BIOAEROSOL MONITORING – A PRACTICAL EVALUATION OF METHODOLOGY

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Abstract

Bioaerosols are airborne particulate constituents that include microorganisms, cell components (e.g. endotoxins) and other inhalable particles (e.g. pollen). Whilst ubiquitous in ambient air, industrialised processes can increase bioaerosol levels which raises concerns for people working within the industry as well as local residents and businesses. Particular processes have been associated with specific bioaerosol constituents (e.g. *Aspergillus fumigatus* with composting and Legionella with waste water). Health concerns associated with inhalation/ingestion of particular bioaerosol components justify the need to evaluate risk to human health through monitoring of bioaerosol levels. Biomonitoring methods commonly use impingement, impaction and filtration methods. This study evaluated these different methods by direct comparison of bioaerosol sampling undertaken at different industrial process sites. Data from these case studies is presented and critically evaluated. The impingement method used was significantly more frequently effective in yielding higher counts than the filter method. It is concluded that the effectiveness of the method used is likely to be influenced by the nature of the environment of the industrial process being analysed.

Key words

Bioaerosol, monitoring, impingement, filter, air sampling, respiratory health, Aspergillus, gram-negatives,

Introduction

Naturally present in the environment, suspended particulates of biological origin such as bacteria, fungi, viruses or protozoa and their constituents such as toxins, spores, allergens, pollen, mycotoxins and β(1→3)-glucans are referred to as bioaerosols (Taha *et al.,* 2005). They can be suspended in the air as clumps, aggregates, single cells or cell fragments and due to their small size (typically less than 10µm in diameter), they are easily transported via wind. Their small size means that they can easily penetrate the lungs as they are not filtered out by hairs or specialised cells that line the inside of the nose (Taha *et al.,* 2006).

Many respiratory illnesses have been attributed to be caused or worsened by bioaerosols to include aspergillosis and asthma. Endotoxins, which are fragments of lipopolysaccharide from gram negative bacterial cell walls, can produce airway inflammation when inhaled as they are potent proinflammatory agents (Deacon *et al.,* 2009). Whilst some bioaerosol related illnesses can be acute, the main groups of people most likely to be affected are the very young, elderly, immuno-compromised, those exposed to high levels on a regular basis or people already suffering from some form of respiratory illness.

There are many sources of heightened outdoor bioaerosol levels associated with activities including wastewater treatment plants (Grisoli *et al.,* 2009) livestock breeding and farming (Herr *et al.,* 2003) and composting (Sanchez-Mondedero and Stentiford, 2003). Due to their variety in size when clumped together or attached to organic dust particles, plumes of bioaerosols can occur when levels are high whereby levels are super-concentrated in a particular timeframe. The source of bioaerosols will determine the key microorganisms present i.e. green waste composting and *Aspergillus fumigatus*, food waste composting and coliforms such as *Escherichia coli* and the wastewater industry, coliforms and potentially *Legionella pneumophila*.

Whilst most recent publicity has been concentrated on how current waste-based processes have an effect on the general public, more attention is now focused on people who regularly work at very close proximity to sources of bioaerosols such as compost facility operators (especially at in-vessel composting sites). Whilst there are Health & Safety concerns for the people working in industries associated with heightened bioaerosol concentrations, there are no dose-response levels in place to date making it difficult to assess the immediate risk to the health of the worker. However, with research on-going in trying to get closer to realistic threshold limits for bioaerosols, this paper looks at the effectiveness of different monitoring units to assess the levels exposed to. The most commonly used methods are impaction, filtration and impingement.

i. Impaction. This is where a known volume of air is impacted directly onto a nutritive agar plate via the use of equipment such as an Andersen sampler. The agar plates used are selective for the growth of the microorganism/s of interest e.g. Malt Extract Agar (MEA) for fungi and Nutrient Agar (NA) for bacteria (AfOR, 2009). The main advantage for this type of monitoring is that, assuming the plates are stored correctly before and after use until transferred to an incubator to allow growth of countable colonies, there is no subsequent laboratory based manipulation of the samples required which greatly reduces the chance of accidental contamination. Disadvantages for this method of monitoring are that sampling times and hence sample volumes, need to be low to achieve countable results and at extreme levels yield uncountable results. Additionally, unless many impactors are set up, only one media type can be used at a time making it difficult to look for correlations between different types of microorganisms (e.g. fungi and bacteria).

ii. Filtration. This method involves the drawing of a known volume of air directly on to a polycarbonate or quartz filter. A small sampling pump is used and the samples are collected on the filter housed in a sampling head such as that designed by the Institute of Occupational Medicine (IOM). Once the filter has been 'washed' in the laboratory, a solution is then available to process on multiple agar types so a total picture of microorganisms for a particular sampling timeframe becomes available. Longer sampling times can be used up to 60 minutes but any longer than this is not recommended so as to avoid in a loss of viability due to desiccation. Research into the survival of microorganisms on filters is scarce so more research should be undertaken into this survival issue.

iii. Impingement. Whilst filtration focuses on a sample collected onto a filter, impingement refers to a sample being collected directly into a fluid. Whilst longer sampling periods are an option with this method, there is a potential loss of viability due to evaporation of the

collection fluid so a maximum of 60 minutes is recommended for this method (Dart & Thornburg, 2008). As with the filtration method, a collected sampled can be processed on to multiple agar plates to provide additional data on the microorganisms present. If not stored at the correct temperature, an increase or decrease in viable cell numbers could give incorrect results so again more research is required in this area.

In order to facilitate comparison of data of similar sites from different assessors, a method should be standardised to provide some form of guarantee that the results are directly comparable. An example of this is the Environment Agency and Association for Organics Recycling publication, the Standardised Protocol for the Monitoring of Bioaerosols at Open Composting Facilities (AfOR, 2009). Similar approaches are needed for other types of sites and especially with regard to obtaining samples from around the breathing zone of workers exposed to high bioaerosol levels. To ascertain the most reliable methods to be used, a direct comparison of these methods is required that encompasses a range of bioaerosol levels and diversity of sampling sites. Whilst in its early stages, this research aims to provide a preliminary insight into three different sampling methods; impaction via Andersen samplers, filtration via a Casella Apex monitor fitted with an IOM sampling head and impingement via a CIP 10M monitor.

Methods

Sites

Bioaerosol sampling took place at four sites in England between November 2010 and September 2011. Site 1 was a medium sized open air windrow composting site that accepts green waste only. Monitors were set up on tripods at an approximate height of 1.7m. Site 2 was an industrial site that used refuse derived fuel (RDF) for energy. The monitors were again set up on tripods at around 1.7m in height. Site 3 was a small scale Country Estate green waste composting site and the monitors were worn by site operatives. Site 4 was an in-vessel composting facility where feedstock consisted of municipal solid waste. The monitors were worn by site operatives in addition to being set up on tripods. For all samples obtained, a Casella monitor was running at the same time as the CIP 10M so that a direct comparison of the results from each monitor could be made (Andersens were used for the first site only – site 2).

Impaction sampling

Andersen samplers were used to enable samples to be drawn directly onto agar plates. Two Andersens were fixed to a tripod to enable duplicate samples to be run concurrently and agar plates were positioned inside. Running at 28.3l/min, timeframes ranged from one minute to ten minutes. After each timeframe, the agar plates were removed and placed immediately into refrigerated storage where they remained until incubation at the laboratory. Sabouraud Dextrose Agar (SDA) plates for fungi/yeasts were incubated at 25°C for five days and Half-Strength NA plates for mesophilic bacteria and MacConkey No. 3 Agar (MAC3) for Gram Negative bacteria at 37°C for 48 hours.

Filtration sampling

Samples were obtained directly onto Whatman Quartz QM-A filters with the use of a pump operating at 2l/min. The filters were autoclaved prior to aseptically being transferred to an IOM

housing unit. The pump was secured onto the waistband of a site operative and IOM head onto the lapel of a shirt or jacket or mounted on a tripod. Sampling times were around 30 minutes after which the filters were aseptically removed from the IOM housing unit, placed into a sterile Petri dish and put into refrigerated storage.

In the laboratory, filters were individually transferred into 5ml of 0.9% sterile saline solution with 0.01% Tween 80 and shaken for a minimum of 20 minutes at 37°C. Each sample was then serially diluted and spread plated onto agar plates (MEA for *A. fumigatus*, Half-Strength NA for mesophilic bacteria, MAC3 Agar for Gram Negative bacteria and SDA for fungi/yeasts).

Impingement sampling

This method involved the collection of samples directly into 2ml of collection fluid (0.9% sterile saline solution with 0.01% Tween 80). A lightweight chest harness was worn and the CIP 10M sampler sat inside at the centre of the chest or was mounted on a tripod. The collecting fluid is housed within a 'cup' that when switched on, rotates at 7000 RPM. Air was drawn into the fluid at 10l/min and the sampling times were kept at around 30 minutes as per the filtration sampling. After the sampling timeframe, the cup contents were swilled out with an additional 4ml of collection fluid into a sterile container and placed into refrigerated storage. Prior to processing in the laboratory, each sample was removed from the refrigerator and allowed to reach ambient temperature for 2-3 minutes to avoid excessive temperature stress before serially diluting and plating onto agar plates as per the filtration method.

Viable counting procedures were carried out in accordance with the Standardised Protocol for the Monitoring of Bioaerosols at Open Composting Facilities (AfOR, 2009). For the filtration and impingement sampling, Half-Strength NA and MAC3 plates were incubated at 37°C for 7 days, MEA plates at 40°C for 48 hours and SDA plates at 25°C for 5 days prior to presumptive enumeration of the visible colony growth. Results were calculated as the number of colony forming units per cubic metre of air (cfu/m^3).

Results

Initially, samples were collected with the use of Andersen Samplers directly onto the relevant agar types but the concentrations of bioaerosols at the locations where sampling took place were too high for this type of monitoring. Flooding of the plates occurred which made it impossible to count the number of colonies present. In addition to this, there appeared to be contamination of the samplers due to the excessive bioaerosol levels thus making subsequent repeat sampling results inconclusive. Therefore, the Andersen sampling method was regarded as unsuitable for this level of sampling and the research was restricted to a direct comparison of the CIP impingement method and the quartz filter method.

Fifty seven bioaerosol samples were collected from four different sites that potentially posed a microbial risk to the health of on-site workers. Samples were enumerated for total viable mesophilic bacteria in addition to total viable fungi or *Aspergillus fumigatus*, and / or viable Gram Negative bacteria. The results for each site are presented in Tables 1-4. The RDF using site (site 2) and the Municipal Waste Composting site (site 4) yielded the highest microbial counts, an expected outcome

for indoor bioaerosols. Similarly expected, the smaller scale Country Estate composting site (site 3) gave lower results than the larger scale green waste composting site (site 1). The smaller scale Country Estate composting site (site 3) was the only site to give consistently lower results for the CIP impingement method compared to the filter method.

The data from all four sites was combined for comparative evaluation of the two sampling methods. Of all the 57 bioaerosol samples obtained from all sites, only three (5%) yielded no detectable microorganisms from either method. Whilst neither monitor showed consistently higher results than the other, the general trend was that the CIP impingement based monitor yielded higher bioaerosol counts than the filtration based method. Thus, 67% of the CIP microbial enumeration results were higher than the corresponding filter results (Figure 1a).

Tables 1-4: Comparison of bioaerosol data from two different samplers collected from various sites. Shaded background highlights the higher level of growth detected. NG = No (detectable) Growth

Table 1: Site 1 – Medium sized open air composting facility (green waste only)

	Mesophilic bacteria (cfu/m^3)		Total fungi (cfu/m^3)		*A. fumigatus* (cfu/m^3)		Gram -ive bacteria (cfu/m^3)	
	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter
Outside site office – no site activity	4.2×10^3	1.3×10^3	-	-	8.3×10^1	NG	-	-
Outside site office – no site activity	3.2×10^4	1.7×10^3	-	-	5.0×10^2	NG	-	-
Outside site office – during shredding operation	1.9×10^3	1.1×10^3	-	-	5.6×10^1	NG	-	-
Outside site office – during shredding operation	4.7×10^4	3.8×10^4	-	-	NG	NG	-	-
Outside site office – during shredding operation	3.8×10^3	2.6×10^3	-	-	8.9×10^1	NG		
Adjacent to windrows - no site activity	7.6×10^4	6.3×10^2	-	-	1.3×10^2	NG	-	-
Adjacent to windrows – no site activity	1.5×10^5	2.5×10^3	-	-	1.7×10^4	NG	-	-
Adjacent to shredder whilst in operation	6.2×10^4	2.6×10^4	-	-	6.2×10^4	4.0×10^4	-	-
Adjacent to shredder whilst in operation	8.0×10^4	1.2×10^5	-	-	3.1×10^4	7.4×10^4	-	-

Table 2: Site 2 – Industrial site using refuse derived (RDF) fuels

Sample type	Mesophilic bacteria (cfu/m^3)		Total fungi (cfu/m^3)		*A. fumigatus* (cfu/m^3)		Gram -ive bacteria (cfu/m^3)	
	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter
Loading bay during non-continuous RDF offloading	4.6×10^5	1.2×10^5	9.1×10^4	2.1×10^4	-	-	2.6×10^4	6.7×10^3
Loading bay during a period of no activity	1.2×10^5	6.6×10^4	2.7×10^4	3.6×10^4	-	-	1.0×10^4	7.0×10^3
Loading bay during offloading RDF and manual sweeping	1.6×10^6	7.2×10^5	7.7×10^5	3.1×10^5	-	-	1.3×10^4	1.1×10^3
Outdoors during minimal offloading (under cover)	1.2×10^3	5.6×10^3	3.3×10^2	5.5×10^2	-	-	NG	NG
Outdoors during minimal offloading (under cover)	8.9×10^2	2.1×10^4	3.2×10^2	5.0×10^3	-	-	NG	NG

Table 3: Site 3 – Small scale composting site (green waste only)

Sample type	Mesophilic bacteria (cfu/m^3)		Total fungi (cfu/m^3)		*A. fumigatus* (cfu/m^3)		Gram -ive bacteria (cfu/m^3)	
	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter	CIP 10M	Filter
In cab of front loader - moving windrow material	5.1×10^3	3.4×10^4	-	-	6.5×10^2	3.8×10^3	-	-
In cab of front loader - moving windrow material	5.5×10^3	1.4×10^4	-	-	9.9×10^2	1.0×10^3	-	-
Whilst manually applying final product to flower beds	1.9×10^4	7.2×10^4	-	-	2.6×10^3	3.6×10^3	-	-

Table 4: Site 4 – In-vessel municipal solid waste composting site

Sample type	Mesophilic bacteria (cfu/m^3)		Total fungi (cfu/m^3)		*A. fumigatus* (cfu/m^3)		Gram -ive bacteria (cfu/m^3)	
	CIP 10M	Filter	CIP 10M	Filter	CIP10M	Filter	CIP 10M	Filter
Reception area where waste mechanically sorted	7.2×10^4	3.5×10^3	1.2×10^4	1.3×10^3	-	-	5.0×10^3	1.1×10^3
Reception area where waste mechanically sorted	4.5×10^4	5.3×10^3	7.7×10^3	3.3×10^3	-	-	4.7×10^3	1.4×10^3
Maturation area - front loader moving material	2.6×10^6	4.7×10^5	6.2×10^3	1.1×10^3	-	-	2.3×10^3	1.3×10^2
Packaging area - front loader moving material	9.2×10^5	1.2×10^5	7.5×10^3	7.7×10^3	-	-	1.2×10^3	NG
In cab of front loader in maturation area	6.1×10^4	2.5×10^4	6.2×10^2	1.8×10^2	-	-	1.7×10^1	5.0×10^1
In cab of front loader in packaging area	7.2×10^4	5.0×10^4	1.2×10^3	8.5×10^2	-	-	1.0×10^2	1.5×10^2

For mesophilic bacteria testing, 74% of the CIP results were higher than the filter method (Figure 1b), with the most noticeable difference at Site 1 when the monitors were set up adjacent to windrowed material during a period of no site activity. For both timeframes monitored, the results for the CIP were 100-fold higher than the filter; 7.6 $x10^4$ and 1.5 $x10^5$ cfu/m^3 for the CIP compared to 6.2 $x10^2$ and 2.5 $x10^3$ cfu/m^3 respectively. When the filter results were higher than the CIP, the most noticeable instance was an outdoor sample taken at Site 2 where the results were 2.1 $x10^4$ for the filter method and 8.9 $x10^2$ for the CIP (Table 2).

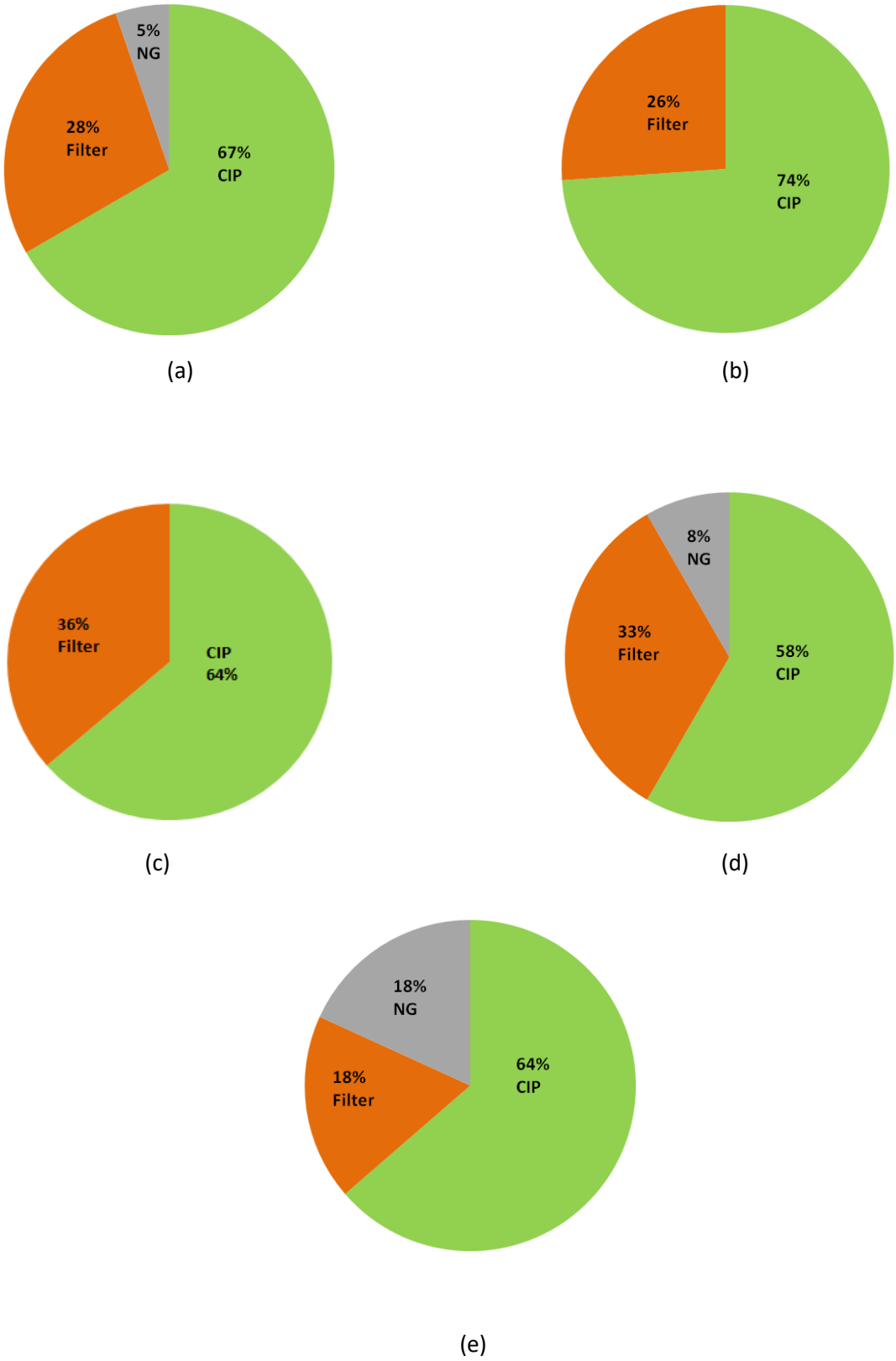

Figure 1: **Overall direct comparison of the CIP and filter methods for bioaerosol sampling. Percentage values represent proportion of samples that gave the higher viable count result for : (a) Overall comparison of all microbial analyses; (b) Mesophilic bacteria; (c) Total fungi; (d) *Aspergillus fumigatus;* (e) Gram Negative bacteria. NG= No (detectable) Growth**

The CIP results for total fungi were higher than the filter method on 64% of sampling occasions. 11 samples were collected in total and seven CIP samples yielded higher results than the filter and four filter samples higher results than the CIP (Figure 1c). No more than a 10-fold difference was noticed between the methods, e.g. at site 2 an outdoor sample for the filter method resulted in a count of 5.0×10^3 as opposed to the CIP result of 3.2×10^2 (Table 2) and in the reception area of Site 4, a result of 1.2×10^4 was recorded against a filter result of 1.3×10^3 (Table 4).

Results for *A. fumigatus* were more variable despite 58% of the CIP results being higher than the filter (Figure 1d). This was predominantly due to seven of the nine filter samples resulting in no detectable *A. fumigatus* growth at Site 1 yet six of these sampling periods did result in growth via the CIP method. Of the five *A. fumigatus* samples where growth was detected on the filters, only one CIP result, from a timeframe when the monitors were adjacent to an operational shredder, was higher than the corresponding filter result; 6.2×10^4 for the CIP and 4.0×10^4 for the filter (Table 1). All other recordable *A. fumigatus* levels were higher via the filter method than the CIP.

Of the 11 sampling locations where levels of gram negative bacteria were measured, two results (18%) detected no growth for either monitor. For the remaining nine locations, 64% of CIP results were higher than the filter method and 18% vice versa (Figure 1e). The highest difference in results was from the packaging area at Site 4 where no growth was detected via the filter method yet a level of 1.2×10^3 resulted from the CIP (Table 4).

Discussion

Although it is recommended that personnel bioaerosol samplers should be compared to common static systems such as the Andersen sampler (Kenny *et al.,* 2008) after the first monitoring exercise at site 2, the use of the Andersen samplers was discontinued. This was due to the high bioaerosol levels present which flooded the agar plates making the counting of individual colonies an impossibility after incubation even after a one minute sampling timeframe. Subsequent samples were also contaminated due to large particulate matter that had obstructed the inlet holes of the Andersen samplers.

Based on the use of the filter and impingement methods using the CIP monitor and IOM sampling head this preliminary research on these methods has revealed that in 67% of the sampling timeframes, the CIP yielded higher microbial results than the filter method. Whilst it could be argued that a sample collected directly into a liquid may encourage microbial growth due to the presence of organic dust particulates as a nutrient source, this possibility was substantially reduced by ensuring that as soon as samples were collected, they were stored under refrigeration conditions until processed (Li and Lin, 2001). This step would have significantly slowed down any metabolic reactions of the microorganisms. It is more probable that the lower filter results were due to desiccation during the sampling and subsequent storage (Gorner *et al.,* 2005). It is also feasible that entrapment of some microorganisms within the filter may mean that they were not released during the processing stage. It has been argued that desiccation concerns should prevent the general use of the IOM method with respect to bacteria if a culture-based analysis technique is used. This is

especially so if gram negative species are the most prominent as these are most susceptible to dessication (Haatainen *et al.,* 2005). In contrast, field trials of the CIP monitor are reported to have a comparative efficiency with existing devices comparable to impaction methods but can cope with much higher concentrations (Gorner *et al.,* 2005).

Whilst the general trend is that the CIP method resulted in higher microbial counts, the exception was at site 3 (a small scale Country Estate composting facility) where mesophilic bacteria and *A. fumigatus* were monitored for and all filter results were higher than the CIP. A possible explanation for this is that the compost feedstock was a more woody base and therefore a relatively dry product. Identification of specific fungal or bacterial species was beyond the scope of this research but it is possible that the bacterial presence was due to spore-formers such as *Bacillus spp* which are renowned for resisting desiccation. Similarly, the dry conditions may have preselected fungal spores that are resistant, or have adapted, to desiccation. Site 2 provided a variation in sampler efficacy, as determined by higher microbial counts, and this may be a consequence of resultant microbial variation caused by the diverse nature of the RDF materials being processed combined with the presence of high dust concentrations at this site. For this particular source of elevated bioaerosol levels, future work should concentrate on individual RDF sources so as to gauge more specific correlation between the two monitoring methods used in this study.

It is the view of the authors that the environmental conditions at the site of sampling influence the microbial state present. It is known that relative humidity and sampling times can affect the survival of airborne microorganisms. Whilst fungal spores are less affected by these factors, bacterial endospore (e.g. *Bacillus subtilis*) survival appears to decrease with decreasing humidity. Bacterial cells (*Pseudomonas fluorescens* and *Serratia marcescens*) are reported to be only culturable if sampled for ten minutes or less via the filtration method (Wang *et al.,* 2001). As only 26% of mesophilic bacteria results yielded higher results via the filter method, it would appear that bacterial viability is increased when samples are collected into a liquid due to reduced chances of desiccation. In the fungal results, there was a 10% increase in the filter method yielding higher results (36%). Using *Escherichia coli* and *B. subtilis* as representative bacterial cells and endospore formers, a previous study (Jensen *et al.,* 1992) found that *E. coli* viability was greatly reduced via a filtration method when compared to an impaction method, yet *B. subtilis* endospores resulted in comparable results for these two methods. Fungal spores and bacterial endospores are known to be more resistant to environmental stresses such as desiccation than vegetative bacterial cells, which could explain this finding (Maus *et al.,* 2001).

The extent of desiccation on filters is time dependent and a sampling time of less than one hour has been suggested so as to reduce the chance of loss of viability via the filter method (AfOR, 2009). In contrast a study concentrating on the detection of *Legionella pneumophila* (Chang *et al.,* 2010), it concluded that longer sampling times via the impingement method was a preferred method of quantifying *L. pneumophila*.

Conclusions

1. Overall, The CIP impingement based method gave higher results than the IOM filter method for both bacterial and fungal growth.

2. The success of the monitoring method is very much dependent on a range of factors to include but not limited to; humidity, dust levels, concentration of bioaerosols and the feedstock/source of bioaerosols.

3. Whilst the Andersen impaction method alleviates many concerns of subsequent contamination via analytical requirements, it is not a suitable method to use where bioaerosol and dust levels are high.

4. Loss of viability due to monitoring via the filtration method is a concern that should be explored further in more detail.

5. Further/extended research needs to be carried out into the effectiveness of personnel bioaerosol monitoring methods in order to ascertain the most reliable method to use.

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