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Support vector regression model of wastewater bioreactor performance using microbial community diversity indices: Effect of stress and bioaugmentation

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ABSTRACT

The relationship between microbial community structure and function has been examined in detail in natural and engineered environments, but little work has been done on using microbial community information to predict function. We processed microbial community and operational data from controlled experiments with bench-scale bioreactor systems to predict reactor process performance. Four membrane-operated sequencing batch reactors treating synthetic wastewater were operated in two experiments to test the effects of (i) the toxic compound 3-chloroaniline (3-CA) and (ii) bioaugmentation targeting 3-CA degradation, on the sludge microbial community in the reactors. In the first experiment, two reactors were treated with 3-CA and two reactors were operated as controls without 3-CA input. In the second experiment, all four reactors were additionally bioaugmented with a *Pseudomonas putida* strain carrying a plasmid with a portion of the pathway for 3-CA degradation. Molecular data were generated from terminal restriction fragment length polymorphism (T-RFLP) analysis targeting the 16S rRNA and *amoA* genes from the sludge community. The electropherograms resulting from these T-RFs were used to calculate diversity indices – community richness, dynamics and evenness – for the domain Bacteria as well as for ammonia-oxidizing bacteria in each reactor over time. These diversity indices were then used to train and test a support vector regression (SVR) model to predict reactor performance based on input microbial community indices and operational data. Considering the diversity indices over time and across replicate reactors as discrete values, it was found that, although bioaugmentation with a bacterial strain harboring a subset of genes involved in the degradation of 3-CA did not bring about 3-CA degradation, it significantly

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affected the community as measured through all three diversity indices in both the general bacterial community and the ammonia-oxidizer community ($\alpha = 0.5$). The impact of bioaugmentation was also seen qualitatively in the variation of community richness and evenness over time in each reactor, with overall community richness falling in the case of bioaugmented reactors subjected to 3-CA and community evenness remaining lower and more stable in the bioaugmented reactors as opposed to the unbioaugmented reactors. Using diversity indices, 3-CA input, bioaugmentation and time as input variables, the SVR model successfully predicted reactor performance in terms of the removal of broad-range contaminants like COD, ammonia and nitrate as well as specific contaminants like 3-CA. This work was the first to demonstrate that (i) bioaugmentation, even when unsuccessful, can produce a change in community structure and (ii) microbial community information can be used to reliably predict process performance. However, T-RFLP may not result in the most accurate representation of the microbial community itself, and a much more powerful prediction tool can potentially be developed using more sophisticated molecular methods.

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1. Introduction

Wastewater treatment (WWT) plants are increasingly tasked with addressing removal of specific contaminants like xenobiotics as a result of ever more stringent discharge regulations, and because these contaminants may disrupt regular process performance. For example, the state of Oregon in the United States recently revised water quality standards for toxic pollutants in surface waters (Oregon Department of Environmental Quality, 2011). This action will require WWT plants discharging into surface waters in the state to remove a wider range of contaminants to lower levels. However, current practice in WWT system design is more suited to the removal of broad category constituents like biochemical oxygen demand (BOD), which represents general organic substrates. The approach assumes that the microbial community involved in the biological treatment process has sufficient functional redundancy and diversity so that knowledge of the microbial community structure and its specific metabolic capabilities is unnecessary. This redundancy has been shown for broad-range processes like BOD removal and nitrification (Hien et al., 2011; Reid et al., 2008; Siripong and Rittmann, 2007; Wang et al., 2012). For example, ammonia oxidation, crucial to the nitrification process common in most wastewater treatment systems around the world, was functionally stable in reactors where the ammonia oxidizing community was dynamic (Siripong and Rittmann, 2007; Wang et al., 2012). This has also been shown in the case of BOD removal, where reactors with high community diversity performed similarly (Reid et al., 2008; Valentin-Vargas et al., 2012; Wang et al., 2011). However, functional redundancy may not be apparent in the case of specific processes in WWT, like the response to perturbations (Kaewpipat and Grady, 2002) and the mineralization of xenobiotics (Falk and Wuertz, 2010). Engineers would therefore benefit from a more detailed and specific knowledge of the role of microbial communities in biological wastewater treatment. An overall understanding of reactor dynamics must originate in understanding three components: (a) operational, environmental and design conditions of the reactor, (b) the microbial community in the reactor, and (c) reactor treatment performance in response to (a) and (b) and to their possible fluctuations.

While parameters that fall under (a) are often easily controlled or monitored, (b) is not as straightforward to control or monitor, requiring a reliable method to describe microbial communities. Several cultivation-independent methods exist to qualitatively describe microbial communities, of which terminal restriction fragment length polymorphism (T-RFLP) has historically been an effective method in terms of base pair resolution and lower labor intensity (Osborn et al., 2000; Schutte et al., 2008). A simple yet effective way to quantitatively describe microbial communities based on T-RFLP data is through the use of diversity indices. For example, the three diversity indices, richness (R), community dynamics (Dy) and functional organization (Fo), defined here as evenness, can be used to describe a microbial community using data obtained from any established fingerprinting or sequencing technique (Marzorati et al., 2008).

Once a microbial community has been adequately described, its relationship to function can be investigated. In this case, diversity indices may be used to relate the microbial community in bioreactors to process performance. Traditional statistical analyses in wastewater systems are gradually being replaced by artificial intelligence soft computing approaches such as artificial neural networks (ANN), fuzzy logic and adaptive neuro fuzzy–fuzzy interference system (ANFIS) and can be used to identify complex relationships between community structure and function. Such models are useful in modeling biological systems because they are able to account for nonlinear relationships that are not yet completely understood in theory (Chen et al., 2003; Huang et al., 2010). They have been used extensively to model full-scale WWT systems (Aguado et al., 2009; Civelekoglu et al., 2009; Hamed et al., 2004; Hanbay et al., 2008), and it has been demonstrated that these techniques are generally more satisfactory than those given by traditional regression equations (Civelekoglu et al., 2009; Hamed et al., 2004; Karthikeyan et al., 2005). Recently, Singh and colleagues used support vector regression (SVR) to predict the removal efficiency of

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