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Insights into microbial diversity in wastewater treatment systems: How far have we come?

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ABSTRACT

Biological wastewater treatment processes are based on the exploitation of the concerted activity of microorganisms. Knowledge on the microbial community structure and the links to the changing environmental conditions is therefore crucial for the development and optimization of biological systems by engineers. The advent of molecular techniques occurred in the last decades quickly showed the inadequacy of culture-dependent methodologies to unveil the great level of diversity present in sludge samples. Initially, culture-independent technologies and more recently the application of *–omics* in wastewater microbiology, have drawn a new view of microbial diversity and function of wastewater treatment systems. This article reviews the current knowledge on the topic placing emphasis on crucial microbial processes carried out in biological wastewater treatment systems driven by specific groups of microbes, such as nitrogen and phosphorus removal bacteria, filamentous and electrogenic microorganisms, as well as Archaea. Despite the recent *–omics* has offered substantial insights into the diversity and ecophysiology of these bacteria never envisioned before by providing millions of sequence reads at an unprecedented scale, studies based on high-throughput sequencing are still scarce. In order to obtain significant gains in the analysis of structure–function relationships, a greater sequencing investment is needed, particularly to uncover gene expression patterns of functionally relevant genes.

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1. Introduction

Through the history of mankind, water availability has played an essential role in human development. In this sense, global demand for water has been continuously rising due to population growth and increasing socioeconomic activities; in a hundred years the world population has tripled while water consumption has increased six-fold. This circumstance is particularly critical in areas where deficiency in hydric resources prevails, a situation that has prompted the need for wastewater treatment and subsequent reuse. The quantity and quality of waste generated and discharged into natural water bodies are a topic of

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serious concern in our world today, and the wealthiest nations invest a considerable effort for mitigating the pollution of dense populations (Oh et al., 2010). In this context, knowledge of the wastewater source is fundamental to determine its physical, chemical and biological characteristics and to establish an appropriate treatment strategy. Composition of wastewaters depends on their origin but in general, major contaminants found include organic compounds, xenobiotics, metals, suspended soils, nutrients (mainly nitrogen and phosphorus) and pathogenic microorganisms (Bitton, 2005). Depending on the final use of the water (drinking, recreation, irrigation, etc.), several technologies can apply to remove these compounds. Among them, biological wastewater treatment systems rely on the use of microorganisms; the unique abilities of microbes to degrade organic matter, remove nutrients and transform toxic compounds into harmless products make them the essential players in waste removal.

For a century, biological wastewater treatment plants (BWWTPs) based in activated sludge have been in charge of treating both domestic and industrial wastes and have constituted an essential instrument in environmental protection. Initially, by the end of the nineteenth century, removal of organic matter and suspended soils was the main requirement, but nowadays BWWTPs have additional objectives, such as elimination of toxic metals, odors, nutrients (N, P) and pathogens (Bitton, 2005). However, a drawback of this technology is the high performance costs, given the need for aeration as well as moving biomass between treatment tanks (Oh et al., 2010; Sheik et al., 2014). Additionally, powerful greenhouse gases such as CH_4 and N_2O are generated in BWWTPs (Sheik et al., 2014). Giving an answer to this problem, Sheik et al. (2014) proposed a bottom-up design approach for BWWTPs based in the concept of "wastewater biorefinery column", which relies on the engineering of distinct ecological niches to guarantee the targeted enrichment of specific functional groups for sustainable production of bioenergy, bioplastic and fertilizers.

Currently, besides BWWTPs, other ecologically engineered systems are becoming alternative solutions to conventional processes due to their efficiency, low establishment costs and low operation and management requirements. Under the denomination of non-conventional technologies, different strategies can be distinguished. For instance, green filters use land planted with trees flooded with the wastewater to be treated; purification is the result of physical, chemical and biological factors (Fahd et al., 2007). Also, constructed wetlands are passive treatment systems constituted by lagoons or shallow ponds or channels planted with characteristic wetland vegetation (aquatic macrophytes), which mimic natural wetlands under a more controlled environment. Different categories of constructed wetlands exist according mainly to hydrology (open water-surface flow and subsurface flow), type of macrophytic growth (emerged, submerged, free-floating) and flow path (horizontal and vertical) (Vymazal, 2011). Other constructed systems simulate the natural processes of purification that occur in rivers and lakes (Middlebrooks et al., 1982) or are based on wastewater filtration through beds using peat (Couillard, 1994) or sand (Lian et al., 2014). An additional sustainable option that has gained thrust in the last decade includes the use of microbial fuel cells (MFCs), i.e. bioelectrochemical systems where bacteria oxidize organic compounds and the electrons generated in this process are converted into electrical energy (Kelly and He, 2014; Mathuriya and Yakhmi, 2014). The energy produced from wastewater treatment could recover the cost of the process, a promising perspective for this alternative.

A variety of lab-scale systems, such as particulate biofilm reactors or membrane bioreactors (MBR) among others, have also been used as pilot systems for wastewater treatment (Ballesteros Martín et al., 2011; Nicolella et al., 2000). They have the advantage of being compact and well-controlled systems with small area requirements which minimize the excess of sludge production; they can also maintain a high biomass age (of several weeks), while the high biomass concentration and mass transfer area result in high conversion capacities to specifically degrade key pollutants. Besides, they have the advantage that changes in the design can be made more economically. However, biofilm formation on carriers can lead to long start-up times, and scaling-up from laboratory scale reactors to full-scale applications exhibit some unknown aspects, since it is difficult to predict the behavior of a complex process (Nicolella et al., 2000).

In all these technologies, performance of the wastewater treatment process is based on the exploitation of the concerted activity of the microorganisms involved. Knowledge on the microbial community structure and the links to the changing environmental conditions is therefore crucial for the development and optimization of biological systems by engineers. Before the development of cultivation-independent techniques, the key drivers of pollutant removal processes were hardly known. This scenario overturned in the last decade of the 20th century with the development of a suite of molecular methods that allowed the study of these organisms circumventing cultivation. Further, the *–omics* revolution occurred in the last years has boosted our understanding on the biology of these biotechnological processes. In this review, we provide an outline on the current scene of the diversity and genomics of prokaryotes in various wastewater treatment systems highlighting the findings of some crucial microbial processes in this field and discussing some unresolved questions.

2. Approaches to study wastewater microbiology

The advent of molecular methods such as Polymerase Chain Reaction (PCR) techniques revolutionized the study of microbial diversity previously constrained by our inability to grow the majority of microorganisms in culture (Rappé and Giovannoni, 2003). PCR amplification and sequencing (Fig. 1) have been used now for decades to study the presence, diversity and expression of ribosomal and protein-coding genes in these constructed environments, allowing the identification of the key microorganisms involved in processes such as carbon-degrading and ammonia oxidation (Gilbride et al., 2006; Wagner and Loy, 2002). These PCR approaches shed light on the diversity of main players in BWWTPs and how genetic diversity is structured in relation to certain environmental parameters (Wang et al., 2012; Wells et al., 2011). Yet, there are inherent PCR amplification- and primer-related biases that limit the quantitative information that can be extracted from these approaches. Perhaps the most critical step from these is primer design, which may be remarkably challenging for the highly diverse functional genes. However, fluorescent in situ hydridization (FISH) using group-specific rRNA-targeted oligonucleotide probes circumvents the PCR bias and has been largely applied to wastewater microbiology providing quantitative information on the dominant groups involved in the different processes of waste removal (Joss et al., 2011; Nielsen et al., 2009a; Wagner et al., 1996).

After the initial application of these approaches, the rise of the "–*omics* era" has resulted in a turning point in studying phylogenetic and functional diversity of wastewater treatment systems (Fig. 1). The development of the so-called high throughput sequencing (HTS) techniques, i.e., 454-pyrosequecing and Illumina among others, has allowed producing millions of sequence reads at an unimaginable low cost. Whole genome sequencing of isolated microorganisms was the first step taken to elucidate the functional potential of wastewater relevant microorganisms (Table 1). Beyond the initial genome sequencing of cultures, metagenomics (the massive DNA sequencing of all genes present in a microbial community) and metatranscriptomics (sequencing of all genes being transcribed, and therefore, likely expressed in a microbial community) have been applied to unveil the genomic potential and transcripts of whole microbial communities circumventing isolation and PCR (Venter et al., 2004; Yu and Zhang, 2012). However, these approaches have also the limitation that most of the information is derived from the most abundant members of the community. Despite treatment systems present in general less diversity than natural ecosystems, significant gains in the analysis of gene expression patterns require a greater sequencing investment and the combination of both metagenomics