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Roles of bacterial and epistylis populations in aerobic granular SBRs treating domestic and synthetic wastewaters

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HIGHLIGHTS

- AGS1 cultivated in SBR using real wastewater (WW) possessed high bacterial richness.
- Less bacterial diversity and richness found in AGS2 cultivated using synthetic WW.
- A high population of epistylis dwelled in the AGS1 favored granulation process.
- Epistylis population achieved organics degradation and removal from WW in AGS1.
- High bacterial and epistylis growth in AGS1 was due to particulate matters from WW.

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ABSTRACT

Aerobic granular sludge (AGS) has been recently studied and developed as a way to circumvent the poor settling and biomass retention capacity of conventional activated sludge. While it is known that AGS allows the simultaneous presence of oxic and anoxic zones on the surface or within granules, respectively, the actual composition dynamics of those granules in relation to their underlying wastewater influent have receive little attention. The main goal of this study is thus to assess the relationship between wastewater composition, microbial community structure and epistylis abundance. Two SBRs (sequencing batch reactors) comprising the same inoculum and fed with either domestic or synthetic wastewater were used in this regard. The more complex composition of domestic wastewater promoted a higher bacterial richness than their synthetic counterparts. Indeed, the vast majority of the bacterial community in the SBR fed with synthetic wastewater was dominated by the genus *Thauera*. The SBR fed with domestic wastewater also showed a more thorough granulation and greater treatment efficiency. Surprisingly, the abundance of epistylis was positively correlated with remaining suspended solids, hinting that ciliates might be responsible for SS (suspended solids) removal and would be a desirable trait to include in wastewater treatment plants. In sum, our study gives insight into the differing population dynamics shaped by domestic and synthetic wastewaters inoculated with the same initial consortium, along with their overall pollutant removal efficiency.

1. Introduction

AGS (aerobic granular sludge) has been widely studied in recent years due to its advantages over conventional AS (activated sludge), such as denser and stronger microbial structures coupled with a great settling capacity, high biomass retention, tolerance to toxic compounds and ability to withstand high organic matter loading rates [1–3]. AGS are distinct from biofilms as they develop by self-aggregation of microbes rather than by attaching themselves to carriers [4]. Previous studies have shown that most aerobic granulation is performed in lab-scale SBRs (sequencing batch reactors) with the use of synthetic substrates consisting mainly of acetate and glucose as carbon sources, rather than real wastewater. Studies reporting successful granule's formation using real wastewater include brewery, dairy, swine slurry, soybean-processing and domestic wastewaters as substrates [5]. This observation points out that aerobic granulation kinetics strongly vary between synthetic and real wastewaters [6–8]. Part of the explanation might lie in the higher diversity of carbon substrates, including organic and inorganic compounds, found in real wastewater. A higher diversity of carbon sources could thus support a more complex ecosystem as well

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as a higher biomass growth [9]. In fact, since synthetic wastewaters are typically composed of labile carbon sources, they preferentially favor copiotroph microorganisms with high growth rates. On the other hand, real wastewaters have fluctuating physicochemical characteristics, which imply that microbial communities can be composed of an heterogenous mix of metabolic types, ranging from oligotrophic autotrophs to heterotrophic copiotrophs. Granulation protocols must therefore be tailor-made for each and every WWTP (wastewater treatment plant).

Studies have shown that aerobic granule's formation is positively correlated with the proliferation of EPS-forming (extracellular polymeric substances) bacteria [10,11]. Reactor operating conditions as well as dominant microorganisms strongly influence granulation mechanisms [12], whereas microbial selection pressure is not mandatory for sludge granulation [13]. The microbial diversity observed in aerobic granules has been reported as strongly related to the composition of culture media as well as the observed granular structure [2]. Anaerobic microorganisms, including dead cells, were also found in aerobic granule's core [14].

AS of WWTP are diverse ecosystems, including various protozoa and metazoan populations such as vorticella, rotifers and epistylis in addition to a vast majority of bacteria [15,16]. Such microscopic eukaryotes ingest small particles in effluents such as SS, thus their presence has been used as an indicator of wastewater treatment performance [17,18]. While also dominated by bacteria, AGS contain a wide range of other microorganisms, including archaea, fungi, protozoa and metazoan [1,14]. Dominant bacterial populations are linked to overall granular sludge structure and pollutant removal potential [19], hence most previous work has focused on bacterial populations in AGS. However, Weber et al. [20] has shown that ciliates such as epistylis are able to attach to AGS when fed with malthouse, brewery or artificial wastewater. Similarly, Li et al. [21] has shown that populations of vorticella and rotifers played a key role in the establishment of aerobic granules in an SBR treating domestic wastewater. These studies have highlighted that the role of protozoa and metazoan in sludge granulation is crucial yet that it has been overlooked. Studies taking both bacteria and small eukaryotes communities into account are currently lacking.

Therefore, the goal of this study is to investigate the relationship between wastewater characteristics (synthetic or real wastewater) and microbial community composition, using 454 pyrosequencing for bacterial populations and SEM (scanning electron microscope) for epistylis populations.

2. Material and methods

2.1. Operation of SBRs

Two lab-scale SBRs using distinct wastewaters as substrates were operated during 200 days with a mean temperature of 25 ± 5 °C. The first reactor, R1, had a working volume of 11 L (0.5 m height, 0.2 m diameter) and was fed with domestic wastewater in order to cultivate AGS, or AGS1. Domestic wastewater was obtained from a septic tank located and operated in community of Zhejiang University of Technology. The second reactor, R2, had a working volume of 4 L (1.0 m height, 0.09 m diameter) and was fed with synthetic wastewater to cultivate AGS, or AGS2. Synthetic wastewater consisted of sodium acetate, NH_4Cl, KH_2PO_4, $FeSO_4{\cdot}7H_2O$, $CaCl_2$, $MgSO_4{\cdot}7H_2O$ and other necessary nutrients as specified in Garny et al. [22]. Characteristics common to both wastewaters are presented in Table 1. Both reactors, R1 and R2, operated six cycles per day, 4 h per cycle. Details on the distribution of operating time in each cycle are given in Table 2. Air pumps was used to diffuse oxygen and kept at 1.2 cm/s in both SBRs. Reactor pumps and valves were controlled by PLC (programmable logical controllers). Both SBRs were inoculated with the same activated sludge collected from an aeration tank of Qige domestic WWTP in Hangzhou city, China. The initial sludge had a fluffy, irregular and loose-structure of flocs. Mixed liquor suspended solids (MLSS) and sludge volume index (SVI_{30}) values were 1968 mg/L and 106.7 mL/g, respectively.

Table 1
Wastewater characteristics.

	COD[1] (mg/L)	NH_4^+-N (mg/L)	TP[2] (mg/L)	Particulate organic matter (%)
Domestic wastewater (R1)	350–450	20–30	4–8	48.7
Synthetic wastewater (R2)	800–1000	50–55	10–15	0

Note: Different range of COD, NH_4^+-N and TP values (fluctuations) was due to variations of domestic wastewater characteristics. Synthetic wastewater consisted of sodium acetate, NH_4Cl, KH_2PO_4, $FeSO_4{\cdot}7H_2O$, $CaCl_2$, $MgSO_4{\cdot}7H_2O$ and other necessary nutrients were readily biodegradable in the SBR.

[1] COD stands for chemical oxygen demand.

[2] TP stands for total phosphorus.

Table 2
Distribution of operating time in SBRs.

	Fill (min)	Aeration (min)	Settle (min)	Effluent (min)	Idle (min)
R1	5	180	3	15	37
R2	10	180	3	10	37

2.2. Bacterial community analysis in AGS

Mature and stable aerobic granules were selected randomly on day 70 from both SBRs (R1 and R2) in separate 2 mL sterile micro tubes. Samples were promptly stored at −20 °C for DNA extraction. Total genomic DNA from AGS1 and AGS2 was extracted using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, CA, USA) according to the manufacturer's instructions. V1-V3 hypervariable regions from bacterial 16S rRNA genes were PCR amplified from DNA using universal primers F (5′-AGAGTTTGATCCTGGCTCAG-3′) and R (5′-TTACCGCGGCTGCTGG CAC-3′). PCR amplification was performed using a Gene Amp PCR System 9700 thermal cycler (Applied Biosystem Inc., CA, USA) with 25 μL reaction volume per sample [(5 U Taq DNA polymerase (Takara Biotechnology Co., Japan), 1 × PCR reaction buffer, 2.5 mM dNTPs, 1 μL of each primer and 2 μL of DNA template]. The PCR amplification consisted of an initial DNA denaturation step at 94 °C for 5 min, followed by 27 cycles of denaturation step at 94 °C for 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for 60 s, and then a final elongation step at 72 °C for 7 min. PCR products were then purified using a Mini Elute PCR Purification Kit (Qiagen, Germany) and separated by electrophoresis on a 1.5% agarose gel. Bands corresponding to V1-V3 regions of 16S rRNA genes were then purified using a QIA quick Gel Extraction Kit (Qiagen, Germany). Purified PCR products were quantified using Nano Drop ND-1000 fluorescence spectrophotometer (Thermo Scientific, USA) and then pooled together to obtain a final concentration of 20 ng/μL. Pyrosequencing was performed using a Roche GS-FLX system at Personal Biotechnology Co. (Shanghai, China).

Sequencing data were analyzed using both Qiime (V1.9.1) (Quantitative Insights Into Microbial Ecology) and Mothur software (V1.30.1). Sequences shorter than 200 bp, longer than 1000 bp or containing ambiguous bases were first removed from the dataset. Following reads quality control (Any sequence with a length < 200 bp or > 1000 bp, and ambiguous bases were removed from the dataset), sequences were clustered into OTUs (Operational Taxonomic Units) with Qiime using a 97% sequence identity threshold. Diversity indices (Simpson and Shannon) and estimators (ACE and Chao1) were