007). Particles were collected on polycarbonate filters (SKC Ltd.) with 0.8 µm pore size and 25 mm diameter. Air was drawn through the sampling heads at a flow rate of 2 ±

100 0.2 L min^{-1} (SKC, 2002). After sampling the cassettes and filters were placed in a
101 sterile 30 mL Nalgene vial (121 ºC, 15 min) and stored in an ice-box at 4 °C for
102 transport. The filters had an effective exposed diameter of 15 mm.
103
104 **2.2. Scanning electron microscopy protocol**
105 The filters were mounted onto a 25.3 mm (diameter) SEM stub prior to gold coating
106 within 24 hours of sample collection (Polaron Equipment Ltd., SEM gold coating unit
107 ES100). The coated filters were examined with a high-resolution SEM (XL30SFEG,
108 Phillips; 10-12 kV beam size, 3-4 spot size) according to standard SEM practices.
109 Nine pairs of coordinates were selected for analysis (Figure 1) using a systematic
110 sampling design. Initial focus of the microscope was on the upper right edge
111 (x=6000, y=6000) of the filter at a magnification of x30 and then increased to x2000
112 when particles of interest (0.5 - 10 µm in size) were found. New viewing fields were
113 selected at each of the nine pairs of coordinates until ten fields containing at least
114 one particle were found for each pair of coordinates with 20 viewing fields around the
115 central set of coordinates to account for 100 viewing fields in total (Heikkilä et al.,
116 1988). The magnification was adjusted to ensure the visual properties of the particles
117 were sufficiently clear to analyse and record their number, size, shape, type of
118 particle, and aggregation status. Blank viewing fields, defined as fields with no
119 particles of interest, were also considered and recorded to calculate the total area
120 examined per filter. Blank viewing fields were scanned at magnifications of x500,
121 x1000 and x2000.
122
123 **Figure 1 here**
124

125 **2.3. Statistical analysis**

126 Description of the data was performed by arithmetic mean values and standard error
127 to measure variability, and a correlation analysis where required. One-factor ANOVA
128 and, where applicable, Fisher tests were used to analyse the differences between
129 independent data groups, using STATISTICA 8 (StatSoft Ltd.).

130

131 **3. Results and Discussion**

132 All SEM results shown correspond to the total area of 100 viewing fields (0.252 mm^2)
133 plus blanks scanned per filter as explained above. Filters (total area 490.8 mm^2
134 each) with low numbers of particles had a larger area analysed than those heavily
135 populated, resulting in an average of 0.19% of the filter being analysed.

136

137 **3.1. Particle size distribution and characterisation**

138 In this study, particles observed were classified as small (0.5 - 1 μm) and large cells
139 (2 - 3 μm). These were further classified into 8 different small cells and two major
140 large cell types according to their physical appearance (Table 1). Particles such as
141 filamentous and pollen-like particles (>10 μm), or those with no structure, were
142 considered to result from structural defects of the filters according to additional
143 analyses of filters that were not exposed to composting emissions (Tamer Vestlund,
144 2009).

145

146 **Table 1 here**

147

148 A wide variety of microorganisms is present in and released from compost. Michel et
149 al. (2002) identified over 94 species of microorganisms in green waste compost.

150 Similarly, Epstein (1997) listed 16 species of bacteria, 16 of actinomycetes and 35
151 species of fungi derived from compost. Although the presence of the bioaerosols
152 typically associated with composting (e.g. *Aspergillus fumigatus*) was confirmed by
153 culture (see Tamer Vestlund, 2009), difficulties in identifying particular species could
154 arise as sample preparation for SEM analysis results in the dehydration of the
155 sample that causes collapse and distortion of the image (Heikkilä et al., 1988).
156 Therefore, this research focused on the observable properties of bioaerosols (size,
157 shape and aggregation status), irrespective of the bioaerosol species.
158
159 Figure 2 shows the dominant cell types according to sampling position at the
160 composting facility. Cell type A was the most commonly occurring at all distances,
161 with types B and D also found in the samples taken at source. Cell type G was also
162 found in high proportions at 100m downwind of the composting facility. The overall
163 tendency was for small cells to occur in higher frequencies than the large cells in all
164 experiments, with the majority of particles present in the 100 viewing fields examined
165 from compost samples are in the 0.5-1 µm size range. The dominance of smaller
166 particles reflects previous research from compost facilities using Andersen 6 stage
167 samples. Reinthaler et al. (1997) found that 56-73% of all particles were smaller than
168 3.4 µm. Kamilaki and Stentiford (2001) found that 80% of all the *A. fumigatus*
169 captured on stages 3, 4 and 5 of an Andersen sampler were in the size range of 1.1
170 to 3.3 µm. Byeon et al. (2008) examined bioaerosols in a municipal composting
171 facility and reported concentrations of 10^8 CFU/m^3 total airborne particles sized 0.3
172 µm, which drastically decreased as the particle diameter increased. While not
173 directly comparable, these studies provide the only other published indications of the
174 size range of bioaerosols emitted from composting.

Figure 2 here

3.2. Aggregate size distribution and characterisation

Airborne microorganisms have been found in aggregates consisting of 2-6 spores in various environments (Bell et al., 2000; Karlsson and Malmberg, 1989; Lacey, 1991; Lacey and Dutkiewicz, 1976a, b; Levetin, 1995; Madelin and Johnson, 1992; Trunov et al., 2001). However, Figure 3 demonstrates that in all cases, single cells dominated over aggregates. The majority of cells observed for all sampling locations were small cells (66-99%); while their aggregates accounted for 1.4-30%. The proportion of single large cells and their aggregates are 1.3-6 % and 0.7-1.4 %, respectively. In addition, no aggregate structures were observed at 100 m downwind from the compost source, suggesting that aggregates drop out from the pollutant plume. Although, with a sampling height of 1.8 m, there is the possibility that the full pollutant plume was not sampled and aggregates may have disintegrated during the sampling process.

Bioaerosol survival rates within aggregates exceed that of single cells due to the protective effect of the outer layer for the inner cells (Carrera et al., 2005; Duncan and Ho, 2008; Lighthart and Schaffer, 1994; Marthi et al., 1990; Thomas et al., 2008; Tong and Lighthart, 1997). As most of the particles studied here consisted of single cells, it is conceivable that even if the particles were dispersed further downwind due to their small size, they will be less protected from environmental factors, and therefore cell viability could be reduced. This suggests that traditional culture

techniques often used for sampling downwind of composting facilities may underestimate the actual concentration of particles in the plume.

Bioaerosols have various release mechanisms. Filamentous structures or mycelia that extend above the growth substrate can become airborne as short chains, single spores or as fractions of mycelium (Gregory, 1973; Jankowska et al., 2000; Kanaani et al. 2008; Lacey, 1997; Madelin and Madelin, 1995; Pillai and Ricke, 2002). These can disintegrate into smaller sections and single spores, either due to release mechanisms or during sampling (Madelin and Johnson, 1992; Trunov et al., 2001). Single particles could also aggregate once airborne to make larger units (Calleja, 1984).

Based on the results, aggregates of cells were classified into clusters and chain-like structures, depending on either width or length. The vast majority of aggregates were clusters indicating that either a larger proportion of non-filamentous microorganism aggregates become airborne, or that cells are clustering into aggregates subsequent to release (Figure 4). Furthermore, small aggregates dominated over large ones regardless of their shape. Approximately 50% of the small aggregates had a diameter of < 2 μm in size, equating to aggregates of 2-3 cells based on the assumption that single cells ranged from 0.5 to 1 μm. Agitation produced more aggregates than static windrows ($p=0.005$; Figure 4). Aggregates of three or more cells were more abundant in samples from the source than in any downwind sample (Figure 4). No aggregates were identified in upwind samples, suggesting that the composting activities may have an impact on the formation of aggregates. It is also

223 possible that the sampling technique has impacted on the number and formation of
224 aggregates.
225
226 Several studies suggest that particles can be released as single cells, aggregates
227 and as cells attached to other particles such as dust or wood fibres (Swan et al.,
228 2003; ADAS/SWICEB 2005; Wittmaack et al., 2005). The results here do not
229 suggest that the release of bioaerosols is dependent on the release of matter such
230 as dust or wood fibres. However, only a small portion of each filter (maximum of
231 1.1%) was examined. There is therefore the possibility that these particles could
232 have existed in areas that were not examined or that the filters did not effectively
233 sample or retain wood fibres.
234
235 3.3. **Particle morphology**
236 The majority of the particles, both single and aggregated cells, were spherical in
237 nature with an aspect ratio of 1 (Figure 5). Gregory (1973) showed that the falling
238 rate of a particle due to gravitational forces is proportional to the square of its radius.
239 Furthermore, non-spherically shaped particles might fall more slowly due to an
240 increased surface drag that would result in a delay in deposition (Lacey, 1991;
241 McCartney, 1994; Levetin, 1995). Therefore, as the majority of particles observed in
242 this study were spherical or almost spherical (aspect ratio 1 to 1.5), the effects of
243 surface drag on bioaerosols is proposed to be minimal.
244
245 **Figure 5 here**
246
247 **3.4. Limitations of methodology**

248 SEM is able to provide accurate and detailed information on particle surface and physical
249 particle size; however the samples are prepared and scanned under vacuum conditions,
250 which causes dehydration, collapse and distortion of particles that might bias the actual size
251 and surface characteristics of the particle (Heywood, 1969; Skujiņš *et al.*, 1971; Gwaze *et al*.,
252 2007). Furthermore, due to the fact that only a very small percentage of the overall filter was
253 analysed, the results here are only a representation rather than absolute values of the overall
254 bioaerosol concentrations. The classification of the shape and nature of particles of interest
255 was based on subjective assessment. Similar limitations have been reported due to the
256 tendency of the operator to focus on more interesting particle features (Gwaze *et al.*, 2007;
257 Shekunov *et al.*, 2007)
258
259 **3.5. Implications**
260 Bioaerosol dispersion modelling could be an invaluable tool to estimate downwind
261 concentrations, particularly for regulatory compliance and in the design of control strategies.
262 Knowledge on the physical attributes of bioaerosols is thus crucial to provide confidence in
263 model outputs for composting facilities. A key decision for modellers is whether to model as a
264 particle or as a gas (Drew et al., 2007). However, there is currently insufficient information
265 available to fully define the particle properties within dispersion models. The results here
266 suggest that modelling as a gas would suffice, as the majority of particles found were small
267 enough for this to be a suitable option.
268
269 Studies on the health impact of airborne pollutants have shown that smaller particles
270 (<2.5 μm) are more likely to negatively affect sensitive receptors as they can
271 penetrate deeper into the lungs (Dockery et al., 1993; Levy et al., 2000; Schwartz et
272 al., 1996; Spengler and Wilson, 1996; Sturm, 2011). Thomas et al. (2008) argued

273 that a lower dose of aggregate particulates is required to initiate an adverse health
274 impact compared to non-aggregate particles, because aggregates contain higher
275 number of individual cells. This has important implications in determining a dose-
276 response relationship for bioaerosols.
277
278 **4. Conclusions**
279 To the authors' knowledge, this is the first study that has classified bioaerosols
280 emitted from compost according to shape and size. The results suggest the following
281 conclusions regarding bioaerosols from composting sites:
282 • The majority of bioaerosols released in this study were single cells, shaped
283 spherically or almost spherically, suggesting that they may disperse further than
284 heavier aggregate structures.
285 • Eight types of small (0.5-1 μm) cells and 2 types of large (1-2 μm) cells and their
286 aggregates were released from both static (i.e. compost windrow) and active (i.e.
287 agitation) compost sources.
288 • The majority of all aggregates consisted of 2-3 cells and were smaller than 10
289 μm. Again, these are more likely to disperse further downwind, but would not
290 benefit from the protection that larger aggregates would provide from
291 environmental factors.
292 • Aggregate structures were primarily released in clusters as opposed to chains.
293 • There were no aggregate structures observed at 100 m downwind from compost
294 source, or upwind, suggesting that composting facilities impact on the formation
295 of aggregates.
296
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