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Mini Review: Cytochrome P450BM-3 in the Remediation of Semi-Volatile Fatty Acids Associated with Feedlot Odors

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Abstract. *A sound understanding of Animal Feeding Operations (AFOs) air emissions and their effects require the expertise of many disciplines. These disciplines may include a broad representation of academia, industry and local communities. Bioengineering is an emerging field that can provide new remediation methodologies by addressing the problem of chemical odor emissions from CAFO's. Development of methods for the reduction of organic compounds has the ability to move from monitoring and detection to air quality improvement. Utilization of metabolic pathways and the heterologous protein expression of Saccharomyces cerevisiae can yield cost effective methods for odor management at CAFOs. The metabolism of volatile fatty acids (VFAs) using Saccharomyces cerevisiae produced cytochrome P450BM-3 could possibly reduce downwind odors and assist in beneficial biogas production in wastewater effluents. The purpose of this review is to discuss a multiple disciplinary approach to air quality combining a variety of approaches which can assist federal agencies in successfully implementing a long term plan for this issue.*

Keywords. *Volatile Fatty Acids, Bioengineering, CAFO, Cytochrome P450,*

Introduction

Odor emissions from Concentrated Animal Feeding Operation (CAFO) wastewater storage ponds have been targeted as concern due to the air quality of local communities. Despite the lack of EPA air quality odor emission standards for CAFOs, monitoring and remediation of offensive animal odor emissions is an urgent priority for the livestock industry and the neighboring communities. A sound understanding of Animal Feeding Operations (AFOs) air emissions and their effects require the expertise of many disciplines. These disciplines may include a broad representation of academia, industry and local communities. Agricultural engineers, economists, biologists and chemists will be key in integrating and interpreting the implications of this recalcitrant issue (NRC, 2003). Abatement and air quality efforts lag behind those in odor detection and monitoring. There is a general paucity of credible scientific information on the effectiveness of mitigation technologies on concentrations, rates, and fates of air emissions from AFOs (NRC, 2003).

More than 7 million cattle are fed annually in Texas Panhandle feed yards, representing thirty percent of the nation's fed beef. These feed yards produce 18 million tons of wet manure annually, and generate $1.4 billion in annual income to the Texas Panhandle. The long-term sustainability of the cattle feeding industry is critical to the economy of the Texas Panhandle. Large AFO facilities are generally geographically concentrated, as well; almost 80% of the largest Texas feedlots are located in the Texas Panhandle. Almost one third of the cattle produced in confinement in the U.S. are fed within a 150 mile radius of Amarillo, Texas (TASS, 2000). Likewise, almost 75% of all hogs produced in Texas are also raised in the Texas Panhandle, (TASS, 1998) concentrating enormous quantities of animal waste in one geographical area.

The use of bioengineering and bioremediation techniques can yield an effective method for control of CAFO associated odors. *Saccharomyces cerevisiae* coupled with the heterologous expression of Cytochrome P450 can hydroxylate hydrophobic molecules, like VFAs, to less volatile products. This family of detoxifying enzymes catalyzes hydroxylation reactions using NADPH and O_2.

The specific purpose of this paper is to propose an application that addresses ambient air quality using a multidisciplinary approach. The use of agricultural engineering techniques in conjunction with principles from biochemistry and organic chemistry in a system approach can yield novel bio-remediation management practices. A multiple disciplinary approach combining a variety of approaches can assist federal agencies in successfully implementing a long term plan for this issue.

Characterization and Identification

Odor abatement and remediation from municipal, industrial and agricultural facilities have been a research focus for decades with few signs of success (Nicell, 1993; Wagner, 2002; Aitken, 1994; Stroot, 2001; McMahon, 2001). Human olfactometry has been augmented with gas chromatography/mass spectrometry-olfactometry (GC/MS-O), to quantify accurately the chemical compounds that are present in odor samples (Wright *et al.*, 2004, 2005). The use of solid phase microextraction (SPME) has been used to identify and characterize nuisance odor emissions surrounding CAFOs (Wright et al., 2005; Cai, 2005; Koziel, 2004). Sterilization techniques and removing chemical compounds that contribute to the overall odor over long distances have been the focus of odor management. Complete sterilization could have merit but would be cost prohibitive for most CAFOs. The possibility of removing only those odorants that have long-distance contributions appears to be a more achievable and cost-effective means of controlling downwind odor nuisance (Parker et al., 2005).

Beef cattle and manure produce volatile fatty acids (VFAs) through the process of fermentation of starches and proteins in their digestive systems or excreta. All VFAs are semi-volatile organic compounds (SVOCs). Because of the polar nature of VFAs they are more likely to stay in aqueous solution. From an air quality regulatory perspective, most VFAs meet the definition of volatile organic compounds (VOCs), as they have not been listed as exempt by the U.S. EPA in agriculture applications. Volatile fatty acids identified in CAFOs include acetic acid, propionic acid, butyric acid, isobutyric acid, hexanoic acid, and isovaleric acid (ASABE, 2005; Spinhirne *et al.,* 2002). These chemical compounds are listed as VOCs for regulatory purposes and are simular structure and behavior. VFAs are very water soluble and, as such, are found at high concentrations in aqueous media such as manure and liquid solution.

Novel Method

Bioengineering is an emerging field that can provide new remediation methodologies by addressing the problem of chemical odor emissions from CAFOs. Development of methods for the reduction of organic compounds has the ability to move from monitoring and detection to air quality improvement. Utilization of metabolic pathways and the heterologous protein expression of *Saccharomyces cerevisiae* can yield cost effective methods for odor management at CAFOs. Yeast cell surface display technology is a recent innovation that allows a protein to be produced inside a cell and attached to the outer surface of the same cell (Boder, 1997). The most important advantage of this technique is that the protein can be directly exposed to the targeted chemical compounds. Diffusional barriers that are inherent to cytoplasmic production of proteins and target compound accumulation inside the cell are not of concern using this technology.

Oxygenase enzymes catalyze the hydroxylation of organic substrates which can be applied to many potential applications in environmental remediation, toxicology and gene therapy (Nelson, 1996). The oxygenases (including the di-iron enzymes such as methane or toluene monooxygenases, the ubiquitous cytochrome P450 heme monooxygenases, as well as a large number of dioxygenases (Butler, 1997) involved in degradation of xenobiotics) nearly always function as large, multi-meric protein complexes. The ability to tailor the substrate specificities and to improve the activities, stabilities and expression of these enzymes using recombinant DNA techniques will greatly expand their utility in chemical synthesis, bioremediation, drug discovery and medicine (Joo et al, 1999).

Application of Cytochrome P450BM-3

P450 monooxygenases from microorganisms display a rather narrow substrate specificity. Substrates such as fatty acids and indolyl-fatty acid derivatives have been reported (Li, 2000). Li and Poulos (1997) discovered a self-sufficient P450 monooxygenase from Bacillus megaterium (P450 BM3) which the catalytic heme domain and the FAD containing reductase domain are naturally fused. P450 monooxygenase has been functionally expressed in *Escheriachia coli* and is conveniently prepared and purified in gram quantities (Schwaneberg et al., 1999).

The cytochrome P450 monooxygenase (P450) superfamily of hemoprotein enzymes catalyzes the oxidation of a wide variety of organic molecules in both prokaryotic and eukaryotic organisms. The self-sufficient cytochrome P450 BM-3 enzyme from *Bacillus megaterium* catalyzes subterminal hydroxylation of saturated long-chain fatty acids and structurally related compounds. P450s have numerous physiological roles, including the metabolism of fatty acids (Porter and Coon, 1991; Ortiz de Montellano, 1986). Cytochrome P450 BM3 from *Bacillus megaterium* holds a unique position in the P450 superfamily. It is the only known prokaryote or eukaryote P450 system that is catalytically self-sufficient, possessing the heme-containing P450 domain which was isolated cytochrome P450 monooxygenase domain of P450 BM3 (N-terminal) fused to the flavin-containing reductase domain (C-terminal) in a single 119-kDa polypeptide. It is also the only known bacterial P450 that utilizes a single NADPH-dependent reductase system similar to microsomal systems (Narhi and Fulco, 1986).

B. megaterium P450 BM-3 is water-soluble in contrast to most other members of the P450 family which are multi-protein complexes attached to membranes and can be incorporated in a bioreactor using zinc instead of expensive NADPH as an external electron donor (Schwaneberg, 2000). The heme is bound on the proximal face by a cysteine and on the distal face by a weakly bound water molecule, creating a low-spin hexacoordinated ferric center. Distal face access can only occur along a long hydrophobic channel which has been shown to be the substrate binding site (Ravichandran et al., 1993; Hasemann et al., 1995). The known function is subterminal hydroxylation of saturated medium and long-chain fatty acids and the specific activity in this reaction is about 1000-fold higher than eukaryotic P450 monooxygenases of a similar specificity.

Future work needs to include implementation and testing of this system, then engineering the enzyme to improve performance. Innovative strategies for generating diversity and high-throughput screening technologies will make it possible to evolve multifunctional enzymes and even whole biosynthetic pathways (Petrounia and Arnold, 2000).

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Conclusion

Odor contributes to the nuisance experienced in areas surrounding livestock facilities. The microbial treatment of wastewater effluents for the remediation of odorous chemical compounds can improve air quality. The metabolism of VFAs using *Saccharomyces cerevisiae* produced P450BM-3 could possibly reduce downwind odors and assist in beneficial biogas production in wastewater effluents. The acid formation phase of an anaerobic effluent is key in converting some organics and inorganics to methane and usable nutrients for cropland. However, if a wastewater storage pond is stationary in this phase; many odorous compounds can be generated, to include VFAs. This interruption in the wastewater cycle can force the release of nuisance odors and decrease the nutrient potential of the wastewater and sludge in the pond.

The use of a cross disciplinary approach to AFO emissions is needed for long term success in air quality. The National Research Council (NRC, 2003) suggests USDA and EPA have not devoted the necessary financial or technical resources to estimate air emissions from AFOs and develop mitigation technologies. They also state the scientific knowledge needed to guide regulatory and management actions requires close cooperation between the major federal agencies (EPA and USDA), the states, industry and environmental interest, and the research community, including universities (NRC, 2003). Novel monitoring and detection systems still need development, and test applications of current abatement and management methodologies should be employed in the present.

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