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Bait and scrape: An approach for assessing biofilm microbial communities on organic media used for gas-phase biofiltration

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ABSTRACT

Gas-phase biofilters offer effective pollution control for agricultural effluents, but a better understanding of microbial communities responsible for capture and degradation is needed to improve process control. In this study, we developed a wood bait and optimized microbial biofilm sampling for monitoring microbial biomarkers (microbial C, ergosterol, DNA) in a full-scale biofilter. Results demonstrated that targeting biofilm dynamics required removing the biofilm from the wood substrates prior to biomarker extraction. We identified a sampling threshold for these biofilms of ≥100 mg for accurate and low variability biomarker measurement, a threshold that can inform analyses in other systems or using other approaches (i.e. DNA sequencing). Using this approach in a full-scale biofilter revealed that the fungal contribution (as ergosterol) to total microbial biomass was greatest in the most desiccation-prone area of the biofilter. This observation is in-line with results from previous lab-scale studies and could be due, in part, to connectivity between fungal hyphae growing in biofilms and the wood baits, shown by confocal microscopy. This work provides a targeted sampling strategy for microbial biofilms in gas-phase biofiltration, adaptable to other pollution control bioreactors and that can be used to study microbial community dynamics in full-scale systems.

1. Introduction

1.1. Focused microbial ecology can guide and improve biofilter performance

Bioreactors are an efficient alternative to traditional physical and chemical pollution control technologies (Delhoménie and Heitz, 2005). Unlike other technologies, bioreactors harness bacterial and fungal microbial communities to capture and degrade pollutants. The use of a diverse and specialized microbial consortium enables the simultaneous treatment of pollutant mixtures of variable concentrations, giving bioreactors great promise for aqueous (Schipper et al., 2010), and gaseous agricultural effluents (Nicolai et al., 2008). Despite their efficacy, low cost, and compatibility to most U.S. (Liu et al., 2014) and European farming systems (Hamon et al., 2012; Ubeda et al., 2013), bioreactor deployment has been hampered by unpredictable performance. Developing more reliable bioreactors for agricultural pollutant treatment will require an improved understanding of the microbial dynamics that underpin stable performance (Briones and Raskin, 2003; Cabrol and Malhautier, 2011).

For mitigating livestock emissions, biofilters are typically used. Biofilter are bioreactors which use microbial growths and their extracellular products attached to stationary reactor media surfaces – biofilms – to capture and degrade pollutants from a passing effluent (Mudliar et al., 2010). These systems can simultaneously treat volatile organic compounds (VOCs), hazardous odors (e.g. H_2S, NH_3), and greenhouse gas emissions (e.g. CH_4), but mitigation is highly variable with efforts to improve control hampered by inadequately understood, complex biofilm processes (Chen and Hoff, 2009). As an example of this complexity, degradation of odors VOCs by biofilm heterotrophic bacteria is facilitated by their nourishment with nitrite and nitrate, byproducts of ammonia-oxidizing bacteria (Kristiansen et al., 2011). But, it has also been shown that ammonia-oxidizing bacteria are spatially restricted in biofilters due to inhibition by their own byproducts and by competition with

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VOC-degrading heterotrophs for oxygen (Juhler et al., 2009). Thus, biofilter function is dictated by the same nutritional cross-feeding considered esential to the stability of microbial communities (Seth and Taga, 2014), suggesting the whole biofilm community may be more important to biofilter performance than the study of individual functional groups.

1.2. Fungal biomass in biofilms may significantly influence biofiltration

The role of fungi in supporting biofilter performance and resilience have been particularly overlooked (Ralebitso-Senior et al., 2012), despite their abundance in organic (Cardenas-Gonzalez et al., 1999) and synthetic media biofilters (Kristiansen et al., 2011; Xue et al., 2013). Several fungal attributes give these organisms unique potential to affect biofilter performance. These include: (1) their tolerance to desiccation (Kennes and Veiga, 2004) and acidification (Devinny and Ramesh, 2005), both common stresses to biofilter stability; (2) their specialized oxidative capacities, in many cases driving cometabolism of aromatic pollutants and lignin (Estevez et al., 2005; Jorio et al., 2009; Spigno et al., 2003), (3) their filamentous (hyphal) growth, which can extend into media pore space to increase the effective surface area interfacing with the effluent (Arriaga and Revah, 2005; Ralebitso-Senior et al., 2012 and van Groenestijn et al., 2001); and (4) their unique hyphal surface properties, which enable improved capture of emissions like hydrophobic volatiles (Kennes and Veiga, 2004; Rene et al., 2013).

In the presence of hydrophobic VOCs, fungal biomass has been shown to increase in hydrophobicity and surface area (Vergara-Fernandez et al., 2006). The fungal: bacterial ratio has also been shown to correlate with the capture of emissions with low dissolution (Prenafeta-Boldú et al., 2012). These characteristics of fungi could be harnessed to filter livestock emissions in confined housings,where high exhaust rates reduce residence time and increase the role for capture, not oxidation. To date, the importance of fungal biomass has not been explored in full-scale livestock emission biofilters and an adaptable biofilm monitoring approach tunable to various full-scale designs is required to enable such investigations.

1.3. Adapting monitoring general microbial biomarker approach for full-scale biofilters

Comprehensive monitoring of biofilter microbial communities demands high-throughput, to detect small spatial/temporal biofilm changes in large, heterogeneous bioreactors; this heterogeneity the result of bioreactor size, fluctuating emissions loading, and non-uniform organic media. Wood mulch is one of the most widely used media in US livestock production emission biofilters (Chen and Hoff, 2009). It is preferred for its low-cost, wide availability, moisture holding capacity, bulk porosity, and ability to support a diverse microbial community (Schmidt et al., 2004). Although wood mulch promotes robust biofilm formation, its heterogeneity complicating microbial sampling (Cabrol et al., 2010), so that large sample numbers are required to ensure abiotic and biofilm representativeness.

Furthermore, monitoring strategies must target the surface biofilm communities where effluents and biocatalysis are at an interface. Unlike biofilms on impervious media, biofilms on wood mulch are accompanied by active microbial populations within the medium. Thus, while some members of these communities are significant to biofiltration (i.e. facilitating pollutant sorption, biodegrading pollutants, supporting/antagonizing growth of biocatalytic groups), the significance of others inside the wood may be less direct (i.e. wood degrading saprobes). Separation of biofilm and wood microbiota would enable independent assessments of the impacts of their growths on pollutant biocatalysis and the interactions of these distinct populations.

One rapid, low-cost microbial monitoring approach suitable for high-sample numbers, biofilms, and wood microbiota is the detection of microbial biomarkers. Like any measure of microbial structure, biomarkers are not free of caveat and can only minimally resolve complex microbial consortia. However, microbial biomass measures can provide relevant functional information, as has been shown in many natural systems (Joergensen and Wichern, 2008) and in engineered biofilters, to isolate the role of fungi in capturing hydrophobic emissions (Prenafeta-Boldú et al., 2012). Moreover, rapid biomarker sampling to complement or target other deeper microbial community assessments has been demonstrated in lab-scale biofilter research (Cabrol et al., 2009; Prenafeta-Boldú et al., 2012).

1.4. Aims

There were three main intentions of this research. Our first objective was to design a bait system that would provide a relevant substrate for monitoring bacterial and fungal biofilms colonizing biofilter packing media. Our second objective was to optimize the harvest and sampling strategies for these baits to ensure repeatable, low variability extraction of biofilm microbial biomarkers. Our third objective was to evaluate the approach in a full-scale biofilter to verify our ability to track key biofilter microbes in the field. By developing a bait and retrieve method, a more reproducible monitoring of biofilms should be achievable than what has been accomplished using the typical 'grab' sample approach. By sampling in various areas of a biofilter, our study was geared to capture biofilm variability across biofilter packing. Biomarkers (microbial carbon (C), ergosterol) were chosen to monitor bacterial and fungal dynamics in biofilters for their functional significance in other systems, low-cost, high-throughput, and their potential to target more detailed molecular assessments.

2. Materials and methods

2.1. Design of wood biofilm baits

Wood baits were developed to enable uniform biofilm sampling in the heterogeneous biofilter media, similar to the "coupon" baits used to monitor biofilms in aqueous systems (e.g. Deines et al., 2010). Baits consisted of 3 birch (*Betula papyrifera*) wafers ($6.5 \times 2.0 \times 0.5$ cm) cut from dried lumber with the largest face tangential, strung to a numbered aluminum tag with fishing line (Fig. S1 in the Supplementary material). The wafer dimensions resembled the average biofilter media size distribution, and replicate wafers enabled replicate biomass measures.

2.2. Optimizing the harvest and sampling of biofilms

To identify a harvest strategy that targeted biofilms and to identify required biofilm sample sizes for biomarker assessment, a microcosm was used to generate sample material. The microcosm consisted of a gas-tight plastic tub packed with sieved wood mulch media (1 cm mesh) from a biofilter treating swine odor. This was plumbed to a 55 gal swine manure storage which generated a polluted effluent for a lab-scale biofiltration system (Fig. S2 in the Supplementary material). Average inlet emissions were 23.5 ± 13.5 ppm NH_3, 565 ± 660 ppb H_2S, 518 ± 53 ppm CO_2, 38 ± 26 ppm CH_4, and 404 ± 80 ppb N_2O ($n > 500$). Baits were incorporated into the tub and the contents were mixed and watered weekly to promote homogenous biofilm growth and to maintain wood moisture content between 40 and 60 wt.% dry (100 °C, 48 h).