The toilet malodor challenge

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Abstract

Globally, 2.5 billion people lack access to adequate sanitation. To help address this issue, Firmenich partnered with the Bill & Melinda Gates Foundation initiative: Reinvent the Toilet Challenge.

A receptor-based discovery program was developed to identify malodor antagonists and to bring affordable, novel and effective toilet cleaning and freshening products to global markets. When integrated into cleaning products, and used as part of a regular hygiene and maintenance regime, our malodor counteracting technologies aim to promote sanitary environments.

This presentation will focus on human waste and toilet malodor analysis. We will explain how pit latrine headspace analyses were performed in crowded slums in Africa and India. Based on the diverse volatiles found, rigorous sensory analysis allowed us to develop fecal reconstitutions using only four molecules. Hedonic appreciation of odors (like or dislike) is driven by diverse factors including cultural heritage. We therefore validated the authenticity of our reconstitutions via sensory surveys of more than 400 subjects in Switzerland, Africa and India. In the meantime, the four key malodorant molecules were used to identify odor receptors which were expressed and screened using a library of volatile organic compounds to identify potential antagonists. We recreated the exact toilet conditions in terms of temperature, humidity and ventilation in model latrine cabins. These cabins were equipped with devices that delivered malodors, including H_2S and methyl mercaptan. We concurrently monitored perfume release by solid supported delivery systems, analyzed the concentrations of antagonists in the air and conducted sensory analyses.

Introduction

Offending toilet malodors resulting from the action of environmental microbiota on human waste to produce volatile organic chemicals (VOCs) makes toilet/latrine use undesirable to populations more accustomed to defecating in the open.

The compounds responsible for fecal malodor have been well-known since 1878 when a Swiss doctor distilled 50 kg of human fecal material to yield a crystalline compound he named skatole [1]. The first analysis of odorant compounds from feces was published in 1987 [2]. Subsequently, Sato et al reported the first exhaustive analysis of odorant compounds from human waste sludge [3] and the analysis of fresh fecal odorants in Western style toilets [4]. Unfortunately, these studies were insufficient to help us answer key questions related to the toilet malodor challenge, specifically, what are the odor differences between conventional and urine diverted toilets and how different is the smell of an African pit latrine compared with an Indian toilet. Additionally, we couldn't find any documented quantitative headspace analysis of key latrine odorants.

The project was based on two approaches to the control of malodor. The first was a traditional fragrance engineering approach, where perfumers used psychophysical data and their experience to build a fragrance which combined with the malodor to produce an acceptable or even pleasant smell. The second approach relied on a new breakthrough

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technology based on the identification of molecules that antagonize temporarily but very specifically the odor receptors (OR) involved in the perception of the malodorous chemicals. Humans express an estimated 360 ORs in their noses, and according to the experiments performed within this project, only a small fraction of these receptors are activated by fecal malodors. This second approach was riskier, because it required the development of new technologies, but we anticipated that the final perfume including malodor antagonists would be considerably more performant. For the second approach it was critical to find the key malodorant compounds to identify which ORs were activated. It was also critical to simplify the screening protocols and for this reason we focused on four key malodorant molecules.

This presentation reviews the odors associated with different toilet systems in India and Africa. The analytical challenges of working with potentially pathogenic materials, in crowded informal settlements, is explained. The development of new tools to evaluate our new malodor counteractant technologies and their validation process are also discussed.

Experimental

The qualitative and quantitative analyses of latrine sludge VOCs by SPME and SPE was described in J. Environ. Sci. Technol. 2013 [5]. The quantification of H_2S, CH_3SH and other selected VOCs in toilet headspace was described in the same journal [6]. The sensory survey of reconstituted latrine malodors was described in Flavour Fragr. J. [7].

Results and discussion

1. Challenges to the analysis of human excrement and sludge in informal settlements

Analyzing urine is easier compared to feces because it is generally sterile, and, if not, can be easily sterilized using membrane filters [8]. Conversely, feces contain gut bacteria remains, proteoglycans, cellulosic fibers debris and can be contaminated by pathogenic species. While fresh stools can vary in consistency, they can usually be suspended in water and sterilized by ultra-filtration. Waste sludge, however, was a colloid and the membrane fouling made processing for analysis almost impossible. Consequently, SPME was gauged to be the most suitable analytical technique. Human waste, urine and feces were collected in a bucket which was covered with a lid into which a small hole had been drilled to allow insertion of a SPME fiber. The draw back was that the composition of VOCs was evolving. The action of ureases caused an increase in sample pH resulting in deprotonation of the organic acids which could consequently not be detected in the headspace. When fresh stools were analyzed without urine the VOC profile changed. Dynamic headspace was performed using Tenax cartridges but this technique had two drawbacks: we could not smell the extract, the rapid breakthrough of highly volatile compounds and finally we noticed an important diffusion of volatiles over time, even in closed tubes, which makes this technique not suitable for shipping Tenax tubes from Africa or India. Static headspace analysis using Porapak resin was better but the absorption process was slow, and it was not convenient to leave a device containing the resin hanging in the toilet without attendance overnight. In fact, many were stolen from the sampling sites. Finally, the analysis of the exposed Porapak resin, extracted with Et_2O was very instructive. It was possible to smell the headspace extract back in the lab with perfumers and to perform GC-olfaction to determine that four classes of compounds were key sludge odorants, namely sulfur-containing compounds, short chain fatty acids, phenols, indole and skatole (Figure 1).

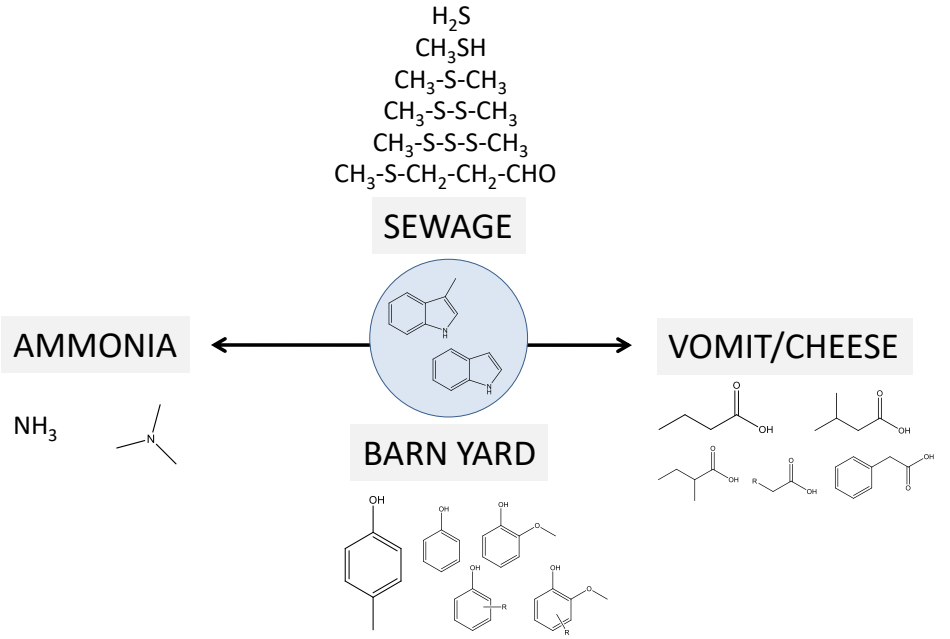

Figure 1: Schematic summary of VOC impairing toilet smell

Finally, sludge samples spiked with deuterated standards were analyzed in closed vials using SPME. The SPME fibers were thermally desorbed and analyzed by GC-MS in our labs in the USA.

2. Learning from the first analytical campaign

Latrine odors can be classified as resulting from anaerobic and aerobic microbial degradation. Typically, anaerobic latrines produced a strong H_2S, CH_3SH sewage odor. This was mainly the case for toilets in India connected to sewage pipes or in South Africa when the sludge was covered by rain or infiltrated water. Latrines equipped with efficient ventilation ports didn't smell much. Urine-diverted toilets systems smelled more barn-yard and stale urine.

Based on the VOCs detected during our study, the malodor was reconstituted using pure chemicals. It was possible to simplify the formula to four chemicals while retaining its authenticity. Dimethyltrisulfide was used as a proxy for methylmercaptan, which was difficult to handle, and indole was used instead of skatole which was declared carcinogenic [9].

The logical next step was to confirm that the reconstitutions were representative of toilet malodors. To achieve this goal, sensory surveys were conducted in Africa, India and Switzerland. Three bad smells, one complex fecal odor reconstitution, one fecal odor containing only the four compounds and one urine odor reconstitution were submitted to panelists along with three pleasant smells: banana, citrus, lavender.

From the sensory surveys the conclusion was that the fecal reconstitutions were both evaluated as latrine malodor and unpleasant. From these results we were confident that identifying ORs activated by the four key malodorant compounds, and subsequently

antagonists to the activation, would help us to design the first malodor counteractant prototypes based on antagonists.

3. Toilet headspace analysis

For the sensory analyses, we used smelling sticks that had been impregnated with the reconstituted latrine malodor. The real latrine smell was quite different from what we could smell from the smelling sticks. The reason was that H_2S and CH_3SH are very important contributors to the real odor and the partition coefficients of short chain fatty acids is related to sludge pH and matrix interactions. During our second campaign we attempted to precisely quantify key malodor molecules in Indian and African latrines.

This was achieved by bubbling 350 L of latrine air through water in order to trap the VOCs. H_2S and CH_3SH were derivatized using N-ethylmaleimide (NEM). The aqueous extract was loaded on a SPE-Oasis cartridge which was shipped to Switzerland. To abide by flight regulations, the water eluted from the first SPE-Oasis cartridge was not acidified with HCl but with acidic sulfonic resin and reloaded onto a fresh SPE-Osasis cartridge to quantify butyric acid. Recovery factors, reproducibility, limits of detection and limits of quantification were established and verified using olfactometers and certified diluted H_2S and CH_3SH standards (Figure 2).

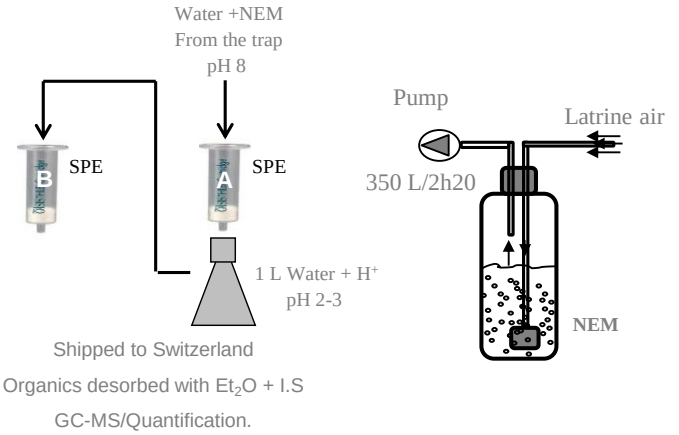

Figure 2: Schematic analysis of toilet headspace and quantification of H_2S and CH_3SH in addition to p-cresol, indole and butyric acid.

4. Recreating latrine conditions in the laboratory to assess perfume performance

Delivery systems can be used for extended release of and enhanced fragrance performance. When we obtained the first generation of malodor control prototype fragrances, we committed to evaluate their performance under realistic conditions. In the field many problems emerged. When cellulosic pads were used as delivery systems they were stolen and the citrus fragrance-containing pads were partially eaten. The air flows were totally different for different latrines, even those in the same block. When we conducted a sensory survey with local subjects we experienced all kinds of problems. For example, the toilet cleaning person cooked a curry in the ablution block, therefore the hedonic results were confounded, not due to the perfume but due to his cuisine.

For these reasons it was decided to build model toilet system using climate-controlled chambers where exact concentrations of malodors, at constant humidity and

temperature, were injected. Under these conditions the perfume prototype-containing delivery system performance was precisely evaluated by sensory analyses or by quantification of the VOCs in the headspace (Figure 3).

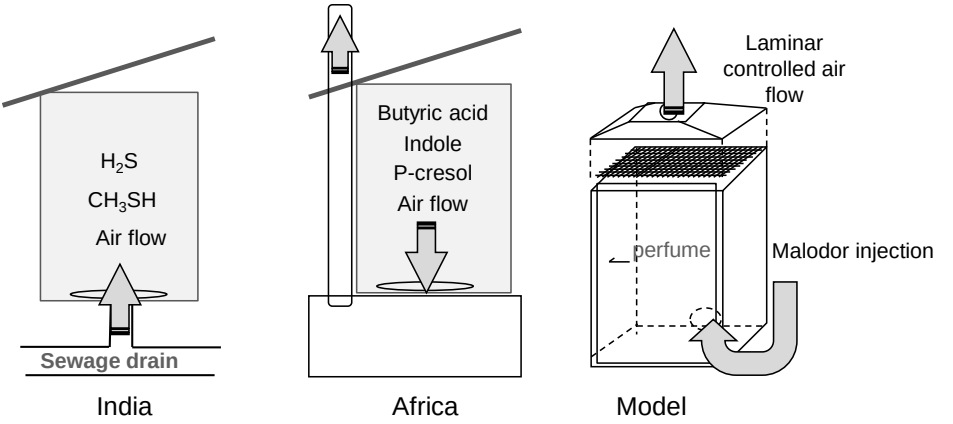

Figure 3: Trends in toilet systems visited. Arrows explain air flows.

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