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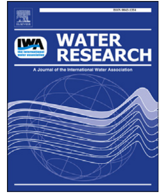
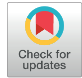


Insights into Feast-Famine polyhydroxyalkanoate (PHA)-producer selection: Microbial community succession, relationships with system function and underlying driving forces



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A B S T R A C T

The Feast-Famine (FF) process has been frequently used to select polyhydroxyalkanoate (PHA)-accumulating mixed cultures (MCs), but there has been little insight into the ecophysiology of the microbial community during the selection process. In three FF systems with well-defined conditions, synchronized variations in higher-order properties of MCs and complicate microbial community succession mainly including enrichment and elimination of non-top competitors and unexpected turnover of top competitors, were observed. Quantification of PHA-accumulating function genes (*phaC*) revealed that the top competitors maintained the PHA synthesis by playing consecutive roles when the highly dynamic turnover occurred. Due to its specific physiological characteristics during the PHA-accumulating process, *Thauera* strain OTU 7 was found to be responsible for the fluctuating SVI, which threatened the robustness of the FF system. This trait was also responsible for its later competitive exclusion by the other PHA-producer, *Paracoccus* strain OTU 1. Deterministic processes dominated the entire FF system, resulting in the inevitable microbial community succession in the acclimation phase and maintenance of the stable PHA-accumulating function in the maturation phase. However, neutral processes, likely caused by predation from bacterial phages, also occurred, which led to the unpredictable temporal dynamics of the top competitors.

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1. Introduction

Using microbial communities comprising multiple types of microorganisms as biocatalysts or producers, mixed culture (MC) biotechnology has made significant advances in the conventional environmental field and may play an increasingly practical role in the industrial arena (Kleerebezem and Van Loosdrecht, 2007; Dionisi and Silva, 2016). The MC polyhydroxyalkanoate (PHA) production process is one example of such a potential biotechnology. The MCs in the production system are capable of converting suitable carbon sources into PHAs to form granules, which can be used as raw materials for biodegradable plastics and fuels.

The core of the MC PHA production process is the enrichment of PHA-accumulating bacteria. Different strategies have been proposed to realize this goal; among these, the Feast-Famine (FF) process has proved to be the most effective (Dias et al., 2006). MCs under FF conditions are subjected to a series of alternating excess and limited external substrate conditions (feast and famine, respectively), and bacteria with robust carbon storage abilities ultimately dominate in the system. The MC PHA production process under FF conditions has been intensively studied over the past several decades. The benefits, including pollution reduction and resource capture (Albuquerque et al., 2010; Jiang et al., 2009, 2012), as well as high-value by-product production and energy conservation (Frison et al., 2015; Scherson et al., 2013), follow a basic trend: endowing the process with more economic and environmental benefits, then advancing it for industrial-scale application. However, there are still obstacles limiting its effective industrial application although the maximum PHA level in MCs has been obtained as high as 89% (Johnson et al., 2009). Besides the reduction in production cost, the overall function robustness (such as PHA production performance and physical properties) and resiliency (Coats et al., 2016) of the MCs are also key issues for the industrial

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utility of the process. A PHA-accumulating microbial community with higher-order properties (Konopka et al., 2015) is the functional core of the MC-FF process, and the regulation and stabilization of such a community must be studied in-depth at the microbial level (Rittmann et al., 2016). Valuable microbial ecological studies of the competition between PHA- and non-PHA-producers (Jiang et al., 2011) and the substrate preference specificity of PHA-producers (Albuquerque et al., 2013) have been performed; however, there are still important questions remaining to be answered:

(1) What is the succession pattern of the microbial community in an MC-FF system? Both stable (Lapara et al., 2002) and highly dynamic microbial communities (Wittebolle et al., 2008) have been observed in bioreactors with complex food webs; however, robust information concerning population succession dynamics at various time points in an MC-FF system with rather simple trophic levels is lacking.
(2) What is the relationship between microbial community succession and overall system function? For further optimal regulation of the MC-FF process, information concerning the relationship between community succession and system function is necessary. However, due to insufficient temporal information regarding the dynamics of the microbial community throughout the process, these links are currently unknown. In addition, the physical characteristics of the bacterial species in the MC-FF system must be considered, as their stability is crucial to the long-term robustness of an operational system, as well as downstream processing.
(3) What is the underlying mechanism guiding the assembly of the microbial community in an MC-FF system? Similar processes have been intensively studied in other natural and artificial microbial ecosystems, and two mechanisms have been identified: deterministic and stochastic (neutral) processes (Nemergut et al., 2013; Vanwonterghem et al., 2014). The majority of MC-FF analyses have been based solely on observations at the physiological level, which are mainly derived from macroscopic kinetic and stoichiometric investigations of the MCs. Robust information at the ecophysiological level supporting the identification of the microbial community assembly mechanisms in an MC-FF system is lacking.

The overall function robustness of MC-FF system was deeply investigated in this study. Substrates simulating three typical kinds of fermentation liquid products of waste-activated sludge were used to enrich PHA-producers from the active sludge of a local wastewater treatment plant (WWTP). The enrichment was conducted in three sequencing batch reactors (SBRs), which were operated in parallel under FF conditions for more than 6 months. The temporal variation in the microbial community and higher-order properties of the MCs, in terms of PHA-producing performance and physical properties of bacterial flocs, were synchronously monitored during the operation starting from the initiation of the FF system. The microbial community succession of the MCs in the three reactors was investigated, as well as their relationships to the overall system function. The underlying mechanisms driving the temporal dynamics of the microbial community, especially the dominant species, are discussed.

2. Materials and methods

2.1. FF process

Three SBRs, each with working volumes of 4 L, were operated for the long-term enrichment of PHA-accumulating MCs (Fig. S1). Activated sludge taken from a local WWTP with Anaerobic-Anoxic-Oxic (AAO) process was used as the inoculum. The activated sludge was screened (screen size of 0.25 mm) to remove undissolved particles before inoculation. The substrates utilized simulated three different kinds of liquid products of waste-activated sludge fermentation: an acetic acid-type (Yuan et al., 2006), a propionic acid-type (Peng et al., 2009) and a butyric acid-type (Liu et al., 2008). The main parameters of the substrates and the operation are shown in Table 1. The resulting applied organic loading rate (OLR) for each reactor was 1.6 g COD/L/d, which belongs to the low-OLR enrichment strategy (Albuquerque et al., 2010; Dias et al., 2006). Each reactor was operated with a 12 h cycle comprising four periods: feeding (10 min), aeration (620 min), sedimentation (80 min) and withdrawal (10 min). All three SBRs were operated at room temperature (21 ± 2 °C). Additional details of the operation of the three SBRs are described in Supplementary Information S1 and Fig. S1.

2.2. Batch assays

The maximum PHA level of the MCs was evaluated by fed-batch PHA accumulation assays (Fig. S1). The batch reactors were inoculated with MCs collected from the FF reactor at the end of the operating cycle and fed with equal volumes of VFA mixture with the same composition as the substrate used in the corresponding FF reactors. The concentration of the fed VFA was approximately 4.0 g COD/L. Each time that the substrate was exhausted (indicated by a rapid increase in DO concentration), the supernatant was removed to half of the working volume after settling, and then the same volume of substrate was added. Feed pH was adjusted to 7 (± 0.1) by adding 2 M NaOH and was uncontrolled during the reaction. Air was supplied through a ceramic diffuser from a compressed-air pump. Tests were performed at room temperature (21 ± 2 °C).

2.3. Microbial community analysis

Biomass samples were taken from the SBRs at the end of the aeration phase and stored at −80 °C in 40% (volume fraction) glycerol. Total genomic DNA was extracted from the activated sludge samples using an E.Z.N.A. bacterial DNA kit (OMEGA, United States). 16S rRNA gene high-throughput sequencing was performed using a Hiseq 2500 (Illumina, USA) platform. The V3-V4 regions of the 16S rRNA genes were amplified with Primers 341F and 806R (Roggenbuck et al., 2014). Quality control of the raw data was assessed using UPARSE (Edgar, 2013), then USEARCH was used to filter chimeras and cluster the remaining sequences into operational taxonomic units (OTUs) at identity thresholds of 0.97 (Edgar, 2010). QIIME v1.5.0 software was used to select representative sequences for each OTU and assign them to the specific taxonomic level in the RDP database with the aid of an RDP classifier (Maidak et al., 1997). OTU tables were generated after datasets were rarefied to 40,000 reads per sample. Raw sequence data is available at BioProject (PRJNA360897).

2.4. phaC gene quantification

The relative abundance of *phaC* genes in the microbial communities was determined by real-time PCR. The primer pair CF1/CR4 (Sheu et al., 2000) was used to quantify the Type I and II *phaC* gene-encoding PHA synthases. The primer pair 1369F/1492R (Edwards et al., 2008) was used to quantify the total bacteria level in the FF system. The information for each primer used in this study is listed in Table 2. The relative abundance of the *phaC* genes compared to the total bacteria was calculated by dividing the absolute copy number of *phaC* genes by the total number of 16S rRNA copies. Details of the real-time PCR process are listed in Supplementary Information S2.

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