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Direct oxygen uptake from air by novel glycogen accumulating organism dominated biofilm minimizes excess sludge production

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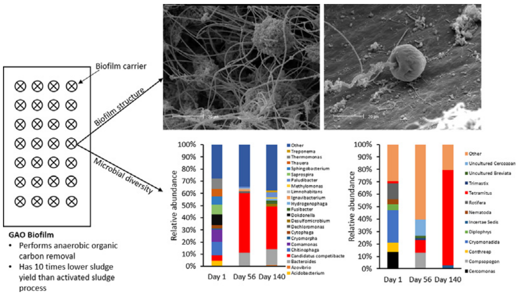
HIGHLIGHTS

- Reduced sludge production in novel GAO dominated biofilm system was elucidated.
- Sludge age and direct exposure of the biofilm to air facilitated low sludge yield.
- Role of microbial communities on minimized sludge production was discussed.
- High density of *Tetramitus* was shown to be associated with lower sludge production.

GRAPHICAL ABSTRACT



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ABSTRACT

The cost associated with treatment and disposal of excess sludge produced is one of the greatest operational expenses in wastewater treatment plants. In this study, we quantify and explain greatly reduced excess sludge production in the novel glycogen accumulating organism (GAO) dominated drained biofilm system previously shown to be capable of extremely energy efficient removal of organic carbon (biological oxygen demand or BOD) from wastewater. The average excess sludge production rate was 0.05 g VSS g^{-1} BOD (acetate) removed, which is about 9-times lower than that of comparative studies using the same acetate based synthetic wastewater. The substantially lower sludge yield was attributed to a number of features such as the high oxygen consumption facilitated by direct oxygen uptake from air, high biomass content (21.41 g VSS L^{-1} of reactor), the predominance of the GAO (*Candidatus competibacter*) with a low growth yield and the overwhelming presence of the predatory protozoa (*Tetramitus*) in the biofilm. Overall, the combination of low-energy requirement for air supply (no compressed air supply) and the low excess sludge production rate, could make this novel "GAO drained biofilm" process one of the most economical ways of biological organic carbon removal from wastewater.

1. Introduction

The conventional activated sludge process is by far the most widely used system to treat municipal and industrial wastewater. Despite its high organic carbon and nutrient removal efficiency, the activated sludge process has a major drawback such as generation of excessive sludge which contains active and inactive microorganisms and must be treated prior to its disposal to prevent adverse impacts on public health and the environment (Guo et al., 2013). Currently, excess sludge management is a rising concern for wastewater treatment plants around the world due to the increasing costs and restrictions associated with sludge treatment and disposal (Huang et al., 2014). The treatment of excess sludge is expensive and may take up to 60% of the plant's total operational cost (Campos et al., 2009). Therefore, the interest in sludge minimization is steadily increasing.

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Minimization of excess sludge volume can be done by several dewatering techniques. However, these processes do not reduce the actual solids content of the sludge. Sludge solids minimization strategies can be classified into two major categories: (i) sludge reduction through post-treatment process, and (ii) *in-situ* reduction of excess sludge during the wastewater treatment (Mahmood and Elliott, 2006; Wang et al., 2017). A number of different approaches, relying on single or a combination of mechanical, physical, chemical and biological methods have been employed to minimize the sludge generated in wastewater treatment facilities (Semblante et al., 2017; Wang et al., 2017). Mechanical sludge treatment methods such as high-pressure homogenizer (150–600 bar) and ultrasonic treatment (at 9–41 kHz for several seconds to 2.5 h), enhances the sludge degradation rate, but the amount of sludge reduced is often limited to <20% (Boehler and Siegrist, 2006; Wang et al., 2017). Thermal treatment (carried out at 165–180 °C for 30 min) of sludge improves both the sludge volume and its anaerobic digestibility (Wang et al., 2017). In addition to these technologies, chemical treatment methods such as ozone (O_3), hydrogen peroxide (H_2O_2) and alkali (NaOH) treatment can also enhance sludge degradability, thereby leading to improved sludge minimization. However, these sludge disintegration technologies significantly increase operating costs (Guo et al., 2013; Mahmood and Elliott, 2006; Semblante et al., 2017), and energy footprint of the wastewater treatment plant.

Anaerobic and aerobic digestions are the most common biological post-treatment methods which are implemented between the activated sludge and dewatering processes. Due to high operational complexity and expenses, anaerobic digestion is usually used in large wastewater treatment facilities where biogas co-production can recover the energy used. In contrast, aerobic digestion is typically applied in smaller treatment plants because of its operational simplicity. However, both treatment options suffer from disadvantages such as high initial investments and operational costs (Khursheed and Kazmi, 2011).

To save costs associated with the excess sludge management, it is preferable to reduce sludge production during the wastewater treatment processes (*in-situ*) rather than relying on post-treatment of the sludge produced. One approach that leads to lower sludge production is achieved by extending the solids retention time (SRT, also known as the sludge age) (Ghyoot and Verstraete, 2000; Tandukar et al., 2007). Several studies have shown that at longer SRT conditions, microorganisms use oxygen mainly for cell maintenance (endogenous respiration) rather than cell growth. Such endogenous respiration leads to sludge reduction (Sun et al., 2007). The SRT in biofilms is particularly high, which explains the lower sludge production in biofilm-based wastewater treatment systems compared with activated sludge processes. Furthermore, the long SRT of biofilms enables the growth of slow-growing microorganisms such as nitrifying bacteria (Bassin et al., 2015), anaerobic ammonia oxidizing (Anammox) bacteria (Tsushima et al., 2007). Additionally, biofilms have also been found to encourage the increased abundance of higher organisms such as eukaryotic predators in the active biomass components (Hao et al., 2010; Wang et al., 2017).

The presence of predators in the activated sludge and on biofilms has been known since the beginning of activated sludge technology. Predatory microorganisms are at the top of the food chain in the ecological system of wastewater treatment plants, and their concentration depends on the sludge retention time, food sources and wastewater composition. (Revilla et al., 2016). Among the predators commonly found in wastewater treatment plants, protozoa are the most abundant types which may constitute approximately 5% of the total dry-weight of activated sludge (Curds, 1982) and their abundance and diversity are considered as an indicator of process performance (Madoni, 2011). Protozoa help to shape the bacterial community within the niche by releasing mineral nutrients (carbon mineralization) and growth-stimulating compounds that can promote bacterial activity (Ratsak et al., 1996). In addition to these indirect effects, protozoa effectively graze on bacteria and inert particles; in this way, they have a significant role in sludge reduction and improving the wastewater treatment efficiency (Miyaoka et al., 2017; Ratsak et al., 1996).

The improved nutrient removal performance and stability of the biofilm-based wastewater treatment process such as the sequencing batch biofilm reactor (SBBR) are associated with its higher biomass concentration and increased SRT. The alternating anaerobic and aerobic phases used in a typical SBBR promotes the development of storage bacteria (e.g., polyphosphate accumulating organism, glycogen accumulating organism) which are able to uptake organic carbon (biological oxygen demand or BOD) from wastewater under anaerobic conditions and store intracellularly as poly-hydroxyalkanoates (PHAs). In a previous study, a passively aerated glycogen accumulating organism (GAO) dominated biofilm process was developed for energy efficient removal of organic carbon from wastewater (Hossain et al., 2017). In addition to stable performance, the described biofilm system showed little excess sludge production. However, it is not clear what factors contributed to this reduced sludge production.

The objective of this study is to quantify and explain the low excess sludge production observed in a GAO dominated, drained biofilm which is operated sequentially with anaerobic conditions (submersed biofilm) followed by aerobic exposure of the biofilm directly to air (passive aeration). The low sludge yield is verified by measuring the oxygen utilization by the biomass during the aerobic stage. Moreover, microbial community structure analysis and their potential role in reduced sludge production are also discussed.

2. Materials and methods

2.1. Experimental setup and operation

A cylindrical reactor with a working volume of 0.255 L was operated in this study (Fig. 1). The reactor was completely automated; with all pumps, airflow valves and phase lengths controlled by National Instruments Instrumentation Control Software LabVIEW™ (version 9.1). The reactor was filled with packing material (AMB™ Biomedia Bioballs), whose specific surface area for biofilm growth and support is 500 m^2/m^3. The carrier material is made from polyethylene - a non-porous polymer. These carrier materials have a cylindrical shape with 7 mm height and 11 mm diameter. The volume occupied by the empty carrier materials was about 20% ($V_{carrier}/V_{reactor}$).

Prior to operation, the described biofilm reactor was inoculated with activated sludge from a local wastewater treatment plant (Subiaco, Western Australia). After seeding, the biofilm reactor was operated automatically in a sequencing batch mode by specifically timed phases. The reactor was filled with synthetic wastewater (within 5 min through a peristaltic pump), then maintained under anaerobic condition for about 2 h. The anaerobic phase was followed by gravity drainage (10 min), and exposure of the biofilm directly to air, which was recirculated within the reactor for 1 h.

2.2. Synthetic wastewater

Synthetic wastewater was used to maintain reproducibility and enable direct comparison with many studies using the same wastewater. The use of this common synthetic wastewater has not been shown to result in lower sludge production. On the contrary, as all its BOD is readily bio-degradable and its composition provides all elements necessary for biomass growth a potentially higher cell yield than with real wastewater could be expected. The standard composition of the synthetic wastewater was (mg L^{-1}): CH_3COONa 660, NH_4Cl 160, KH_2PO_4 44, $NaHCO_3$ 125, $MgSO_4$. $7H_2O$ 25, $CaCl_2$. $2H_2O$ 300, $FeSO_4$. $7H_2O$ 6.25, yeast extracts 50, and 1.25 mL L^{-1} of trace element solution, which contained (g L^{-1}): EDTA 15, $ZnSO_4$. $5H_2O$ 0.43, $CoCl_2$. $6H_2O$ 0.24, $MnCl_2$. $4H_2O$ 0.99, $CuSO_4$. $5H_2O$ 0.25, $NaMoO_4$. $2H_2O$ 0.22, $NiCl_2$. $6H_2O$ 0.19, $NaSeO_4$. $10H_2O$ 0.21, H_3BO_4 0.014 and $NaWO_4$. $2H_2O$ 0.050.