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Total and active microbial communities in a full-scale system treating wastewater from soy sauce production

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ABSTRACT

Microbes within activated sludge and their potentially metabolic activity are important factors in contaminants removal during actual wastewater treatment. In this study, microbial community structures and metabolic activity in an anaerobic-anoxic-oxic (A_2O) system treating soy sauce-producing wastewater were studied using 16S rDNA and rRNA high-throughput sequencing. At DNA and RNA level, microbial communities harbored distinct biodiversity. *Proteobacteria* was the most abundant and active phylum in activated sludge. Abundant *Megasphaera*, *Thauera* and *Azoarcus* were in slow metabolism, while *Psychrobacter* with low abundance had the highest potential for activity in the anaerobic tank. As for archaea, *Cenarchaeales* was the most abundant and active order and a large amount of hydrogenotrophic methanogens kept syntrophic with *Megasphaera*. The rare taxa, such as *Methanosarcinales* and *Nitrosopumilales*, had a high potential for activity in the anoxic tank. Furthermore, the total and active microbes were quantified via DNA and RNA-based qPCR and the 16S rRNA: rRNA gene ratios of bacteria and archaea decreased along with the treatment process. Altogether, the findings provided novel insights into the evolution of microbial activity in an integrated soy sauce-producing wastewater treatment process.

1. Introduction

Soy sauce, one of the widely accepted condiments in China, is produced from steam-cooked defatted soybeans and roasted wheat flour cultured with koji mould (Cheng et al., 2014). Currently, increasing production of soy sauce generates large volumes of wastewater with complicated compositions, representing an important source of pollution. It contains lots of nutrients (e.g. polysaccharides, proteins and fat), food residues, detergents, disinfectants, salt, various microbes and their secreted enzymes and metabolites, etc., which is rich in organic concentration and high in color, as well as salinity, leading to the complexity and difficulty of wastewater treatment for beneficial reuse (e.g., aqua farming, irrigation, surface water discharge).

Many physical, chemical and biological methods have been applied in practice for treatment of soy sauce-producing wastewater (Lu et al., 2010). Among biological methods, anaerobic-anoxic-oxic (A_2O) activated sludge system is the most commonly applied technology because of its simple flowsheet, low operational cost and energy requirement, as well as the stability of simultaneous nitrogen and phosphorus removal (Abu-Alhail and Lu, 2014). Aerobic and anoxic zones are responsible for nitrogen removal through nitrification and denitrification, respectively; phosphorus is removed through coupling of aerobic and anaerobic zones; and, the aerobic zone is used for COD removal (Mujtaba and Lee, 2017). Activated sludge microbiome is commonly comprised of prokaryotes, eukaryotes and viruses, in which bacteria is predominant and plays a pivotal role in the degradation and removal of pollutants (Ye et al., 2012; Ferrera and Sánchez, 2016; Bassin et al., 2017).

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Phyla of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Chloroflexi* had been recognized as dominant communities in activated sludge, which mainly contributed to transformation of organic matters and involved in biogeochemical cycling of primary elements such as nitrogen, sulfur and phosphorus (Xia et al., 2010; Girard et al., 2012; Hu et al., 2012; Jena et al., 2016). However, to date, still comparatively little is known about the real microbial activity at each stage of treatment in a full-scale wastewater treatment plant (WWTP).

Nowadays, high-throughput sequencing has been a prevalent technique to investigate microbial diversity and structures in sewage treatment process. Nevertheless, the majority of amplicon sequencing focused on ribosomal DNA (rDNA) representing the total microbial communities, which cannot distinguish between the dead, dormant or metabolically active portions within a sample (Kearns et al., 2016). Recent report indicated that ribosomal RNA (rRNA) is a better marker itself to accurately estimate the potentially active part of microbial communities at the time of sampling (CarinaSundberg et al., 2013). The high ratios of RNA/DNA for some microbial taxa indicate their high metabolic activity (Baldrian et al., 2012). The comparison of phylogenetic clustering in both DNA and RNA-based microbial communities can further enhance our understanding of the factors that shape complex microbial structures in nature (DeAngelis and Firestone, 2012). Romanowicz et al. (2016) unraveled that pH and soil moisture could influence the compositions of total and active microorganisms in forest soils. Currently, some studies have performed DNA and RNA-based methods to evaluate the metabolic activity of diverse microbial communities. Smith et al. (2014) reported that the biofilm's metabolic diversity increased as temperature decreased from 15 °C to 3 °C in response to a greater flux of complex organics into the biofilm in a psychrophilic anaerobic membrane bioreactor (MBR) treating domestic wastewater. Maza-Márquez et al. (2016b) revealed the activity evolution of fungal community in a full-scale MBR operated throughout four experimental phases by targeting the DNA and RNA molecules. However, limited investigations depicted microbial communities and their activity dynamics in a full-scale system treating soy sauce-producing wastewater at both DNA and RNA level. Thus, it is worthy to detect microbial activity at each stage of the system by co-analysis of DNA and RNA.

In this study, total communities of bacteria and archaea and their active members in a full-scale A_2O system treating soy sauce-producing wastewater were analyzed at DNA and RNA level using the combination of Illumina HiSeq sequencing and quantitative PCR (qPCR). Principal component analysis (PCA) and clustering analysis were used to appraise distinctions between the total and active microbial compositions. Subsequently, the potentially metabolic activity and dormancy of taxa were assessed by comparing DNA and RNA-derived relative abundance. The biomass of total and active microbes was further estimated via DNA and RNA-based qPCR.

2. Materials and methods

2.1. Sampling

Mixed liquor samples were collected from the regulating tank (TJ), anaerobic tank (AT), anoxic tank (QT) and aerobic tank (OT) of a full-scale system treating wastewater from soy sauce production in Guangdong, China (22°46′0″N, 112°57′0″E) in Jan 15, 2016. This system treated nearly 2×10^3 m^3/d of soy sauce-producing wastewater. A coagulation–flocculation process was occurred in TJ to pre-treat soy sauce-producing wastewater prior to a subsequent biological treatment by the units of AT, QT and OT, successively. The mixed liquor samples were collected from the four corners and middle section of each tank using an organic glass hydrophore, 3–4 m depth, then individually kept in sterile polyethylene containers after mixing each tank's samples thoroughly and transported to laboratory within 3 h on ice. Immediately after arriving at the laboratory, 10 mL mixed liquor was centrifuged at 6000 rpm for 10 min at 4 °C, then the supernatant was decanted, and the collected residue was stored at −80 °C for further analysis.

2.2. Nucleic acid extraction and reverse transcription

Microbial genomic DNA and RNA were extracted from the collected residue of each sample in triplicate using E.Z.N.A.® Soil DNA Kit and Soil RNA Kit (Omega Bio-Tek, Norcross, GA, USA), respectively, in accordance with the manufacturer's instructions. DNA and RNA concentration and purity were determined using 1% agarose gel electrophoresis and Nanodrop spectrophotometer ND-1000 (ThermoFisher Scientific, USA). A PrimeScript™ RT Reagent Kit (Takara Bio Inc., Japan) was used to remove residual genomic DNA from RNA samples completely and to synthesize cDNA. A PCR test targeting 16S rRNA was conducted to verify whether genomic DNA was clear. Henceforth, the term cDNA was used to refer to the RNA-based samples. DNA and cDNA extracted from the four separate units were stored at −80 °C until further processing.

2.3. PCR amplification and Illumina HiSeq sequencing

The specific primers 515F/806R (Bassin et al., 2017) and 519F/915R (Wei et al., 2015) with the Illumina barcodes were used to amplify the V4 regions of bacterial and archaeal 16S rRNA from the genome DNA and cDNA, respectively. Triplicated PCR reactions were carried out using 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 mM of each primer and 10 ng DNA or cDNA templates. The PCR program for both bacterial and archaeal amplifications was: 98 °C for 1 min, followed by 30 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 60 s, and finally elongation at 72 °C for 5 min. PCR products were mixed in equimolar ratios, then purified with Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were constructed using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index adaptors were added. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Ultimately, the library was sequenced on an Illumina HiSeq 2500 platform by applying the 250 bp paired-end strategy at the Beijing Novogene Bioinformatics Technology Company.

2.4. Bioinformatics and statistical analysis

Paired-end reads from the original DNA and cDNA fragments were aligned and merged using FLASH (v1.2.7) and then assigned to each sample according to the unique barcodes. The splicing sequences were called raw tags. Then the raw tags were analyzed to trim off the low quality reads, adaptors, barcodes and primers using QIIME standard pipeline (Caporaso et al., 2010). The chimera sequences were removed using Gold Database with the UCHIME algorithm (Edgar et al., 2011) to obtain the effective tags. The effective tags with ≧97% similarity were assigned to the same Operational Taxonomic Units (OTUs) using Uparse v7.0.1001 (Edgar, 2013). Then, the obtained OTUs were annotated and taxonomically assigned via GreenGene Database v. May 2013 and RDP classifier (v2.2) (Cole et al., 2005) with more than 80% confidence. From the cluster file, alpha diversity was evaluated by calculating indices of Shannon, Simpson, Chao1, ACE and Good's coverage in QIIME (v1.7.0) (Schloss et al., 2009). Indices of Shannon and Simpson represented the community diversity, Chao1 and ACE indicated the