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# Rapid formation and characterization of aerobic granules in pilot-scale sequential batch reactor for high-strength organic wastewater treatment

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ABSTRACT

This work presents the first attempt in the treatment of high-strength organic wastewater at organic loading rate (OLR) up to 30 kg/m$^3$ d in an aerobic granular sludge sequencing batch reactor. The reactor was operated for 100 days, divided into two main periods according to the applied OLR. In the first period, aerobic granules were cultivated and allowed to stabilize at an OLR of 10.2 ± 2.1 kg COD/m$^3$ d (from the reactor start-up until day 41). In the second period (from day 42 to day 100), the applied OLR was 27.0 ± 3.5 kg COD/m$^3$ d. Stable aerobic granules of average diameter of 1–2 mm dominated the reactor after 30 days, improving the settleability of the biomass. The COD removal efficiency was 98.4 ± 1.1% during the first 45 days of operation. However, after increasing the OLR, the COD removal efficiency decreased drastically to 64.4 ± 13.7% from days 46 to 64. Thereafter, the reactor recovered from the shock load and the removal efficiency increased to 96 ± 2.7% until the end of the 100 days. The effect of COD/N ratio was investigated for the treatment of nitrogen-deficient wastewater. It was shown that under severe nitrogen-deficient conditions (COD/N ratio > 70), the COD removal efficiency was 64.4 ± 13.7%. A COD/N ratio of 25–30 should be attained to ensure that there is no limitation in heterotrophic growth. The results from this study indicate that aerobic granulation can provide a promising high-strength organic wastewater treatment technology.

## 1. Introduction

With the continuous industrial development, massive quantities of high-strength organic wastewater are produced, which could cause a major threat to human and environmental health. The definition of high-strength organic wastewater is not clear cut and it is widely accepted that high-strength organic wastewaters were identified as those of COD (chemical oxygen demand) concentration greater than 4000 mg/L [1–3]. The treatment of high-strength organic wastewaters proves challenging due to the presence of excessive amounts of organics. Moreover, high-strength organic wastewater typically requires nutrients adjustment, and higher removal efficiency processes to meet the constantly rising environmental standards on treated effluents.

For many years, high-strength organic wastewaters were preferably treated in an anaerobic reactor producing low surplus sludge, and at the same time utilizing the elevated level of organic content for energy generation [3,4]. However, in practical applications, anaerobic treatment suffers from low growth rate of the microorganisms, high sensitivity to toxic loadings, low temperatures, pH changes and fluctuations in environmental conditions, a low settling rate of biomass, and the need for post treatment of the noxious anaerobic effluent which often contains ammonium ion ($NH_4^+$) and hydrogen sulfide ($HS^-$) [2,3,5,6]. Moreover, complete stabilization of high-strength organic matter cannot be achieved anaerobically; and, this results in effluent quality that usually fails to comply with the standards [7]. An aerobic post-treatment is usually required to bring the water quality within regulations, depending on the desired end use [8].

During the last 25 years, aerobic granulation has evolved and proved to be one of the most efficient biological wastewater treatment technologies. Such granules are spherical aggregates of microorganisms, without any media for attachment, offering dense and strong microbial structure, good settling ability, high biomass retention, tolerance to toxicity and resistance to shock loading, and can achieve rapid treatment of wastewater in a smaller footprint, when compared to floccular sludge cultures [9–25,53]. Aerobic granulation offers the unique advantage of the ability to be developed in a much shorter time, compared to 2–8 months for anaerobic granules [26], and the potential for simultaneous organics and nutrients removal [16,22,27,28]. This was attributed to the spatial structure of aerobic granule allowing for the co-existence of aerobic and anaerobic populations [22,29–31]. Aerobic granulation has been reported to withstand OLR up to 15 kg COD/m$^3$ d in sequencing batch reactors (SBRs) [22,32,33]. With the

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utilization of support material such as shell carriers, granules could withstand OLR up to 15 kg COD/$m^3$ d [34]. In a 330-day study using aerobic granular sludge in treating effluent from a seafood industry at OLR of 2–13 kg COD/$m^3$ d, it was reported that aerobic granules could withstand OLR only up to 4.4 kg sCOD/$m^3$ d without disintegration [35]. Adav et al. [19] reported that aerobic granules disintegrated at OLR of 21.3 kg sCOD/$m^3$. Long et al. [36] reported that aerobic granules lost stability at OLR of 18 kg/$m^3$ d due to the increase of the granule size, which resulted in the formation of massive dead cells inside the core of the granules, causing disintegration of the granular structures.

In addition, despite the report that the formation of aerobic granules is independent of the substrate concentration, the size of aerobic granules slightly increased with an increase in substrate concentration, while granule strength decreased with substrate concentration [37]. It was indicated that aerobic granules can withstand 5000 mg COD/L; however, high aeration rate of 3.2 cm/s is required at OLR 15 kg/$m^3$ d while aeration rate of 2.4 cm/s can provide stable granules up to 9 kg/$m^3$ d OLR [38]. Adav et al. [19] reported that the critical COD values for granule disintegration was 3000–4000 mg/L and that the tested isolates did not grow in the medium at COD > 3000 mg/L.

The long time required for granule formation and maturation, and granule disintegration remain unresolved problems of the aerobic granulation technology [25,39]. Moreover, the present available information with respect to the favorable operational conditions is not sufficient for predictable startup and operation of aerobic granular sludge reactor for treatment of high-strength organic wastewater. Therefore, this work aimed at examining aerobic granule cultivation, characteristics, and performance for the treatment of high-strength organic wastewater, particularly nutrient-deficient substrate. This work is designed to achieve the following three objectives: (1) to evaluate the performance in terms of treatment efficiency as well as granules physical properties and stability under high OLRs; (2) to investigate the effect of COD/N ratio on reactor performance; and (3) to examine the effect of pulse feeding and starvation conditions on granule stability under high OLRs.

## 2. Materials and methods

### 2.1. Experimental set-up and seed sludge

One cylindrical acrylic reactor with an internal diameter of 150 mm and a working volume of 16 L was used as the SBR to cultivate aerobic granules. Aeration was provided by means of fine air bubble diffusers located at the bottom of the reactor with an air flow rate of 28 L/min, which resulted in a superficial upflow air velocity of 2.8 cm/s. Influent was introduced through a port located at the bottom of the reactor while effluent was discharged through an outlet port placed at intermediate height of the reactor resulting in a volumetric exchange ratio of 56%. The reactor was operated at 4 h per cycle sequentially: influent filling (8 min), aeration, settling, and effluent withdrawal (2 min). The settling time was decreased from 20 min to 8 min in cultivation stage (first week of operation) with the remaining being aeration stage.

Return activated sludge (RAS) from Pine Creek Wastewater Treatment Plant in Calgary was used as inoculum to start up the system. The seed sludge was greyish brown in colour and had a suspended solids (SS) concentration of 5.1 g/L, sludge volume index (SVI) of 155.5 mL/g, and a mean particle size of 115 μm.

### 2.2. Media

The synthetic wastewater consisted of sodium acetate as sole carbon source, and the composition of wastewater was as follows: NaAc anhydrous, 2930 mg/L; $NH_4Cl$, 350 mg/L; $K_2HPO_4$, 30 mg/L; $KH_2PO_4$, 25 mg/L, and other necessary elements were similar to that detailed elsewhere [29]. This resulted in an initial substrate COD concentration of 2600 ± 450 mg/L and an OLR of 10.2 ± 2.1 kg COD/$m^3$ d for the reactor. After 41 days from start-up, the COD concentration was increased to 7500 ± 600 mg/L by proportionally adjusting the concentration of NaAc to attain higher OLRs of 27.0 ± 3.5 kg COD/$m^3$ d. Nitrogen and phosphorus concentrations were kept constant until 60 days of operation. From 60 days to the end of the experiment, nitrogen and phosphorus were supplemented at a COD:N:P ratio of 100:2.5:0.3 to meet the minimal growth requirements. The experiment was conducted at room temperature (18 ± 2 °C).

### 2.3. Analytical methods

Biomass characteristics – mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and $SVI_{30}$ – were analyzed in accordance with the standard methods [56]. For the measurement of sludge $SVI_5$, 5 min settling time was used instead of the 30 min used for the analysis of $SVI_{30}$ as described in Liu et al. [54]. Influent and effluent samples, filtered through 0.45 μm membranes, were measured for soluble COD (sCOD) and ammonia nitrogen ($NH_4^+$-N) by HACH DR/2400 (HACH Company) with HACH Kits following the USEPA reactor digestion method (COD HR and HR plus), and salicylate method TNT Plus 830, 831,832, and 833 (ULR, LR, HR, UHR), respectively. Phosphate ($PO_4^{3-}$) was analyzed using Metrohm Compact IC Flex. Mean particle size and size distribution were measured by a laser particle size analysis system with a measuring range from 0 to 2000 μm (Malvern MasterSizer Series 2000, Malvern Instruments Ltd.).

### 2.4. Microbial community analysis

Genomic DNA was extracted using a DNeasy PowerSoil Kit from QIAGEN, Inc. (MD, USA). Paired-end sequencing based on the 16S rRNA gene was performed at the Research and Testing Laboratory (Lubbock, TX, USA), using the Illumina MiSeq platform [40]. Primers 357wF (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) were used, which covered V3–V4 hypervariable regions [41]. Chimeras and poor quality sequences were removed from the denoised sequence reads. The remaining sequences were clustered into operational taxonomic units (OTUs) with 0% divergence using USEARCH. Taxonomic information was assigned to OTUs based on a database of high quality sequences derived from the NCBI using a distributed. NET algorithm that utilizes BLASTN+ (Kraken BLAST, www.krakenblast.com).

## 3. Results and discussion

### 3.1. Formation and characteristics of granules

#### 3.1.1. Settling property

The settleability of sludge is indicated by the sludge volume index (SVI). In general, flocculent sludge exhibits SVI values > 120 mL/g, while granular biomass offers considerably reduced SVI values (< 50 mL/g) [12,55]. The profile of SVI showed a declining trend during the operation as shown in Fig. 1(a). After 10 days of operation, granules started to be observed in the reactor. However, the biomass settleability fluctuated during the first three weeks of operation. After four weeks from start-up, stable and mature granules dominated, improving the settleability of sludge. SVI dropped to below 40 mL/gSS, and the $SVI_5$ started approaching $SVI_{30}$ (i.e., no compression settling) confirming stable granulation in the reactor. It was highlighted that a granular system is practically identified when the difference between $SVI_5$ and $SVI_{30}$ is within 10% [26,54].

#### 3.1.2. Biomass concentration

The MLSS maintained a rising overall profile during the operation as can been seen in Fig. 1(b). The biomass concentration in the reactor fluctuated between 3000 to 5000 mg TSS/L in the first 25 days of