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Shifts in microbial community in response to dissolved oxygen levels in activated sludge

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HIGHLIGHTS

- Wastewater treatment was analyzed at three DO levels; 1, 2 and 4 mg l^{-1}.
- Degradative efficiency was observed to vary between 60% and 65% at 4 ppm DO.
- Shifts in bacterial diversity were compared across different DO.
- Analytical and bioinformatics tools were used to assess degradative capacity.

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ABSTRACT

This study evaluates the degradative efficiency of activated biomass collected from a Common Effluent Treatment Plant (CETP) under three different dissolved oxygen (DO) levels, 1, 2 and 4 mg l^{-1}. The change in bacterial diversity with reference to DO levels was also analyzed. Results demonstrate that degradative efficiency was the highest, when the reactor was maintained at 4 mg l^{-1} DO, but amplicon library analysis showed a greater diversity of bacteria in the reactor maintained at 2 mg l^{-1} DO. Bacteria belonging to the order Desulfuromonadales, Entomoplasmatales, Pasteurellales, Thermales and Chloroflexales have only been detected in this reactor. Ammonia and nitrate levels in all three reactors indicated efficient nitrification process. Results of this study offer new insights into understanding the performance of activated biomass vis-à-vis microbial diversity and degradative efficiency with reference to DO. This information would be useful in improving the efficiency of any wastewater treatment plant.

1. Introduction

The most common aerobic strategy for treatment of wastewater is the activated sludge process. Activated Sludge comprises of highly complex microbial biomass made of eukaryotes, bacteria, archaea, and viruses, in which bacteria are dominant and play an important role in the removal of organic pollutants (Rani et al., 2008; Yu and Zhang, 2012; Winkler et al., 2013). Parameters related to bacterial growth and degradation are mainly, the availability of nutrients and dissolved oxygen (DO). DO is a relative measure of the amount of oxygen that is dissolved in wastewater, and it usually fluctuates seasonally and varies with water temperature and altitude. DO levels govern the rate of degradation of the organics in aerobic growth physiology of microbial communities in any wastewater treatment plant (WWTP) and also contribute to operational costs (Kapley et al., 2001; Wells et al., 2009). Different levels of DO can also affect the nitrification, denitrification, besides, aerobic reactions, behavior and activity of heterotrophic and autotrophic microorganisms. A low DO adversely affects treatment efficiencies of bulk wastewater volumes, especially in industrial effluents, coming from diverse sources. On the other hand, an excessive DO concentration will lead to unnecessary power consumption. In the aeration tank, the required aeration depends on the actual oxygen demand and the oxygen transfer efficiency. The actual oxygen demand is determined by the amount of pollutants oxidized and biomass produced, while the oxygen transfer efficiency is related to aeration devices, operational DO, temperature, sludge property, and MLSS concentration.

Understanding the bacterial diversity present in a wastewater treatment plant and analyzing community shifts with change in operational parameters, is the key to develop successful treatment strategies. There are a large number of reported methods to study bacterial diversity; amplification and sequencing of 16S rRNA gene, Random Amplified Polymorphic DNA analysis (RAPD); (Kapley et al.,

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2007; Li et al., 2012; Sharma et al., 2013), T-RFLP (Felföldi et al., 2010), PCR-DGGE (Lyautey et al., 2005; Yao et al., 2010; Liu et al., 2012, 2013). Each of these has been used successfully, but next generation sequence technologies have widened the scope of data generation and allow us to view this picture in greater detail.

This study explores the change in microbial community profile at different DO levels in activated biomass collected from a Common Effluent Treatment Plant (CETP) and compares the change in treatment efficiency with change in DO. The microbial diversity of activated biomass was analyzed under three different DO levels to answer the following questions; (1) How much does the wastewater treatment efficiency vary with change in DO? and (2) How does DO affect the microbial community of the activated biomass? Keeping all operational parameters and nutrient levels constant, COD, ammonia and nitrate levels were monitored in all three reactors as a measure of treatment efficiency. 16S amplicon library was prepared and taxonomic analysis carried out to analyze community shifts based on DO variations. The distribution of bacterial phyla under different operational conditions can be linked to performance of wastewater treatment plant. This study aims to generate a knowledge base that can be used to enhance treatment efficiency in industrial wastewater treatment and define optimum DO conditions that correlate to maximum degradative efficiency.

2. Methods

2.1. Source of wastewater and activated sludge

Wastewater and activated biomass used in this study was collected from a CETP, treating wastewater from pharma, chemical and dye industries. This CETP runs at a capacity 2500–3000 m^3/Day. Sampling of activated biomass was carried out from the aerator unit and both the biomass and wastewater (inlet) was immediately brought to the laboratory within 12 h.

2.2. Reactor design and setup

The activated biomass collected from the CETP was added into three reactors having total volume of 5 L and working volume of 3 L each. The addition of biomass in each reactor was 3000 mg l^{-1}, maintaining the MLSS (mixed liquor suspended solids) concentration being operated at the CETP. Air inlet into the reactors was maintained with the help of a rotameter and the reactors were run at 1 mg l^{-1}, 2 mg l^{-1} and 4 mg l^{-1} DO and will be henceforth referred to as Reactor A, Reactor B and Reactor C respectively. The oxygen concentration in the reactors was monitored using an oxygen meter and probe (Yellow Springs Instrument Co., Model 85). All three reactors were fed with wastewater from the CETP and the HRT (hydraulic retention time) was maintained at 3 days to mimic conditions operating at the CETP. Wastewater was collected in bulk so as to maintain a constant influent throughout the period of study. A schematic diagram of the reactor setup can be seen in Supplementary Fig. 1.

2.3. Analytical methods

COD, ammonia and nitrate were monitored every three days in keeping with HRT; samples were collected before the removal of treated wastewater and after addition of fresh wastewater. COD ammonia and nitrate were monitored for both influent and treated wastewater (effluent). 5 ml sample from each reactor was collected in triplicates and centrifuged at 7000*g*. The supernatant was filtered through 0.25 μm membrane filter. 500 μl of filtered sample was used to detect COD using COD detection kit (Merck Germany, 100–1500 mg l^{-1}), as per directions of the manufacturer. Levels of ammonia and nitrate were monitored according to APHA standard protocols (American Public Health Association).

2.4. Metagenomic DNA extraction

10 ml activated sludge samples were collected at 0 h and after 2 months from each reactor, centrifuged at 7000*g* at 4 °C. The samples were washed as per the protocol described earlier (Purohit et al., 2003) and DNA was prepared using the FastDNA SPIN Kit for soil (MP Biomedicals, USA) as per the instructions of the manufacturer. Metagenomic DNA was also prepared from the activated biomass sample that was collected from the CETP, before aliquoting it into the three reactors. This is referred to as 'control DNA'. The individual DNA extracts were visualized using 1.0% gel electrophoresis, and the DNA concentrations and purities of the extracts were determined by microspectrophotometry (NanoDrop-1000, Thermo Scientific, USA). This sample was used for constructing the amplicon library.

2.5. Next generation sequencing of amplicon library

The microbial community of the three reactors and control was identified by amplifying and sequence analysis of the V3 region of 16S rRNA gene from the metagenome. Primers used, target the surrounding conserved region and illumina sequences adapters and dual-index barcodes were added to the amplicon. The primers used to amplify the V3 region were;

Forward primer sequence-5′ CCTACGGGAGGCAGCAG 3′ and Reverse primer sequence-5′ ATTACCGCGGCTGCTGG 3′. Libraries were then normalized and sequenced on the MiSeq platform (Illumina). Sequence data was processed by read trimming and identification of V3 sequences, followed by filtering and assigning the operational taxonomic units (OTUs). The reads from filtered OTUs are processed using Quantitative Insights into Microbial Ecology (QIIME) program (Caporaso et al., 2010), to construct a representative sequence for each OTU. The representative sequence was aligned to the Greengenes core set reference databases using PyNAST program.

2.6. Analyzing sequence data using bioinformatics tools

2.6.1. Sequence pre-processing

After applying quality parameter, mainly base quality, average base content, and GC distribution in the reads, there were total 7,66,619, 7,13,088 and 7,21,221 pair-end raw reads generated for reactors A, B and C respectively, while the control DNA showed 1,088,944 pair-end raw reads. Further applying specific filters as mentioned in Table 1 and 90–92% reads were obtained and it is used for downstream analysis.

2.6.2. Analysis using MG-RAST

In order to study taxonomic abundance in the library, the obtained sequences were analyzed using Metagenomics-Rapid Annotation using Subsystem Technology server (MG-RAST), (Meyer et al., 2008) with default parameters under the accession number 4537850.3, 4545085.3, 4545086.3 and 4545087.3 for the control, reactor A, B and C respectively. Post-processing of pair end sequences are done by MG-RAST using their own QC pipeline, summarized in Supplementary Table 1.

2.6.2.1. Overall comparative taxonomic abundance. Based on the LCA (Lowest Common Ancestor) algorithm implemented in MG-RAST, comparative taxonomic tree study was performed with parameters of 90% identity, minimum alignment length 50 bp for phylum,