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Cultivating granular sludge directly in a continuous-flow membrane bioreactor with internal circulation

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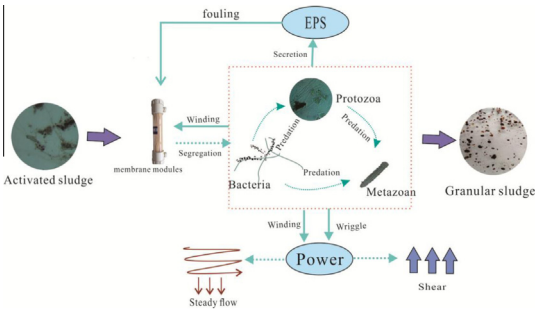
HIGHLIGHTS

- An MBR with internal circulation was used to cultivate granular sludge.
- Granular sludge was cultivated directly in this MBR in a continuous-flow mode.
- The resulting granular sludge was characterized by multi methods.
- Granular sludge remained stable for a long-term operation.
- Essential factors to influence the granulation process were illustrated.

GRAPHICAL ABSTRACT



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ABSTRACT

This research was conducted to cultivate granular sludge directly in a continuous-flow membrane bioreactor and explore the main factors influencing the granulation process. By establishing a suitable internal hydrodynamic circulation in a membrane bioreactor, granular sludge (GS) was successfully cultivated into mature granules with a compact structure and clear shape, in which extracellular polymeric substances played an important role in maintaining its integrity. The results showed that the main factors to determine the cultivation of GS included the total retention of sludge by the membrane module, the internal hydrodynamic circulation, and the entanglement of filamentous bacteria to sludge particles. Filamentous bacteria initiated a granulation process under the action of internal circulation, and maintained the stability of GS for a long period by wrapping biomass aggregates together. Even though filamentous bacteria were the major dominant microbial species in the bioreactor, the microbial community was richly biodiverse, and was responsible for the removal of organic pollutants and nutrients. Overall, the results demonstrated an alternative option for cultivating stable GS directly in a continuous-flow membrane bioreactor.

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1. Introduction

Granular sludge (GS) is a collective of numerous self-immobilized cells in which a variety of functional microorganisms gather together to form microbial aggregates that have a tightly compact structure and diversified microbial communities. These aggregates are the main body that degrade various organic pollutants and convert nutrients from wastewater in a bioreactor [1]. A granular sludge bioreactor (GSBR), mainly composed of GS, possesses many dominant merits [2,3], including abundant microbial biodiversity, high retainable biomass concentration, large relative density, low sludge yield, excellent sludge settling ability, and robust ability to withstand high organic loading rate, which make

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it a promising means for treating both industrial and domestic wastewater [4,5], and attract many academic interests [2,6–8]. The successful implementation of a GSBR totally depends on the cultivation and proliferation of sufficient GS in the bioreactor. However, many factors have different impacts on the formation and the stability of GS, which lead to a very complex mechanism in forming stable and efficient GS [9].

In the past two decades, many studies have focused on exploring the effects of various operational parameters on the granulation process in sequencing batch reactors (SBR) or other reactors; these factors include hydrodynamic shear force (aeration intensity or stirring intensity), hydraulic retention time (HRT), reactor design, settling time, substrate composition, organic loading rate, and seed sludge [1,10,11]. Thus far, anaerobic and aerobic granular sludges have been successfully cultivated in up-flow anaerobic sludge bed reactor (UASB) [7] and SBR [1], and the latter has been regarded as the most successful way [4,8,9,12] to cultivate GS even in a full-scale municipal wastewater treatment plant (WWTP) [5]. From the prospective of an engineering application, the direct cultivation of GS in a continuous-flow reactor is more favorable than in a batch process due to its lower costs and more convenient operation. However, because continuous-flow reactors lack many conditions that are crucial for sludge granulation (such as alternating feast and famine conditions, and hydraulic selection pressure, among others), it is still very difficult to cultivate GS directly and maintain its long-term stability in a continuous-flow operating mode [8].

Combining granular sludge with a membrane bioreactor (MBR) creates a granular membrane bioreactor (GMBR). Such a new type of MBR can be operated in a continuous-flow mode [13] and also is a promising solution to mitigate membrane fouling [14] – a long-standing obstacle that still limits the application of MBRs [15,16]. Recently, Corsino et al. [17] used an MBR to investigate the stability of GS seeded from a column-type SBR, and showed undeniable difficulties for both the granule formation and the maintenance of GS in a continuous-flow mode. For maintaining the long-term stability of GS in a continuous-flow reactor, adding calcium and iron salts to form a strong granular core was verified to be effective [18], but the occurrence of inorganic salts in an MBR might also increase the possibility of permanent membrane fouling. To the best of our knowledge, almost all the reported GMBR must be inoculated with seed granular sludge from an SBR to start up the operation [19]. The direct cultivation of GS in a continuous-flow MBR is highly desirable, but is still quite difficult due to the complex granulation mechanism.

In regards to the formation mechanism and the stability of granular sludge, there still exist considerable disputes about the role of filamentous bacteria. Studying a continuous stirred tank reactor, Morales et al. [20] found that granular-type biomass could form under certain suitable conditions, but the presence of filamentous bacteria caused a failure in the formation of granular sludge. For inhibiting the negative effect of overgrowth of filamentous bacteria on the structural stability of granular sludge during long-term reactor operation, keeping the suspension alkaline was verified to be effective [21]. So far, several reports have demonstrated that the overgrowth of filamentous bacteria can cause poor settleability of granular sludge, resulting in sludge being readily washed out from the bioreactor and eventual failure of a GSBR [22,23]. However, other research has drawn a contradictory conclusion about the effect of filamentous bacteria on the formation of granular sludge. Li et al. [24] successfully cultivated compact granular sludge in an SBR even with the presence of filamentous bacteria, but adding 5% sodium chloride solution in the bioreactor was a prerequisite condition. Wang et al. found that numerous filamentous bacteria wrapped other bacteria together to form granules [25] with inorganic crystal-like material at the center to act as a nucleus. Based on this finding, they proposed a string-bag hypothesis to explain the formation mechanism of granular sludge. Figueroa et al. [26] compared the performance of granular sludge in three SBRs fed with different wastewater as influent, and revealed that filamentous bacteria acted as a structural backbone of granular sludge. Filamentous bacteria were also observed to play a key role in the formation of granular sludge in a conventional, continuous-flow completely mixed activated sludge system [27]. These investigations indicated that, under different scenarios, filamentous bacteria might either initiate the sludge granulation process or cause sludge bulking, with completely opposite effects on the formation of granular sludge and the successful operation of a GSBR.

This research aimed to cultivate GS directly in a continuous-flow MBR and explore the main factors influencing the granulation process. Through an experiment lasting 110 d, two closely related aspects of GS formation were mainly investigated: (1) cultivating GS directly in a continuous-flow membrane bioreactor; (2) characterizing the resulting GS through multiple methods and further illustrating the granulation process. These methods included a conventional optical microscope, scanning electron microscope (SEM), confocal laser scanning microscope (CLSM), and high-throughput sequencing (HTS). We hope the presented results would provide a useful reference for developing a convenient and cost-effective approach to cultivate GS directly and operate this bioreactor steadily in a long-term running.

2. Materials and methods

2.1. Bioreactor configuration and experimental conditions

The MBR used in the experiment was a rectangular bioreactor with an effective working volume of 36 L, which was divided into two chambers. One chamber, with about one-third of the total working volume, was an aeration zone. A membrane module (hydrophilic PVDF hollow membrane with a pore size of 0.22 μm and surface area of 0.5 m^2, MOF-Ib, Tianjin Motimo Membrane Technology Co., LTD, Tianjin, China) was mounted in the middle of this chamber with an aerator fixed below to release air bubbles and push the fluid upward. The other chamber was a mixing zone with a rotator mounted in the middle to provide mixing and drive the mixed liquor downward. With the simultaneous upward flow in the aeration chamber and downward flow in the mixing chamber, an internal hydrodynamic circulation was formed to circulate the fluid within the bioreactor. This configuration constituted a new kind of bioreactor – an internal circulation membrane bioreactor (IC-MBR). The configuration and controlling devices of the IC-MBR are shown in Fig. 1, and the operational conditions are listed in Table 1.

In the experiment, the used synthetic wastewater was prepared as substrate for the bioreactor by dissolving chemically pure glucose (103.42–372.31 mg/L), and other nutrients, including NH_4Cl (19.20–69.10 mg/L), KH_2PO_4 (4.41–15.90 mg/L), $NaHCO_3$ (160 mg/L), $MgSO_4$ (40 mg/L), $MnSO_4$ (12 mg/L), $CaCl_2$ (8 mg/L), and $FeSO_4$ (0.6 mg/L), in tap water. The synthetic wastewater had a carbon (as chemical oxygen demand, COD), nitrogen and phosphorus ratio (COD:N:P) of 100:5:1. Inoculated activated sludge was taken from the secondary sedimentation tank of a local WWTP (Lijiao municipal wastewater treatment plant, located in Haizhu District, Guangzhou, China). The initial concentration of the inoculated activated sludge was controlled at approximately 2500 mg/L mixed liquor suspended solids (MLSS), and the bioreactor was started up by feeding the prepared synthetic wastewater. The whole experimental period lasted for 110 d, which was divided into four phases (Phase 1, days 1–6; Phase 2, days 7–15; Phase 3, days 16–

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