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Effects of a higher hydraulic shear force on denitrification granulation in upflow anoxic sludge blanket reactors

Yaoqi Xue [a], Jianbo Guo [a,b,*], Jing Lian [a], Yuanyuan Zhang [a], Chao Zhang [a], Yue Zhao [a]

[a] Pollution Prevention Biotechnology Laboratory of Hebei Province, School of Environmental Science and Engineering, Hebei University of Science and Technology, Yuxiang Street 26#, Shijiazhuang city 050018, PR China
[b] School of Environmental and Municipal Engineering, Tianjin Chengjian University, Jinjing Road 26, Tianjin 300384, PR China

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ABSTRACT

The objectives of this study were to culture a more stable denitrification granular sludge and to investigate the effects of hydraulic shear force on the stability of the granular sludge. The stability consists of shear stability and removal stability, which are characterized by the shear sensitivity (Kss) and by the relative standard deviation of the specific nitrogen removal rate for three consecutive days (RSD-N_3), respectively. Two upflow granular sludge blanket (USB) reactors under different hydraulic shear conditions were used to culture granular sludge. The Kss of the mature granular sludge in USB_H ($G = 24.7\ s^{-1}$) and USB_L ($G = 14.5\ s^{-1}$) were 0.000024 and 0.0051, respectively. The USB_H only required 56 days to obtain mature granular sludge, whereas the USB_L required 70 days. These results indicated that higher hydraulic shear tended to shorten the granulation time and enhance the shear stability of the granular sludge. The RSD-N_3 of the USB_L during the maturation period was only 3.68%, which is approximately 32.84% of the value for the USB_H, indicating better removal stability for the nitrogen with the USB_L. SEM indicated bacillus bacteria were the largest component of the granular microbial community, and metagenomics using high-throughput sequencing identified *Methyloversatilis* and *Azospira* as the dominant microorganisms. These findings are important for the development of technologies in this field and have extensive applications in the denitrification of granular sludge.

1. Introduction

Nitrate has been identified as a contaminant in various bodies of water worldwide as a result of fertilizer application and animal waste disposal [1]. Nitrate contamination is a concern because it can cause eutrophication [2], which can lead to the growth of phytoplankton, subsequent hypoxia and fish kill. Biological denitrification is the preferred pathway for removing nitrogen pollution in water systems [3,4]. This method can convert nitrate-nitrogen (NO_3^--N) and nitrite-nitrogen (NO_2^--N) into nitrous and nitric oxide gases and eventually N_2 [5]. Over the past thirty years, the feasibility and efficiency of biological denitrification technologies for removing highly loaded nitrogenous effluents have been studied at length, and many novel technologies have been proposed [6,7]. The overall development of biological wastewater treatment is directed toward high efficiency, energy conservation and equipment miniaturization, and granular sludge is an important means for achieving this goal.

The stability of granular sludge is a significant factor in guaranteeing the properties of effluent in practice [8,9]. This parameter has recently attracted more attention in the field of biological wastewater treatment, and the shear and removal stabilities were found to correspond to certain physicochemical characteristics and removal efficiencies. Unstable granular sludge phenomena have also been regularly reported in previous studies [9,10]. However, the complexity of sludge constituents and their chaotic structures make determining the strength and stability of sludge difficult [8]. Consequently, it is important to accurately characterize the stability [11]. Many studies have attempted to confirm the critical factor for sludge stability and its mechanism of action. To characterize the shear stability of sludge, Mikkelsen and Keiding established an adhesion-erosion (AE) model based on the Langmuir adsorption isotherm theory [12], and Chou et al. utilized the relative standard deviation of a specific nitrogen removal rate ($gN\,gVSS^{-1}\,d^{-1}$) for three consecutive days (RSD-3) to characterize the steady state of a sludge [13]. Although our knowledge is still insufficient, it

* Corresponding author at: Hebei University of Science and Technology, Pollution Prevention Biotechnology, Laboratory of Hebei Province, School of Environmental Science and Engineering, Yuxiang Street 26#, Shijiazhuang 050018, PR China. Fax: +86 311 81668439.
E-mail address: jianbguo@163.com (J. Guo).

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is well known that the hydraulic shear force has an important effect on the sludge culture and the reactor's operation [14,15]. In practice, this force can be controlled using engineering approaches [16]. However, unlike floc sludge and aerobic granular sludge studies, previous studies on the effect of the hydraulic shear force on the stability of anoxic granular sludge have only investigated the macroscopic behavior of the material [16], especially under a higher hydraulic shear force environment. Indeed, the majority of these studies only described the minimum shear strength for achieving sludge granulation [17] and only considered the lower hydraulic shear force condition [8,11,16,18]. The effects of hydraulic shear force on the stability of granular sludge in terms of EPS and surface characteristics on the microcosmic level are not well understood for anoxic granulation [19]. In addition, little is known about the effect of the hydraulic shear force on the removal stability.

Accordingly, the primary objectives of this study were to culture a more stable denitrification granular sludge, to investigate the effect and influencing mechanism of higher hydraulic shear forces on the granular sludge's stability, to confirm the specific relationship and the mechanism between the shear stability and the sludge's surface characteristics and to understand the effect of the hydraulic shear force on the removal stability. For these purposes, different hydraulic loads were used to culture granular sludges with higher shear stabilities in two USB reactors, with the other culture conditions held constant. In addition, the effect of the sludge's microbial community structure on its stability was also investigated.

2. Materials and methods

2.1. Reactor, wastewater and seed sludge

Two identical Plexiglas USB column reactors were used to culture the denitrification granular sludge. The USB reactor was 60 cm in height with an internal diameter of 6.8 cm, and it was equipped with a three-phase separator. To investigate the effects of the hydraulic shear force on the sludge, the two USB reactors were operated a low rate ($14.5\,s^{-1}$) and at a high rate ($24.7\,s^{-1}$) and were named USB_L and USB_H, respectively. Each reactor had a working volume of 3.5 L. The influent was synthetic wastewater, which contained the following: chemical oxygen demand (COD) by methanol of 200–2000 $mg\,L^{-1}$ and NO_3^--N by sodium nitrate of 50–500 $mg\,L^{-1}$. The C:N:P was maintained at 40:10:1, and the P source was KH_2PO_4. The organic loading rate (OLR) and the nitrogen loading rate (NLR) were identical in both reactors, and both the COD and the nitrate concentration in the USB_H were half of that in the USB_L at any given time. To achieve the half COD and nitrate concentration in the USB_H, an identical quantity of methanol and sodium nitrate were added to a two-fold greater volume of influent. The other reagent quantities were also identical, except for the microelement solution. A microelement solution ($1.0\,mL\,L^{-1}$) was added to the influent, which contained the following in $mg\,L^{-1}$: $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$, 63.7; $ZnSO_4$, 5.5; $FeSO_4{\cdot}7H_2O$,5.0; $MnSO_4$, 4.3; $ZnSO_4$, 2.2; $CoCl_2{\cdot}6H_2O$, 1.6; $CuSO_4{\cdot}5H_2O$, 1.6; and $Na_2MoO_4{\cdot}2H_2O$, 1.6. Flocculent seed sludge was collected from the return sludge thickening tank at the Qiaoxi Municipal Wastewater Treatment Plant, Shijiazhuang, China, in which the mixed liquor suspended solids (SS) and the mixed liquor volatile suspended solids (VSS) were 12.05 $g\,L^{-1}$ and 4.67 $g\,L^{-1}$, respectively.

2.2. Bioreactor operation

The experiments were conducted over 70 days for the USB_L and 56 days for the USB_H in three periods (i.e., the adaptive, multiplication and maturation periods). The NLR was considered to increase in range of 0.36 $kgN\,m^{-3}\,d^{-1}$ to 3.6 $kgN\,m^{-3}\,d^{-1}$ when the removal rate deviations of the COD and the nitrate stabilized within 5% for three consecutive samples. To obtain the desired hydraulic shear force, the reflux ratio was set to 200%, and the wastewater was recycled from the top effluent. The upflow velocities were 0.87 $m\,h^{-1}$ and 1.67 $m\,h^{-1}$ in the two reactors, respectively. The experiments were performed in a temperature-controlled cupboard at $30\,^\circ C \pm 5\,^\circ C$.

The hydraulic shear rate (G) was used to represent the hydraulic shear force at the reactor level [8,9]. The estimated hydraulic shear rate of the USB reactors was calculated using the following model (Eqs. (1) and (2)):

$$G = \left(\frac{2\psi}{\eta}\right)^{0.5} \tag{1}$$

$$\psi = \frac{\left(\rho_s\epsilon_s(\nu_L+\nu_g) - \rho_L\nu_L(1-\epsilon_L) + \rho_L\nu_g\epsilon_L\right)g}{\rho_L\epsilon_L} \tag{2}$$

where G is the hydraulic shear rate, s^{-1}; ψ is the volume energy dissipation, $W\,m^{-3}$; η is the kinematic viscosity, $m^2\,s^{-1}$; ρ_s is the density of the sludge, $kg\,m^{-3}$; ρ_L is the density of the liquid phase, $kg\,m^{-3}$; ϵ_s is the volume ratio of the solid phase in the reactor; ϵ_L is the volume ratio of the liquid phase in the reactor; ν_L is the superficial liquid velocity, $m\,s^{-1}$; ν_g is the superficial gas velocity, $m\,s^{-1}$; and g is the gravitational acceleration.

2.3. Shear sensitivity analysis

The shear sensitivity of the sludge was characterized by the parameter Kss, which is defined in Eq. (3):

$$Kss = \frac{m_{d,\infty}}{m_T} \tag{3}$$

where $m_{d,\infty}$ is the dispersed particle concentration of the small particles, as defined by Mikkelsen and Keiding [12]. $m_{d,\infty}$ was measured when the shear experiments reached equilibrium, in which the total SS of the granular sludge sample (m_T) was 5 $gSS\,L^{-1}$ at $G = 800\,s^{-1}$. A baffled paddle-mixing cylindrical reactor with dimensions of 58.2 mm × 83.2 mm (inner diameter × height) was used to perform a shear test. This reactor was placed in a stink cupboard at a room temperature of $30\,^\circ C \pm 5\,^\circ C$.

Five-milliliter samples were used for turbidity measurements after the reactor was stirred for per 30 min. The turbidities of the samples were determined from the absorbance of the suspended substances at 650 nm, which was measured after a 2 min centrifugation at 2200 rpm. The dispersed particle concentration was then calculated using the turbidity SS-concentration^{-1} conversion factor of 1.2 $mgSS\,L^{-1}\,FTU^{-1}$, which was reported previously [12].

2.4. Other analysis methods

The COD, nitrate, nitrite, MLSS and MLVSS were measured using standard methods [20]. The EPS of the sludge was extracted using the formaldehyde + NaOH method [21]. Extracellular protein and carbohydrate concentrations were determined using the Bradford method [22] and the anthrone-sulfuric acid method [23], respectively. The extracellular DNA of the EPS was quantified by ultraviolet spectrophotometry at 260 nm [20]. The hydrophobicity of the microbial cells was calculated using the relative hydrophobicity method [11]. The Zeta potential was measured using a Zeta potential instrument (Malvern Zetasizer Nano-S90).