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Response of treatment performance and microbial community structure to the temporary suspension of an industrial anaerobic bioreactor

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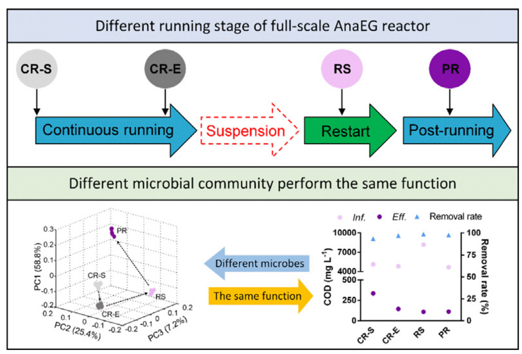
HIGHLIGHTS

- Focused on the stability and microbial response to the suspension of bioreactor
- High-throughput sequencing and qPCR were used for microbial analysis.
- The AnaEG reactor restarts efficiently after the temporary suspension.
- High species diversity ensured the stability of reactor under environmental stress.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a novel type of mesophilic anaerobic bioreactor—an expanded granular sludge bed (EGSB)—was utilized to explore the effect of suspending reactor operation on the treatment performance and the microbial community structure. The parameters of performance and bacterial community before and after a four-week suspension were compared for the starch processing wastewater treatment bioreactor. The results indicate that the removal rate of the organic matter remained higher than 90%, although the biomass significantly decreased after restarting the reactor. However, the relatively stable microbial community structure before the suspension was altered significantly during the restart and post-running stages. This change was primarily due to variability in satellite species and the substitution effect of different dominant bacteria. For example, some non-major carbohydrate-degrading bacteria that were sensitive to nutrition deficiency, such as *Desulfovibrio* and *Geobacter*, were dramatically reduced after the suspension. In contrast, the stress of starvation stimulated the reproduction of hydrolytic bacteria, such as *Macellibacteroides*. However, the high bacterial diversity index (6.12–6.65) and the longstanding core species, including *Chloroflexi*, *Cloacimonetes*, *Ignavibacteriae*, *Thermotogae* and *Euryarchaeota*, maintained the functional stability of the reactor. Consequently, although the total bacteria decreased significantly after reactor operation was suspended, sufficient functional bacteria supported by the high diversity, as well as the longstanding core species, guaranteed the effective degradation after suspension.
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1. Introduction

Compared with aerobic treatment, anaerobic digestion (AD) prevails in industrial wastewater treatment due to several advantages, such as low sludge production and energy requirements, energy recovery from methane production and its ability to process concentrated wastewater (Kleerebezem and Macarie, 2003). However, the temporary suspension of an anaerobic bioreactor is common in industrial wastewater treatment due to the seasonal production of the factories and the need for routine inspection or response to emergency situations. Therefore, a typical running cycle exists amid the industrial application of an anaerobic reactor. It includes several different operation stages, e.g., the start-up stage, continuous running stage, suspended stage caused by malfunction or lockout, restart running stage and post-running stage after restart. In particular, restarting the reactor efficiently is essential for industrial production, and the stability and treatment performance of the reactor during the restart period determines whether it can put into production as soon as possible.

Ye et al. (2018) indicated that the repeated short-term starvation (one to four days) and recovery mode could improve the tolerance and activity of a lab-scale sequencing batch reactor, and the dominant anaerobic ammonium oxidizing bacteria more effectively self-adapted to repeated starvation. They found that short-term starvation benefits the AD, but a longer shutdown period is also common in industrial production. Consequently, Dong et al. (2010) monitored the performance during the restart period of a lab-scale UASB that had been suspended for four months. They claimed that the reactor took 35 days to achieve a stable COD removal rate, but the VFAs and the methane content could stay stable earlier than the COD. Therefore, they believe that these two parameters are good indicators to evaluate whether the reactor is stable at the restart stage.

The performance of the AD depends on the activity of the anaerobic granular sludge (AGS) in the reactor. Therefore, researchers were also concerned about whether the activity of the AGS would be affected during a long-term preservation. J.J Li et al. (2014) found that the methanogenic activity of the AGS was not affected after preservation for two to four months. In addition to a lab-scale reactor, it is more informative to evaluate the performance of the AD during the restart stage during practical industrial applications. A previous study indicated that when the sludge was refilled into the anaerobic reactor after six weeks of storage, significant VFA accumulation (especially propionic acid) was observed during the restart stage (Gallert and Winter, 2008). They hypothesized that syntrophic propionate-oxidizing bacteria appeared to be the most vulnerable bacteria during the storage of the AGS. However, the morphological characteristics and the microbial communities of the granular sludge were not analyzed in their research.

Microorganisms in a bioreactor, which directly determine the performance of anaerobic reactors, would also be affected by environmental changes. Therefore, dissecting the dynamic mechanisms associated with the treatment performance and microbial community in the different operation stages will help us to more effectively manage the anaerobic bioreactor. Jauregui-Jauregui et al. (2014) indicated that an anaerobic fixed-film bioreactor could be restarted efficiently after being shut down for six months during which the inoculum starved; the stable archaeal and resilient bacterial populations could be the primary reason for the high COD removal rate. However, their conclusions were based on the results of the CE-SSCP and qPCR quantification of the total numbers of archaeal and bacterial biomass in the samples, and no additional information on the microbial community could be obtained from their study. In brief, although the previous studies investigated the performance of a suspended anaerobic bioreactor, fewer studies have examined the response of the microbial community.

In addition, as the representative of the third-generation anaerobic reactor, the expanded granular sludge bed (EGSB) type has been widely used due to its outstanding performance in various industrial wastewater treatments. However, its high energy consumption is still one of its major drawbacks, and it would still be affected by variations in pH and upflow velocity (He et al., 2016; Ratanatamskul and Siritiewsri, 2015). Therefore, an advanced EGSB (AnaEG) was developed to overcome the multiple shortcomings of the existing EGSB (Zhang, 2009). Compared with the traditional EGSB, the most salient feature of the AnaEG is its lack of an effluent circulation system. However, the sludge bed can be expanded sufficiently by uniform wastewater distributor and gas production. Therefore, it has the advantages of both energy saving and high efficiency. It has been successfully implemented for coal gasification wastewater treatment (C.J. Li et al., 2014) and palm oil mill effluent treatment (Tabassum et al., 2015). In particular, our previous study showed that when the full-scale AnaEG reactor was utilized to treat starch processing wastewater (SPW), its treatment performance displayed temporal stability and spatial efficiency, which was highly consistent with a stable microbial community structure (Qin et al., 2018). During the long-term monitoring of the AnaEG reactor to treat SPW, the wastewater treatment plant experienced a 4-week suspension. This enabled us to study the effect of the suspension on the anaerobic wastewater treatment in the AnaEG reactor. By evaluating the treatment performance and comparing the microbial community before and after the suspension using high-throughput sequencing and quantitative real-time PCR (qPCR), we aimed to illustrate how the microbial community resisted the external environment and maintained the treatment efficiency of the reactor.

2. Material and methods

2.1. Reactor operation status and sample collection

The surveyed SPW treatment plant is a full-scale mesophilic AnaEG reactor located in Hangzhou, Zhejiang Province of China. It has a diameter of 8 m and a height of 15 m, and its effective volume is 750 m^3. The inoculated sludge was the anaerobic digestion sludge from a municipal wastewater treatment plant, and stable granular sludge was formed in the reactor after being domesticated. The influent of the AnaEG reactor is the wastewater produced during the production of modified starch. Natural starch is used as the raw material for modified starch production. Therefore, the composition of the modified SPW is simple. The characteristics of the raw SPW and the operation parameters of the AnaEG reactor in this study are summarized in Supplementary Fig. S1. Briefly, pH, temperature and the COD of the raw SPW fluctuate in the range of 5.8–8.2, 31–35 °C and 3000–9800 mg L^{-1}, respectively. The flow rate and the OLR of AnaEG vary between 165–360 $m^3\ d^{-1}$ and 1.2–7.2 kg COD $m^{-3}\ d^{-1}$, respectively. Although the raw SPW fluctuates greatly, the AnaEG could always maintain the removal rate of the COD above 90%. It is highly stable when used in industrial production. Three operation stages were investigated in this study. The first one was the continuous running stage (CR) that lasted 15 weeks, including the start (CR-S, March 2016) and the end time point (CR-E, June 2016). Seven weeks later, reactor operation was suspended for four weeks due to the shutdown of the wastewater treatment plant. After restarting the reactor for one week, sampling was implemented at the restart stage (RS, September 2016). The interval between the CR-E and RS stages was 12 weeks. Sampling was conducted at the post-running stage after the reactor restarted for 15 weeks (PR, January 2017). In the four time points of the three operation stages, wastewater and AGS were simultaneously collected from the sampling ports of the reactor. The detailed information of the sample collection strategy is summarized in Supplementary Fig. S2. Samples of wastewater were stored at 4 °C for physicochemical analysis, and the AGS were frozen at −20 °C until further processing and DNA extraction.

2.2. Chemical analysis

Physicochemical analysis of the wastewater (pH, temperature, COD and TOC) and the AGS (SV, MLVSS, SEM and granule size) were

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