1. DoD Grant # W911NF-10-1-0146 ($373,248) (2010-2014)

Title: Characterization of a novel naphthalene metabolic pathway in *R. opacus* M213 and comparative ecology of associated degradative gene(s) in contaminated military sites

Co-PIs and participants:
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Abstract:
Much of what is known of the biochemistry and genetics of bacterial naphthalene metabolism is based on Gram-negative species such as *Pseudomonas putida* G7 and its plasmid, NAH7. Pseudomonads are easily isolated by standard enrichment methods, but may not be representative of dominant pathways present in a given environment. A clear understanding of the fate of organic contaminants in the environment is dependent on an understanding of pathways by which these compounds are degraded by a community, and not merely by those that are easily isolated. Gram-positive *Rhodococcus* strains are present in many soils and have been shown to metabolize a range of aromatic hydrocarbons. Little is known of the pathways or genetics of naphthalene metabolism by *Rhodococcus* strains. Not surprisingly, genes from naphthalene metabolic pathways cloned from *Rhodococcus* strains are generally not closely related to their counterparts from *Pseudomonas* strains. We are interested in the genetics and biochemistry of naphthalene metabolism of *R. opacus* M213, isolated from a fuel-oil contaminated soil in northern Idaho, which exhibits a novel pathway for naphthalene metabolism and harbors a large linear plasmid (pNUO1, 750 kb). Previously described biochemical pathways for bacterial degradation of naphthalene include formation of salicylate as a key intermediate metabolite. Several lines of evidence indicate that this is not true for *R. opacus* M213: 1) M213 does not grow on salicylaldehyde or salicylate, but utilizes o-phthalate, 4 hydroxyphthalate, 3-hydroxybenzoate and protocatechuate; 2) metabolites collected at different time points during growth on naphthalene included o-phthalate and protocatechuate, but not significant amounts of salicylate; 3) oxygen uptake studies on cells induced with naphthalene indicated high activities against some of these intermediates, but not against salicylate; and 4) no salicylate hydroxylase activity was detected in cell free lysates. We propose to characterize this novel pathway through a combination of approaches, driven by four general hypotheses, that are supported by preliminary data. The proposed research, in collaboration with DoD/Army scientists, will facilitate the characterization of degradative microbiota, pathways and processes prior to successful restoration and rehabilitation of contaminated sites.

2. DoD Grant # W911NF-10-R-0006 ($568,472) (2011-2014)

Title: Coupling Phycoremediation of Military Wastewater Pollutants and Nutrients to Generation of Environmentally Sustainable Biobased Products

Co-PIs and participants:
Abstract:
The Obama Administration has projected that the US will need to construct 500 advanced bioenergy plants between 2011 and 2022 to comply with the Renewable Fuel Standard. However, the current bioenergy yielding crops are either not economically or environmentally sustainable. Biofuels production from algae grown in treated wastewater has been proposed as a viable strategy that can address both economic and environmental issues. In this regard, algae can grow and produce biomass rapidly, with doubling times on the order of one day or less. Additionally, algae can remove and sequester nutrients (phycoremediation) from wastewater, and produce biomass in a solid, harvestable form. After recovery of value-added products from algal biomass, the spent algal waste can serve as feedstock for methane and syngas production in an anaerobic digester. Despite the environmental and economic potential of algal products, however, the various stages of the process have not been well integrated. This is due, in part, to the multiple integrated stages of wastewater treatment and algal growth, and the wide range of scientific and engineering expertise needed, and includes topics as diverse as basic cultivation and strain collection, to processing and post-processing technologies. To fully realize the benefits of large-scale algal cultivation however, the growth efficiency of microalgae on wastewater must be improved and more value-added products must be obtained from the algal cultivation. To this end, this proposal will further integrate an ongoing collaboration between Florida Agricultural and Mechanical University (FAMU), Florida State University (FSU) and Army Research Laboratory to bring together expertise spanning the entire bioenergy value chain, including, basic organismal biology, microbiology, molecular biology, environmental, chemical, civil and mechanical engineering, infrastructure and the social dimensions of the sustainable energy economy to obtain a comprehensive understanding of the potential for coupling phycoremediation of military wastewater and the production of next-generation biofuels and other value added products. Our overall objectives will be to (a) perform feasibility, sustainability and optimization studies of an integrated wastewater treatment and algal growth process for wastewater remediation and production of value-added products (lipids, methane) and secondary metabolites for added environmental and economic benefits to the military, (b) study the chemistry and microbial ecology of open algal ponds to develop solutions to key environmental roadblocks that hinder algal growth in wastewater is accomplished, (c) study the use of spent algal sludge for methane production during anaerobic digestion, (d) model the environmental and economic benefit of this technology for deployment at multiple DoD wastewater facilities, and (e) train the next generation of under-represented minority scientists and engineers in DoD-relevant research areas and publish research with students in peer-reviewed scientific journals. This research will meet DoD goals by developing approaches to address two key problems facing the military and society at large: 1) need for a clean, carbon neutral biomass feedstock for biofuel production, and 2) need for a relatively inexpensive nutrient remediation technology. Funding will also facilitate technology building of the environmental biotechnology laboratories at this HBCU that will be beneficial by way of well-trained minority workforce for research specifically aligned towards DoD target areas in STEM.