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Shift in microbial community structure of anaerobic side-stream reactor in response to changes to anaerobic solid retention time and sludge interchange ratio

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HIGHLIGHTS

- The impact of SRT_{ASSR} and IR on the microbial community of an ASSR was investigated.
- A correlation between sludge reduction and microbial community has been discussed.
- A selection of fermenting bacteria was observed.
- An increasing selection of slow growing bacteria was detected.

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ABSTRACT

A laboratory scale nutrient removal activated sludge system coupled with an anaerobic side-stream reactor was operated for 300 days treating real urban wastewater. A significant decrease in sludge production was obtained increasing the anaerobic solid retention time (SRT_{ASSR}) and decreasing the sludge interchange ratio (IR). In this study, the microbial community structure was analyzed and compared with the sludge reduction performance. Quantitative polymerase chain reaction analyses encoding 16 ribosomal RNA and functional genes revealed a wide diversity of phylogenetic groups in each experimental period, resulting from long solids retention time and recirculation of sludge under aerobic, anoxic and anaerobic conditions. However, decreasing SRT_{ASSR} from 10 to 2.5 d and increasing IR from 27 to 100%, an increasing selection of both fermenting bacteria able to release extracellular polymeric substances and hydrolyze organic matter and slow growing bacteria involved in nutrient removal were detected and linked to the sludge reduction mechanisms.

1. Introduction

The more stringent regulatory limits imposed to guarantee high environmental standards caused an inevitable increasing of the production of excess sludge. The EU, the USA, and China each produce 6–11 million tons of sludge as dry solids per year; Australia produces 0.3 million tons each year (Semblante et al., 2016). The handling, treatment and disposal of sewage sludge are challenging waste management problems common to many countries that could account for 25–65% of the total plant operating costs (Chon et al., 2011a,b). In this view, the development of technologies able to reduce the sludge production within the conventional activated sludge (CAS) process inspired an overall rising interest, representing nowadays one of the first priorities in the waste management hierarchy. Among all, the insertion of an anaerobic side-stream reactor (ASSR) in the return sludge line of a CAS system could significantly enhance the sludge reduction. The rate of produced sludge passed through the ASSR is usually expressed as a percentage per day of the total mass present in the CAS, and it is known as sludge Interchange Rate (IR).

Over the last two decades, many studies demonstrated that the CAS-ASSR system can reduce the observed sludge yield (Y_{obs}) by up to 40% (Chudoba et al., 1992), 55% (Chen et al., 2003; Saby et al., 2003), 60% (Novak et al., 2007), 66% (Ferrentino et al., 2016b) as compared to a CAS process. Despite the good sludge reduction performance obtained without any negative effects on effluent quality

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and sludge settleability, the process is still little applied to full scale because the mechanisms leading to sludge reduction has not yet been fully understood (Ferrentino et al., 2016a). The lack of exact information causes uncertainty regarding the appropriate operating and design parameters, and thus a wide variation in sludge reduction efficiency.

A link theory that basically connects all the mechanisms proposed so far, linking the sludge decay, the cell lysis, the extracellular polymeric substances (EPS) destruction and the presence of slow growing microorganisms has been proposed in our previous study (Ferrentino et al., 2016b). In particular, the important role of slow growing bacteria, such as sulphate reducing bacteria (SRB) and denitrifying phosphate accumulating organisms (DPAOs) has been pointed out.

In the literature, several studies had investigated the microbial community in CAS-ASSR systems. The development of a specific microbial community in the ASSR seems to be of prime importance for sludge reduction process (Chon et al., 2011a). Chudoba et al. (1992) was probably the first to find that 60% of the microbial populations in the CAS-ASSR could be classified as phosphorous accumulating organisms (PAOs), contrary to 10% in the reference CAS system. The Authors showed that the periodic passageway of facultative aerobic activated sludge microorganisms through the anaerobic zone in the CAS-ASSR system created conditions of uncoupled growth. In the ASSR, under anaerobic conditions and in the absence of substrate, PAOs use adenosine triphosphate (ATP) and polyphosphates as a source of energy, then, in the water line, under aerobic conditions and in the presence of exogenous substrate, they rebuild their energy reserves at the expense of growth, resulting in a consecutive reduction of activated sludge production.

Wang and Zhao (2011) reported that most bacteria in their ASSR were phylogenetically related to PAOs, denitrifying bacteria and anaerobes. Chon et al. (2011a) showed about 75% similarity for microbial composition between a sequencing batch reactor (SBR) - ASSR system and an anaerobic digester. Recently, Zhou et al. (2015) showed that the insertion of an ASSR could enhance the selection of anaerobic bacteria such as fermentative, hydrogenogenic and acidogenic bacteria that are able to improve the biomass decay and hydrolysis of particulate organic matters. The Authors confirmed also the shift of the main microbial populations from fast growers to slow growers.

However, there is still insufficient literature on the correlation between the sludge production and the microbial community in the ASSR as function of design and operating parameters of the CAS-ASSR system.

For this aim a laboratory scale SBR – ASSR system was established for sludge reduction. The SBR-ASSR system was operated for 9 months under different design parameters: the anaerobic retention time in the ASSR (SRT_{ASSR}) and the sludge Interchange Ratio (IR). The estimation of the sludge reduction and the comparison of the microbial community population have been carried out.

2. Materials and methods

2.1. System operation

The laboratory scale system consisted of an anoxic -aerobic SBR for nutrient removal from real urban wastewater and a continuously mixed ASSR where the produced sludge was treated. A denitrifying side-stream reactor (DSSR) was introduced in the treatment scheme both to increase the solid concentration in the sludge to cycled back to the ASSR and to complete the nitrate removal in order to ensure a tightly anaerobic environment in the ASSR (oxidation – reduction potential (ORP) = −400 mV). Fig. 1 shows the SBR-ASSR experimental set-up and the system operations. The experimental design consisted of three different periods that lasted for about 90 days each: i) 27% IR and SRT_{ASSR} of 10 days; ii) 50% IR and SRT_{ASSR} of 5 days and iii) 100% IR and SRT_{ASSR} of 2.5 days.

The SRT_{ASSR} and the IR were calculated following the Eqs. (1) and (2):

$$IR(\%) = \frac{Q_{IN,ASSR} \cdot X_{IN,ASSR}}{V_{SBR} \cdot X_{SBR}} \quad (1)$$

$$SRT_{ASSR}(d) = HRT_{ASSR} = \frac{V_{ASSR}}{Q_{IN,ASSR}} \quad (2)$$

where $Q_{IN, ASSR}$ is the flow rate of the ASSR influent (L/d), $X_{IN, ASSR}$ is the solid concentration in the ASSR influent (g/L), X_{SBR} is the solid concentration in the SBR (g/L), V_{SBR} and V_{ASSR} are the volume of the SBR and the ASSR (L), respectively.

The SBR was inoculated with the activated sludge obtained from the CAS from the municipal wastewater treatment plant (WWTP) of Trento Nord (Italy), while the ASSR was inoculated with the anaerobic sludge from the ASSR implemented in the WWTP of Levico Terme (Italy). The SBR was fed with the effluent collected from the primary sedimentation tank of Trento Nord municipal WWTP, Italy.

2.2. Estimate of the observed sludge yield

The Y_{obs} was estimated by the ratio of the cumulative generated sludge to cumulative consumed substrate, in terms of soluble chemical oxygen demand (sCOD) (Chon et al. (2011b). Thus, the Y_{obs} values in each period were determined using a regression method with the obtained experimental data.

2.3. Quantification of total Bacteria, total Archaea and sulphate-reducing bacteria (SRB)

Quantitative polymerase chain reaction (qPCR) was used to assess the abundance of total Bacteria, total Archaea in each experimental period. Among Bacteria, specific qPCR-analyses were performed to estimate SRBs. For total Bacteria, a 466-bp fragment of the bacterial 16S rRNA gene (331–797 according to Escherichia coli position) was PCR-amplified with an universal primer set (Nadkarni et al., 2002). For total Archaea, a 417-bp fragment of the archaeal 16S rRNA gene was amplified with the primer pair Arch349F-Arch 806R (Takai and Horikoshi, 2000). For SRBs, a 396-bp fragment of the adenosine 5′-phosphosulphate reductase (apsA) gene was amplified with the primer pair aps3F-aps2R (Christophersen et al., 2011). All PCR reactions were performed in a total volume of 10 μL using the FluoCycleII Sybr reaction mix (Euroclone, Pero, Italy) with 0.3 μM of each primer, and an Eco Illumina thermocycler (Illumina, Inc., San Diego, CA, USA). The amplifications were carried out under the following conditions: 95 °C for 4 min, followed by 40 cycles of 95 °C for 15 s, annealing temperature for 30 s and 72 °C for 30 s, with the acquisition of the fluorescence at the end of each 72 °C elongation step. The annealing temperatures were set as 60 °C, 54 °C and 55 °C for Bacteria, Archaea and SRBs, respectively. The fragments of interest were amplified from reference strains (Escherichia coli K-12 substr. DH10B for bacterial 16S rRNA gene, Methanosarcina acetivorans C2A for archaeal 16S rRNA gene, and Desulfovibrio vulgaris subsp. vulgaris DSM 644 for aprA) and cloned into the plasmid pGEM®-T (Promega Corporation, Madison, WI, USA) to prepare standards for calibration curves. Plasmids were extracted from fresh cultures, and the concentration of plasmidic DNA was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies).