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Microbial community changes during different empty bed residence times and operational fluctuations in an air diffusion reactor for odor abatement

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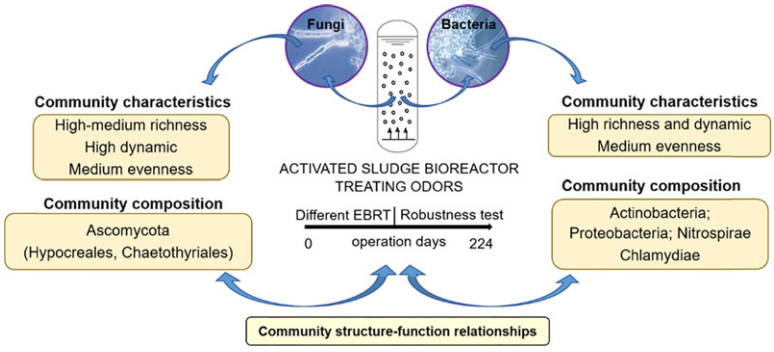
HIGHLIGHTS

- Microbial structure-function relationships evaluated in an odor treating bioreactor
- Evenness and dynamics supported functional stability of the bioreactor
- Actinobacteria and Proteobacteria were highly represented
- Fungi within Hypocreales and Chaetothyriales orders played a role in the process
- Typical H_2S and alpha-pinene degraders were not detected.

GRAPHICAL ABSTRACT



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ABSTRACT

The succession of bacterial and fungal populations was assessed in an activated sludge (AS) diffusion bioreactor treating a synthetic malodorous emission containing H_2S, toluene, butanone and alpha-pinene. Microbial community characteristics (bacterial and fungal diversity, richness, evenness and composition) and bioreactor function relationships were evaluated at different empty bed residence times (EBRTs) and after process fluctuations and operational failures (robustness test). For H_2S, butanone and toluene, the bioreactor showed a stable and efficient abatement performance regardless of the EBRT and fluctuations applied, while low alpha-pinene removals were observed. While no clear positive or negative relationship between community characteristics and bioreactor functions was observed, ecological parameters such as evenness and community dynamics seemed to be of importance for maintaining reactor stability. The optimal degree of evenness of the inoculum likely contributed to the high robustness of the system towards the fluctuations imposed. Actinobacteria, Proteobacteria and Fungi (Hypocreales, Chaeatothyriales) were the most abundant groups retrieved from the AS system with a putative key role in the degradation of butanone and toluene. Typical H_2S and alpha-pinene degraders were not retrieved from the system. The inoculation of *P. fluorescens*, a known alpha-pinene degrader, to the system did not result in the enhancement of the degradation of this compound. This strain was likely outcompeted by the microorganisms already adapted to the AS environment.

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1. Introduction

Changes of microbial communities (microbial succession) in natural and engineered ecosystems (bioreactors) in response to variations in operating or environmental conditions constitute nowadays a main issue in microbial ecology to predict system or ecosystem behaviour (Pholchan et al., 2013). Several characteristics of the microbial community structure seem to play a key role in maintaining functional stability under these variations. Among them, richness, evenness, dynamics, functional redundancy, microbial composition and microbial interactions seem to be of utmost importance for controlling reactor and ecosystem functioning (Bell et al., 2005; Cabrol et al., 2012; Wittebolle et al., 2009). For example, communities exhibiting intermediate evenness values (some species are dominant but most of them are present in decreasing lower amounts) have been shown to better deal with changing environmental conditions, since they have a pool of less dominant species able to replace the leading ones under operating or environmental fluctuations (Marzorati et al., 2008). Species richness effects on ecosystem functioning can decrease when functionally redundant species exist in the community (Bell et al., 2005).

The number of studies addressing this topic in bioreactors treating gas pollutants and malodorous emissions (with harmful effects on both human health and natural ecosystems) has increased during the last decade. Since biofilters and biotrickling filters are by far the most commonly implemented technologies for odor abatement, microbial-based studies have primarily focused on these systems (Cabrol et al., 2012; Lebrero et al., 2012, 2013; Prenafeta-Boldú et al., 2012). In addition to bacteria, fungi have been also investigated in biofilters (Prenafeta-Boldú et al., 2012), due to their ability to degrade complex organic pollutants and their superior performance compared to bacteria when present in media-based odor treatment bioreactors (Estrada et al., 2013; Harms et al., 2011).

However, emerging odor treatment technologies such as activated sludge (AS) diffusion bioreactors (based on the direct sparging of the malodorous emission into the aeration tank in wastewater treatment plants (WWTPs)) have been less studied from both a microbiological and engineering point of view. This technology represents a cost-effective alternative to biofilters and biotrickling filters due to it avoids problems related to packing media compaction, moisture control or accumulation of toxic metabolites. Nevertheless, the lack of reliable data concerning wastewater treatment performance during the abatement of volatile organic compounds (VOCs) from malodorous streams, and the lack of knowledge on the ability of AS systems to cope with process fluctuations and operational failures still limit its widespread application (Bowker, 2000). In this context, unraveling the structure and dynamics of the microbial communities (both bacteria and fungi) governing AS systems treating malodorous emissions under steady or transient operating conditions can contribute to prevent undesirable malfunction events.

This study was thus conducted to assess the temporal variation in the structure and composition of the microbial communities (bacteria and fungi) in an AS diffusion bioreactor treating a mixture of VOCs (toluene, butanone and alpha-pinene) and H_2S at low inlet concentrations (mg m^{-3}), which simulated a simplified odorous emission from a WWTP. The evolution of the microbial populations and their associated ecological parameters were correlated with bioreactor performance during the operation of the system at different empty bed residence times (EBRTs). The system was also evaluated during the analysis of AS robustness versus typical operational fluctuations.

2. Material and methods

2.1. Bioreactor set-up and physical-chemical analysis

The configuration of the AS diffusion bioreactor and the analytical procedures employed to monitor the system were described in detail in Lebrero et al. (2010, 2011). Briefly, the AS system consisted of a jacketed column with a working volume of 8.5 l operated at 20 °C. The reactor was inoculated with 1 l of concentrated (17 g L^{-1}) return municipal activated sludge from Valladolid WWTP (Spain) resuspended in a SO_4^{2-}-free mineral salt medium (MSM) to a volume of 7.5 l. The pH was maintained at 6.3 ± 0.3 by daily addition of a NaOH-Na_2CO_3 solution.

The inlet and outlet concentration of CO_2 and H_2S were analyzed using a GC-TCD (Varian CP-3800) and an electrochemical sensor (Dräger X-am 5000) calibrated in the 0–40 ppm range, respectively. Gas samples for VOC analysis were collected in 250 ml calibrated glass bulbs (SUPELCO) and pre-concentrated by SPME. VOC inlet and outlet concentrations were then determined by GC–MS according to Lebrero et al. (2010). The pH was measured using a pH/conductivity/temperature meter (pH 510 Eutech Instruments, Nijkerk, The Netherlands). Biomass concentration in the AS unit was estimated via culture absorbance measurements (optical density at 600 nm) in a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) and as total solids concentration (Lebrero et al., 2010). Sulfate concentration was determined by HPLC-IC using an IC-Pak Anion HC (150 mm × 4.6 mm) column. Finally, dissolved total organic carbon (DOC), dissolved inorganic carbon (DIC) and dissolved total nitrogen (DTN) were periodically recorded in the AS system using a TOC-V_{CSH} analyzer (Shimadzu, Tokyo, Japan) coupled with a TN module based on chemiluminesce detection (TNM-1, Shimadzu).

2.2. Bioreactor operation: Effect of different EBRTs and alpha-pinene addition

Details about the operation of the AS system under steady conditions can be found in Lebrero et al. (2011). A schematic representation of the different conditions applied to the reactor is shown in Fig. 1. Briefly, a mixture of H_2S, toluene and butanone with pre-humidified and filtered ambient air was fed to the bioreactor at concentrations of 16.9–23.8 mg.m^{-3}, 0.40–0.60 mg.m^{-3} and 4.3–6.3 mg.m^{-3}, respectively. During the first 121 days, the degradation rate of these compounds in the system was evaluated at different EBRTs (day 0 to 29: 94 s; day 29 to 43: 74 s; day 43 to 59: 55 s; day 59 to 95: 48 s; day 95 to 162: 32 s) in the absence of any additional carbon source and at infinite sludge retention time (SRT) (no biomass withdrawal). At day 95 (EBRT of 48 s), after observing a biomass aggregation and compaction in the reactor, glucose was added to the AS system, and the SRT was set-up at 25 days (340 ml of mixed liquor were daily withdrawn and replaced with fresh MSM containing 2 g L^{-1} of glucose) in order to simulate real WWTPs operation. At day 121 (32 s EBRT), alpha-pinene was supplemented to the previous gas mixture to evaluate the degradation rate of a hydrophobic odorant in the bioreactor. To enhance alpha-pinene biodegradation, a culture of *Pseudomonas fluorescens* NCIMB 11671, purchased from the National Collection of Industrial and Marine Bacteria (Aberdeen, Scotland), was inoculated to the bioreactor at day 132. The system was finally maintained at 32 s of EBRT until the end of the experiment (day 162).

The VOCs butanone, toluene and α-pinene were selected as model VOCs representing soluble, moderately soluble and hydrophobic compounds commonly found in WWTP emissions (Lehtinen and Veijanen, 2011; Zarra et al., 2008). H_2S was selected as model sulfur odorant, widely present in malodorous emissions from WWTPs and sewage works.

2.3. Bioreactor operation: Robustness analysis

To systematically evaluate the effect of the operational failures and fluctuations in the robustness analysis, the EBRT was set up at 50 s to ensure this parameter was not a limiting factor for bioreactor performance. The robustness test consisted of process fluctuations and simulated operational failures imposed to the AS system from day 163