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Industrial activated sludge exhibit unique bacterial community composition at high taxonomic ranks

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ABSTRACT

Biological degradation of domestic and industrial wastewater by activated sludge depends on a common process of separation of the diverse self-assembled and self-sustained microbial flocs from the treated wastewater. Previous surveys of bacterial communities indicated the presence of a common core of bacterial phyla in municipal activated sludge, an observation consistent with the concept of ecological coherence of high taxonomic ranks. The aim of this work was to test whether this critical feature brings about a common pattern of abundance distribution of high bacterial taxa in industrial and domestic activated sludge, and to relate the bacterial community structure of industrial activated sludge with relevant operational parameters. We have applied 454 pyrosequencing of 16S rRNA genes to evaluate bacterial communities in full-scale biological wastewater treatment plants sampled at different times, including seven systems treating wastewater from different industries and one plant that treats domestic wastewater, and compared our datasets with the data from municipal wastewater treatment plants obtained by three different laboratories. We observed that *each* industrial activated sludge system exhibited a unique bacterial community composition, which is clearly distinct from the common profile of bacterial phyla or classes observed in municipal plants. The influence of process parameters on the bacterial community structure was evaluated using constrained analysis of principal coordinates (CAP). Part of the differences in the bacterial community structure between industrial wastewater treatment systems were explained by dissolved oxygen and pH. Despite the ecological relevance of floc formation for the assembly of bacterial communities in activated sludge, the wastewater characteristics are likely to be the major determinant that drives bacterial composition at high taxonomic ranks.

1. Introduction

Biological treatment of industrial and municipal wastewater by activated sludge (AS) relies in the self-assembly of an active microbial community, which is able to form flocculent aggregates that are separated from the treated effluent by gravity settling. Past studies have been valuable in showing that AS systems contain highly diverse and dynamic microbial consortia, where bacteria are the dominant organisms responsible for the removal of most of the oxygen-demanding

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pollutants and nutrients. Even though fluctuations in bacterial composition in wastewater treatment processes may occur without affecting performance (Kaewpipat and Grady, 2002), it is generally accepted that bacterial community structure and diversity influences wastewater treatment stability and robustness (Wagner and Loy, 2002; Werner et al., 2011). Yet consensus has still not been reached on the principles underlying the assembly of activated sludge microbial communities. It has been proposed that both neutral processes and species sorting act simultaneously during the assembly of bacterial communities (Ayarza and Erijman, 2011; Ayarza et al., 2010; Langenheder and Szekely, 2012; Ofiteru et al., 2010; Valentin-Vargas et al., 2012).

Recent high-throughput studies showed that bacterial communities of municipal WWTP reactors operated under diverse configurations at different geographic locations are highly similar at the phylum level (Hu et al., 2012; Wang et al., 2012a; Xia et al., 2010; Zhang et al., 2012). The suggestion that similarities in the distribution of bacterial phyla among diverse wastewater treatment plants reflect similar life strategies under most configurations is consistent with the recent proposition that bacterial groups of high taxonomic ranks exhibit ecological coherence (Philippot et al., 2010). This concept refers to the fact that members of a taxon share functional traits, which distinguish them from members of other taxa.

Fewer diversity surveys have been performed in activated sludge systems treating industrial wastewater (Bramucci and Nagarajan 2006; Degenaar et al., 2008; Figuerola and Erijman, 2007; Juretschko et al., 2002). Despite the increasing trend towards combining industrial and domestic wastes for treatment in municipal plants, the need for industrial wastewater treatment remains a critical issue worldwide. Industrial wastewaters comprise a wide spectrum of diverse organic compounds, characteristic of the different industrial categories and their production schemes. Similar to domestic sewage, most industries rely on the activated sludge process to treat their wastewater, after a suitable pretreatment when needed (Orhon et al., 2009). The characteristics of the wastewater can have a decisive influence as a driving force behind the bacterial community dynamics. It was observed that in activated sludge reactors subjected to a gradient of increasing industrial wastewater concentrations the deterministic component of community assembly increased with the concentration of industrial wastewater (van der Gast et al., 2008). Earlier studies of industrial activated sludge showed a numerical dominance of major bacterial phyla, i.e. *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Bramucci and Nagarajan 2006; Degenaar et al., 2008; Figuerola and Erijman, 2007; Juretschko et al., 2002). These patterns do not differ markedly from those found in municipal wastewater treatment plants. However, those early molecular studies of biological industrial wastewater treatment systems have been limited to the analysis of only several dozens of clones, and no attempt has been made to compare bacterial community structures of industrial activated sludge in relation to wastewater characteristics and operational parameters.

Metagenomic approaches based on ‘next generation sequencing’ have the capacity of providing a deeper insight into the participating bacterial populations. In this work we have investigated seven full-scale industrial and one municipal activated sludge plant using barcoded 454 pyrosequencing of 16S rRNA gene and compared our datasets with the data from municipal wastewater treatment plants obtained by three different research groups. The objectives of this work were: 1) to test whether the similarity in the abundance distribution patterns of high bacterial taxa, which have been observed in municipal WWTPs, holds for industrial wastewater treatment systems as well, and 2) to examine the taxa distribution of industrial WWTPs in relation to process parameters.

2. Materials & methods

2.1. *Wastewater treatment plants and sampling*

Activated sludge samples were taken from the aeration basins of eight full-scale WWTPs located in Argentina. Six of the plants were sampled twice, at times separated by periods of two months to almost three years. The other two WWTPs, which treated wastewater from two different pet food industries, were sampled once. The type of wastewater, main operating conditions and times of sampling are given in Table 1 and Table S1. Textile dyeing (TXT) wastewater contained unreacted dyestuffs and other auxiliary chemicals that are used at the various stages of dyeing, such as organic acids, fixing reagents, anti-foaming agents, oxidizing and reducing (sodium dithionite) reagents, sodium hydroxide and diluents. Petroleum refinery wastewater (REF) was characterized by high concentrations of aliphatic and aromatic hydrocarbons, phenolic compounds and ammonia. The pharmaceutical industry evaluated in this study (PHA) manufactures dosage-form products by mixing active ingredients and excipients, such as binders, fillers, flavoring and bulking agents, preservatives and antioxidants. Many different products are manufactured, resulting in large variations in wastewater composition. Compounds present in wastewater are usually found in low concentrations, and include organic acids, carbohydrates, phospholipids, vitamins and inorganic minerals. No antibiotics are manufactured or processed in this industrial facility. Pet food industries (PF1 and PF2) generate high-strength wastewater containing high animal fat concentration, protein, carbohydrates and vitamins, in addition to various cleaning and sanitizing compounds. Organic components of wastewater from the whey processing plant (WHY) consist mostly of lactose and milk serum proteins, generated upon the use of automated cleaning-in-place systems (CIP) for cleaning of equipments and pipelines. The polymer wastewater treatment plant (PLM) receives wastewater-containing monomers (alkanes, alkenes and other aliphatic hydrocarbons) from the manufacturing of basic plastics (polyethylene) and performance plastics (polyurethane).

The operational parameters from each wastewater treatment plant were obtained from the respective staff members. Data were averaged over the month prior to sampling. Phosphorus deficiency in the refinery wastewater and nitrogen deficiency in the textile dyeing wastewater were corrected by the addition of phosphoric acid and urea, respectively. Nutrients were in excess in all the other WWTPs. With only one