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Stability of full-scale engineered ecosystem under disturbance: Response of an activated sludge biological nutrient removal reactor to high flow rate condition



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ABSTRACT

Observations of microbial community changes in full-scale engineered ecosystems such as wastewater treatment plants are rarely available, and it has long been believed that in such large systems it takes time for the microbial community to adapt to new conditions. However, it turns out that the microbial community in such a large system is impressively resilient. In a full-scale activated sludge bioreactor, the flow rate was increased to 135% of the normal average flow and kept constant during a storm event. Based on 454 pyrosequencing results, the microbial community structure was observed to shift significantly within 24 h after the flow rate was increased, but recovered to its initial state within five days. In addition, no significant impact on the bioreactor effluent quality was observed during the five-days at the higher flow rate. With the influent loading, the solid retention time, and sludge settleability carefully controlled to mimic the experimental conditions, a BioWin simulation produced a biomass concentration and effluent quality consistent with experimental results. This indicates that the stability of the microbial community in an activated sludge bioreactor subjects to a flow rate increase can be predicted to some extent.

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1. Introduction

Biological wastewater treatment has now become the largest (by volume) biotechnology industry in the world (Mielczarek et al., 2012). It seeks to protect human health and the environment by removing organic compounds, nutrients (nitrogen and phosphorus) and other pollutants using microbes. The performance of a biological wastewater treatment process is determined by the activity of the microbial community present, and many critical process failures can be attributed to the disruption of this microbial community (Graham and Smith, 2004). Therefore, the stability of the microbial community under disturbances is essential for biological wastewater treatment. However, critical questions remain unanswered, especially in full-scale operation: How robust is the microbial community under disturbance? Are the outcomes after disturbance predictable, reproducible, and controllable? Understanding the relationship between the microbial communities and disrupting conditions should greatly facilitate the maintenance of stable performance under disturbance (Rittmann et al., 2006).

The stability of a microbial community under disturbance is comprised of resistance (no change after disturbance) and resilience (quick recovery after disturbance) (Shade et al., 2012). Stability can be examined in terms of composition and function. In most cases, microbial community is sensitive to disturbance (i.e., the community has low resistance) (Allison and Martiny, 2008). Community function was observed to be more resilient than community composition after a pulse (short-term, instant) disturbance, whereas the recovery of function and composition were almost the same after a press (long-term, continuous) disturbance (Shade et al., 2012). However, most previous evaluations of stability were based on soil microbial communities and experiments under laboratory conditions. In addition, a majority of the studies reported resistance and few studies explicitly measured resilience. Vuono et al. (2015) studied the shift of microbial community in a full-scale activated sludge wastewater treatment plant under press disturbance (changed solids retention time, SRT). However, a knowledge gap still exists with regard to the response of a microbial community to other disturbance in a full-scale wastewater

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treatment plant (WWTP), such as increased flow rate under storm events.

Activated sludge, the most widely used form of secondary wastewater treatment, is a collection of suspended biological flocs that consist of microorganisms, extracellular biopolymers, and organic and inorganic compounds (Metcalf Eddy et al., 2003; Sun et al., 2013). With the development of molecular techniques. especially next generation sequencing technology, it has become feasible to monitor the microbial community in full-scale WWTPs (Hu et al., 2012; Wang et al., 2012; Ye and Zhang, 2013). Variations in microbial communities in activated sludge are thought to be influenced by both deterministic and stochastic properties (Curtis and Sloan, 2006). Deterministic properties include reactor design, environmental and operational conditions (Curtis and Sloan, 2006), and are easier to control and monitor than stochastic properties. Operational parameters affecting microbial community dynamics include influent nutrient loading, dissolved oxygen, pH, temperature, SRT, hydraulic retention time (HRT), etc. (Cydzik-Kwiatkowska et al., 2014; Hong-Yan et al., 2006; Hu et al., 2012; Huang et al., 2008; Kim et al., 2005; Li et al., 2013; Rodriguez-Sanchez et al., 2014; Wang et al., 2014; Xu et al., 2014). The impact on the microbial community of an increased influent flow rate is a combination of changes in nutrient loading, SRT, and HRT. It has been reported that with a fixed SRT and influent concentration, an increased flow rate (i.e., increase in organic loading and decrease in HRT) results in elevated biomass concentration and improved nitrogen removal in a membrane coupled sequencing batch reactor (MSBR) (Xu et al., 2014). With a relative stable nutrient loading rate and uncontrolled SRT, an increased flow rate (i.e., decreased HRT) leads to improved nitrogen removal kinetics (Li et al., 2013). However, the flow rate increase affects various microbial groups differently (Hong-Yan et al., 2006; Li et al., 2013). The fraction of nitrite-oxidizing bacteria (NOB) increases significantly and the increase in nitrite oxidation is also more significant than ammonia oxidation (Li et al., 2013). The effect of increased flow rate on the removal of slowly degradable pollutants, such as antibiotics or other complex chemicals, is less significant than on ammonia removal (Huang et al., 2008; Kim et al., 2005). With a fixed SRT, the effect of a decreased HRT is insignificant, while a decreased SRT significantly reduces pollutant removal. The effect of increased flow rate has rarely been evaluated in full-scale WWTPs because a significantly increased flow rate in a full-scale WWTP cannot be easily maintained during routine operation.

This study examined the response of the microbial community to a significantly increased influent flow rate in a full-scale WWTP. Both reactor performance and microbial community profile were monitored. Dissolved chemical oxygen demand (COD), ammonia, nitrate, and orthophosphate phosphorus, were monitored in a series of locations in the bioreactor. Biomass concentration and sludge settleability were also examined. Also, 16S rRNA gene-based 454 pyrosequencing was undertaken to track variations in the microbial communities in each zone during the flow rate change. A BioWin model was constructed to simulate the performance and biomass dynamics of the full-scale WWTP under increased flow rate. Modeled results were compared with the experimental data.

2. Materials and methods

2.1. Sampling

Samples were taken from Bioreactor #1 in a local Waste Water Treatment Plant (Edmonton, AB, Canada). A single bioreactor has an average designed capacity of 31 ML/d and a peak treatment capacity for a short duration (2-4 h) of 42 ML/d; this is defined as the normal flow condition. A storm event provided the opportunity to maintain the flow rate to Bioreactor #1 at 42 ML/d for five days (24 h/d). Samples were collected at 1:00 p.m. every day during the high-flow test. For tests on seasonal variations, samples were collected on three days in each season. Samples (1 L) were collected from nine locations in Bioreactor #1, as shown in Fig. 1. A depth sampler was used to obtain samples at a depth of 1 m underwater.

2.2. Reactor performance analysis

After 30 min settling, the sample supernatant was filtered with a syringe filter (0.45 μ m pore size) before measurement of dissolved COD, ammonia, nitrate, and orthophosphate phosphorus. The concentrations of COD, ammonia, nitrate, and orthophosphate phosphorus were measured using Hach methods 8000, 10,205, 10,020, and 10,209, respectively. The sludge volume index (SVI), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were measured according to standard methods (Racz et al., 2010).

2.3. Microbial community analysis

Activated sludge samples were collected in duplicates from each zone in the bioreactor and genomic DNA was extracted using a Powersoil[®] DNA Isolation Kit from MO BIO Laboratories, Inc. (Carlsbad, USA).

Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) based on the 16S rRNA gene was performed at the Research and Testing Laboratory (Lubbock, TX, USA), using the Roche Titanium sequencing platform. Primers 28F (5'-GAGTTTGATCNTGGCTCAG-3') and 519R (5'-GTNTTACNGCGGCKGCTG-3') were used, which covered V1-V3 hypervariable regions (Campbell and Kirchman, 2013). Chimeras and poor guality sequences were removed from the denoised sequence reads. The remaining sequences were clustered into operational taxonomic units (OTUs) with 0% divergence using USEARCH. Taxonomic information was assigned to OTUs based on a database of high quality sequences derived from the NCBI using a distributed. NET algorithm that utilizes BLASTN + (Kraken BLAST, www.krakenblast.com). Identity cut-off for classification at the family level was 90-95%. A principal coordinates analysis (PCoA) of microbial community diversity was performed using the QIIME pipeline (http://qiime.org/) with the beta diversity metrics of weighted unifrac (Crawford et al., 2009).

qPCR was used to quantify ammonia oxidizing bacteria (AOB) and NOB. A CFX 96 real-time PCR system with a C1000 Thermal cycler (Bio-Rad Laboratories, Inc.) was used to run the reactions; using 10 μ L of SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Inc.), 6 μ L of sterile water, 10 pmol of each primer, and 2 μ L of DNA



Fig. 1. Configuration of Bioreactor #1 and sampling locations numbered in the order from inlet to outlet. VFA: volatile fatty acids; RAS: return activated sludge.

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