



## Effects of side-stream ratio on sludge reduction and microbial structures of anaerobic side-stream reactor coupled membrane bioreactors



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### HIGHLIGHTS

- Anaerobic side-stream reactor (ASSR) coupled MBR had efficient COD and NH<sub>4</sub>-N removal.
- Rising side-stream ratio (SR) favored nitrogen removal and SCOD release in ASSR.
- Rising SR from 0.2 to 1.0 increased sludge reduction from 6.0% to 49.7% for ASSR-MBR.
- Pyrosequencing analysis showed enrichment of *Chloroflexi* and *Armatimonadetes* in ASSR.
- High SR favored slow growers, and low SR enriched hydrolytic and predatory biomass.

### ARTICLE INFO

#### Article history:

Received 6 February 2017

Received in revised form 9 March 2017

Accepted 11 March 2017

Available online 15 March 2017

#### Keywords:

Sludge reduction

Wastewater treatment

Anaerobic side-stream reactor

Membrane bioreactor

Microbial community

### ABSTRACT

An anoxic/oxic membrane bioreactor (AO-MBR) and three anaerobic side-stream reactor (ASSR) coupled MBRs (ASSR-MBR) were operated to investigate effects of side-stream ratio (SR) on sludge reduction and microbial community structure of ASSR-MBRs. The ASSR-MBR achieved efficient COD and ammonium nitrogen removal. SR increased from 0.2 to 1.0 favored nitrogen removal, and increased sludge reduction from 6.0% to 49.7%. The total released COD in the ASSR increased with the rising SR and was inversely proportional to sludge yield of ASSR-MBR. Pyrosequencing analysis showed that phyla *Chloroflexi* and *Armatimonadetes* surviving in anaerobic conditions were enriched in the ASSR, while *Nitrospirae* was dominant in the MBR. Comparison at the genus level revealed that higher SR favored the growth of slow growers, while lower SR enriched hydrolytic and predatory bacteria. The results suggested that SR has a profound effect on nitrogen removal, sludge reduction and microbial community structure in the ASSR-MBR.

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### 1. Introduction

Activated sludge (AS) process has been successfully applied to both domestic and industrial wastewater treatment plants (WWTPs); however, waste activated sludge (WAS) massively generated from the AS process is continuously puzzling WWTPs with the dramatically increased amount of wastewater and more stringent sludge regulatory requirements in recent years (Khurshheed and Kazmi, 2011). The conventional sludge treatment and disposal processes have become a burden for WWTPs because of their complex process, footprint requirements, operational and transportation costs, and strict control for secondary pollutants (Zhou et al., 2015a). To tackle the pressing problem, various sludge reduction technologies have been developed to directly decrease sludge

production within the AS process rather than struggling with its postal treatment and disposal, which also represents one of the first priorities in the waste management hierarchy nowadays (Ferrentino et al., 2016).

Among these sludge reduction strategies, the biological process with an anaerobic side-stream reduction reactor (ASSR) inserting in the returned activated sludge (RAS) line is considered as a promising way potentially employed in WWTPs (Khurshheed and Kazmi, 2011; Velho et al., 2016). In the ASSR process, a portion of RAS is treated in the anaerobic tank, while the most part of RAS returns to the activated sludge stages without treatment. ASSR has been successfully coupled with various biological wastewater treatment processes, including conventional AS (Rodriguez-Perez and Feroso, 2016; Wang et al., 2015), anoxic/oxic (AO) (Foladori et al., 2015; Velho et al., 2016; Zhou et al., 2015b), sequencing batch reactor (SBR) (Khurshheed et al., 2015; Semblante et al., 2016a,b; Sun et al., 2010), university of captain

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(UCT) (Coma et al., 2013), etc, and significantly reduced sludge reduction without negative effects on pollutants removal except for phosphorus (Velho et al., 2016; Zhou et al., 2015a). Moreover, ASSR also facilitated sludge treatment and management with improved sludge dewaterability (Semblante et al., 2016b) and decreased sludge production. Although many studies reported that the insertion of ASSR does not affect (Velho et al., 2016) and even improves (Rodriguez-Perez and Fermoso, 2016; Ye et al., 2008) sludge settleability, Coma et al. (2013) observed the increase of suspended solids (SS) to 128 mg/L under the optimum sludge reduction of the ASSR even with improving sludge settleability. The combination of membrane bioreactors (MBR) with ASSR (ASSR-MBR) should be a promising method by preventing the deterioration of effluent quality and enhancing the biological maintenance. Saby et al. (2003) has been successfully utilized MBR with the oxic-settling-anaerobic (OSA) technology to achieve precise determination of the sludge reduction, but the systematic investigation of process performance was scarcely conducted for ASSR-MBRs afterwards. Notably, hydraulic retention time (HRT) of the ASSR, which is equal to solid retention time (SRT) by assuming the ASSR as a continuously stirred tank reactor, ranged from 6 to 7 h in the OSA (Ye et al., 2008) to 20 d in the ASSR (Semblante et al., 2016b) for effective sludge reduction, and is comparable to the HRT of main stream. For instance, the volumetric ratio of ASSR and main stream is 0.47 for full-scale application of ASSR-AO in the Levico WWTP (Velho et al., 2016). Therefore, the ASSR-MBR process also provides an alternative for sludge reduction in WWTPs with compact footprint.

Recently, researchers have realized that the lack of knowledge on appropriate design and operational parameters hindered the wide-scale application of the ASSR process (Ferrentino et al., 2016; Semblante et al., 2016b). The influence of oxidation-reduction potential (ORP) level (Saby et al., 2003), SRT (Semblante et al., 2016b; Ye et al., 2008) and aeration condition (Habermacher et al., 2015) of the ASSR on sludge reduction efficiency and process performances were investigated and very helpful to understand the mechanism governing sludge reduction. Previous studies suggested that the sequential exposure of sludge to anaerobic and oxic conditions had a significant influence on sludge reduction, and thus sludge interchange ratio (SIR) is put forward as a key operational parameter for the ASSR-SBR (Ferrentino et al., 2016; Velho et al., 2016). For the continuous flow process, the side-stream ratio (SR), which was utilized by Coma et al. (2013) in a pilot-scale ASSR-UCT, is probably more applicable as it is expressed as a proportion of RAS passed through the ASSR.

$$SR = Q_s/Q_R = Q_s/(RQ_i) \quad (1)$$

where,  $Q_s$ ,  $Q_R$  and  $Q_i$  is flow rate of side-stream recirculation, RAS and influent wastewater, L/d, respectively;  $R$  is the RAS ratio. If an optimum HRT of an ASSR ( $HRT_{ASSR}$ ) for sludge reduction is established, then the volume of an ASSR ( $V_{ASSR}$ ) can be expressed as

$$V_{ASSR} = Q_s HRT_{ASSR} = SR \times RQ_i HRT_{ASSR} \quad (2)$$

Considering  $Q_i$  can be expressed as the volume of main stream ( $V_{MS}$ ) divided by its HRT ( $HRT_{MS}$ ), Eq. (2) can be rewritten as

$$V_{ASSR} = SR \times RV_{MS} HRT_{ASSR}/HRT_{MS} \quad (3)$$

The SIR ( $d^{-1}$ ) can be expressed as

$$SIR = \frac{Q_s X_s}{V_{MS} X_{MS}} = \frac{SR \times RX_s}{HRT_{MS} X_{MS}} \quad (4)$$

where,  $X_s$  and  $X_{MS}$  is sludge concentration in the ASSR and main stream, g/L, respectively. It suggests that SR is also a key factor influencing not only sludge interchange between feast and famine cycles with respect to oxygen and substrate, but also the volume of the ASSR.

The aim of this study was to evaluate effects of SR on sludge reduction and pollutants removal of the ASSR-MBR fed with real wastewater. An AO-MBR was also operated in parallel for the estimation of sludge reduction and the comparison of process performance. The migration and transformation of dissolved organic matters (DOM) and nitrogen species were also analyzed to expound mechanisms of secondary substrate release and nitrogen removal. *Illumina-MiSeq* pyrosequencing analyses were applied to investigate the microbial community structure and population composition in the ASSR-MBR under different SRs.

## 2. Materials and methods

### 2.1. Experimental setup and operating conditions

Four pilot-scale MBRs, an AO-MBR (SR = 0%) and three ASSR-MBRs (Fig. 1), were fed with wastewater from the grit chamber of Dongqu WWTP (Shanghai, China). Three airtight tanks connected to the MBR were employed as the ASSR. Effective volume of anoxic tank, MBR and ASSR was 16.7, 50 and 50 L, respectively. During the experiment, the effluent was obtained by suction using a peristaltic pump connected to four flat-sheet microfiltration membranes with an average pore size of 0.2  $\mu\text{m}$  (300  $\times$  280 mm, Zizheng Environmental Inc., Shanghai, China) in the oxic zone. The membranes were made of polyvinylidene fluoride with polyester non-woven fabric as supporting material. The total effective filtration area was 0.25 m<sup>2</sup> for each MBR. An air diffuser was installed below the membrane module to supply oxygen with air flow rate controlled by a flow meter. The anoxic zone and ASSR were equipped with an agitator each to maintain sludge suspended. Four peristaltic pumps were employed to control the mixed liquor recirculation (MLR), while another three peristaltic pumps were used to deliver AS into the ASSR with a preset SR.

The four MBRs were inoculated with 6.2 g/L AS from Quyang WWTP (Shanghai, China). The wastewater was continuously fed into the four MBRs with permeate flux of 24 L/(m<sup>2</sup> h) for 90 days. Intermittent filtration mode (2 min pause for every 10 min of operation) was employed to mitigate membrane fouling. Mechanical cleaning and in-place chemical cleaning with 0.5% (v/v) NaClO solution were conducted for membrane module when the transmembrane pressure (TMP) monitored by a pressure gauge exceeded 30 kPa. The MLR ratio of AO-MBR was maintained at 250%. The side-stream recirculation rate of the three ASSR-MBRs was controlled at 20% (SR = 0.2), 50% (SR = 0.5) and 100% (SR = 1.0) of the influent flow rate, and thus their MLR ratio was

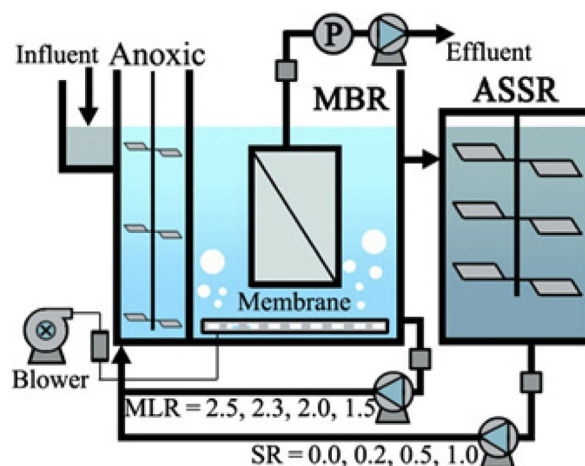


Fig. 1. Schematic diagram of the ASSR-MBR sludge reduction process.

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