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Influence of three selection pressures on aerobic granulation in sequencing batch reactor

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Aerobic granulation is a promising biotechnology for wastewater treatment. The formation and development of granules and the pollutant removal are closely associated with hydrodynamic shear force, settling time and volume exchange ratio (VER), which are proved to be the selection pressures. Three different values of shear force (2, 3 and 4 cm/s), settling time (3, 5 and 10 min) and VER (25, 50 and 75%) have been experimented in sequencing batch reactor (SBR) in seven trials for the optimal performance of aerobic granulation. Higher shear force could develop smaller and more compact granules with better settling behaviour, but chemical oxygen demand (COD) removal is not found to improve beyond a value of 97.9% for shear force above 3 cm/s. A settling time as low as 3 min could result in very good biomass growth and formation of granules with sludge volume index (SVI) as low as 23 ml/g. Aerobic granulation is not successful at a long settling time of 10 min. Higher the VER, the lower is the SVI and the more efficient is the COD removal.

Keywords: Aerobic granulation, Hydrodynamic shear force, Selection pressure, Settling time, Volume exchange ratio.

The wastewater researchers have been focusing on the effective separation of biomass from the treated effluent because it determines the efficiency of a treatment system. Two well-known and conventional forms of microbial immobilization are suspended flocs and attached biofilms[1]. Microbial granules are a more recent form of cell immobilization. Aerobic granular technology is considered to be superior to conventional activated sludge process in terms of efficiency and economy for treating high strength and low strength wastewater[2-4]. Compared to the activated sludge flocs, aerobic granular sludge features many advantages, such as a denser and stronger microbial structure, a better settling ability, more ability to get separated from the liquid media, greater biomass retention and enrichment, and a greater capability to withstand shock loadings[5,6].

Successful development of aerobic granules was first reported by Mishima and Nakamura (1991) in a continuous aerobic upflow sludge blanket reactor[7]. Later many researchers cultivated aerobic granules in sequencing batch reactors (SBRs) using a wide range of substrates under various operating conditions[5,8-12]. To date, most research on aerobic granulation has been done in sequencing batch reactors (SBRs); however, the mechanisms responsible for the formation of aerobic granules are still unclear[13].

Granulation was reported to be affected by a number of operational parameters, such as seed sludge, substrate composition, organic loading rate (OLR), feeding strategy, reactor design, settling time, exchange ratio, and aeration intensity (hydrodynamic shear force). But it was reported by Liu (2008) that only the parameters associated with selection pressure on sludge particles can contribute to the formation of aerobic granules[14].

Aerobic granules have been successfully cultivated with a wide variety of substrates, however, granule microstructure and species diversity appear to be related to the type of carbon source[15]. Although OLR has a significant role in the formation of anaerobic granules, it was reported that aerobic granules can form across a very wide range of OLRs from 2.5 to 15 kg chemical oxygen demand (COD)/(m^3 d)[3,16]. It seems that aerobic granulation is not sensitive to the organic loading rate. Although organic loading rate has not much significance in the formation of aerobic granules, it can affect the physical characteristics of the granules. Similarly it was reported that feeding strategy may influence the characteristics of aerobic granules, but it is unlikely to play a role in triggering aerobic granulation process[17].

Different researchers experimented and established various factors as selection pressures for aerobic granulation. Liu *et al*., (2005) proposed a selection pressure theory by which the three identified key parameters, namely, settling time, volume exchange ratio (VER) and discharge time, can be considered for minimum settling velocity approach[18]. Many researchers concluded by experimental evidences that settling time is a major hydraulic selection pressure for the formation of aerobic granules[13,17,19,20]. VER of SBR could be manipulated as an effective selection pressure for a successful aerobic granulation and the granulation process would be accelerated and enhanced when operated at high VER[21]. Hydraulic shear force in terms of upflow air velocity was proved to be a selection pressure for the successful granulation[12,19,22-25].

Individual studies have been conducted to understand the effect of various selection pressures in the aerobic granulation process, but experimental analysis combining all the possible selection pressures are found scanty. Therefore considering the effects of settling time, volume exchange ratio and hydraulic shear force, it is feasible to apply a combination of selection pressures. This paper attempts to look into the effects of settling time, VER and hydraulic shear force as selection pressures in the aerobic granulation process. This work could contribute to a better understanding of stable operation with aerobic granules at optimal operational parameters.

Experimental Section

Experimental Set-up and Operation

A column type SBR of effective volume of 2 litres was used for the study. The internal diameter and the effective height of the reactor were 6.5 cm and 60.3 cm respectively. Influent was admitted through the bottom and the effluent was withdrawn from the sampling ports provided by means of two peristaltic pumps. Air was introduced from the bottom using an air compressor and a porous stone diffuser. As the first phase, the reactor was operated in successive cycles of 4 hours, which is comprised of 5 min for feeding influent, 225 min for aeration, 5 min for settling and 5 min for effluent withdrawal., The cyclic operation was controlled by a micro controller AT89C51. Schematic of the SBR is shown in Fig. 1.

Influent and Seed Sludge

The synthetic wastewater consisted of sodium acetate as the sole carbon source was used for the study. The composition of synthetic wastewater used for this study is as follows:

Sodium acetate-2.93 g/L, NH_4Cl-350 mg/L, K_2HPO_4-30 mg/L, KH_2PO_4-25 mg/L, $CaCl_2.2H_2O$-30 mg/L, $MgSO_4.7H_2O$-25 mg/L, $FeSO_4.7H_2O$-20 mg/L, and microelement solution 1.0 mg/L. It gives a total influent chemical oxygen demand (COD) of 2000 mg/L and an organic loading rate of 6 kg COD/m^3 d. Microelement solution contained: H_3BO_3-0.05 g/L, $ZnCl_2$-0.05 g/L, $CuCl_2$-0.03 g/L, $MnSO_4.H_2O$-0.05 g/L, $(NH_4)_6\ Mo_7O_{24}.4H_2O$-0.05 g/L, $AlCl_3$-0.05 g/L, $CoCl_2.6H_2O$-0.05 g/L, $NiCl_2$-0.05 g/L.

The seed sludge was collected from the activated sludge processing unit of the Petrochemical Division of Fertilizers And Chemicals Travancore (FACT) Limited, Cochin, Kerala. The sludge had a gray colour with sludge volume index (SVI) of 245 mL/ g and mixed liquor suspended solids (MLSS) of 5050 mg/L.

Operational Strategy

The granulation process in SBR was investigated for three different operational strategies. Hydraulic shear force, settling time and VER were taken as the variables in the operating conditions. Standard operating conditions were decided, shear force as 3 cm/s, settling time as 5 min and VER as 50% based on literature survey. One upper value and lower value for each case were selected for the investigation. Towards the first phase, the SBR was operated for varying degrees of air flow-4 L/min, 6 L/min and 8 L/min with resulting upward velocity of 2, 3 and 4 cm/s respectively. In the next phase, the settling time

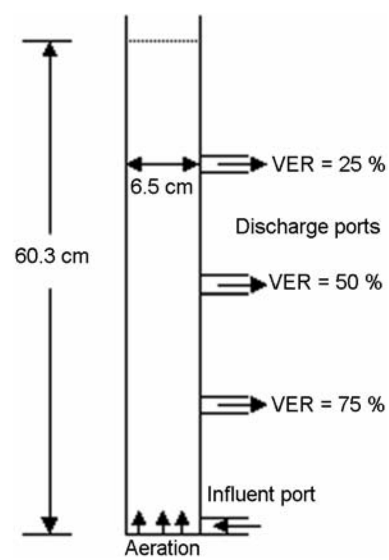

Fig. 1—Schematic diagram of the SBR

was varied from 10 to 3 min. In each run the total cycle time was maintained as 4 h, keeping the feeding time and discharge time as 5 min each, and slight changes in the settling time was adjusted in the aeration time. The effect of VER was studied with three values- 25, 50 and 75%. The operational conditions can be summarized as in Table 1. While experimenting with one parameter, other two parameters were kept constant. The *p*H was maintained in the range 6 to 8.5.

Seven trials were made to study the influence of selection pressures on aerobic granulation. Trial 1, 2 and 3 were used to study the influence of hydraulic shear force, trial 2, 4 and 5 to study the influence of settling time and trial 2, 6 and 7 to study the influence of VER on aerobic granulation. All other operating parameters except rate of air flow, settling time and VER were kept constant for the present study. For each trial the reactor was started with 750 mL of seed sludge.

Analytical methods

Determination of COD, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) were performed in accordance with standard methods[26]. Samples from the reactor were collected and analyzed for pH, COD, MLSS, and MLVSS daily and SVI on alternate days. *p*H and the dissolved oxygen (DO) concentration were monitored using a *p*H meter (Cyberscan *p*H-510) and DO meter (Cyberscan DO-110) respectively. The settling velocity and specific gravity of sludge were measured as reported by Zheng *et al*., (2006)[27]. Olympus BX 51 fluorescent microscope and Olympus FE 220 were used for image analysis of granules.

Table 1—Variables in the operating conditions

Hydraulic shear force (cm/s)	Settling Time (min)	VER (%)	Trial No
2	5	50	1
3	5	50	2
4	5	50	3
3	3	50	4
3	10	50	5
3	5	25	6
3	5	75	7

Results and Discussion

Influence of upflow air velocity

Keeping the settling time as 5 min and VER as 50%, the SBR was operated for three different superficial upflow air velocities, i.e., 2 cm/s, 3 cm/s and 4 cm/s (trial 1, 2 and 3). In a column- type SBR, the superficial upflow velocity of air is a major cause of hydrodynamic turbulence and thereby hydraulic shear force. In each case the reactor was seeded with activated sludge with mean floc size of 0.1 mm. The evolution and growth of granules were monitored by image analysis. In trial 2 with upflow air velocity 3 cm/s, first granule appeared on 17th day and gradually grown and matured by 23rd day with an average size of 2.4 mm. In trial 1 and 3, first appearance of granules was on 21st day and 14th day respectively with matured size of granules as 2.6 mm and 1.5 mm respectively. The development of granules from the seed sludge was observed closely and the morphological variation of the sludge in trial 2 was demonstrated by Fig. 2 (a-c). Figure. 3 showed the comparison of typical granules developed at various shear forces. Performance of the reactor under varying operating conditions is given in Table 2.

The biomass concentration in the reactor was also found to have an increasing trend as the shear force was increased. The ratio of MLVSS to MLSS (MLVSS/MLSS) in the first trial was only 0.83 towards the end of the operation, but for trial 2 and 3, it reached to 0.91 which showed a healthy condition. It shows that the development of biomass is efficient

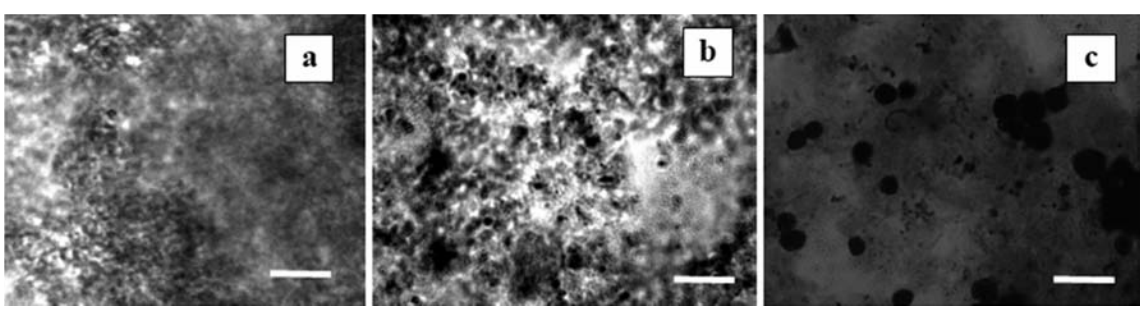

Fig. 2—Morphological variation of the sludge during the experimental period (a) seed sludge at start-up (b) on 7th day (c) on 14th day for trial 2; (bar = 2 mm)

only with higher upflow air velocities. The seed sludge had a SVI of 245 mL/g. In all the three trials, SVI decreased after the start up. After the formation of aerobic granules, it further decreased and reached stable values (40, 25 and 24 mL/g for trial 1, 2 and 3 respectively). Density, and hence the settling velocity of the granules were increased with the upflow air velocity (38.4, 55.2 and 63.6 m/hr for trial 1, 2 and 3 respectively). It is evident from the results that higher upflow velocity favours an early formation of denser and more compact granules in an SBR. Tay *et al.*, (2001) also reported that a certain shear force is necessary for aerobic granulation; and high shear force seems to favor the formation of more regular and compact granules[22]. Other attempts to study the influence of shear force showed that particle size and SVI value were also decreased along with the increase of shear force. It indicated that the high hydraulic shear force was beneficial to develop dense granules and improve sludge settling ability[23, 24].

The influent COD concentration was 2000 mg/L. The effluent COD at steady state was compared with influent COD for determining COD removal efficiency. The COD removal efficiencies for trial 1, 2 and 3 were 93.8, 97.9 and 97.1% respectively. Fig. 4 (a and b) show the graphical representation of the variation of COD removal efficiency and SVI with shear force respectively. Increased shear force enhances the sludge separation in the reactor by forming more compact and smaller granules. The increase of upflow air velocity from 3 cm/s to 4 cm/s increased the MLSS concentration and improved the SVI and settling velocity of the granules, but it did not reflect in the COD removal efficiency. Therefore in the economic point of view, amount of air supplied has to be decided based on

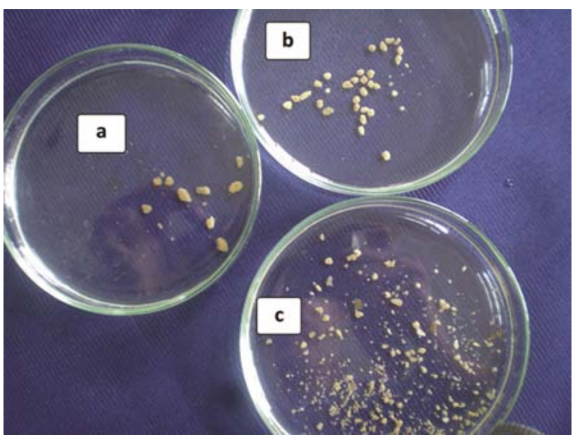

Fig. 3—Typical images of the granules formed at various shear forces (a) trial 1 (b) trial 2 (c) trial 3

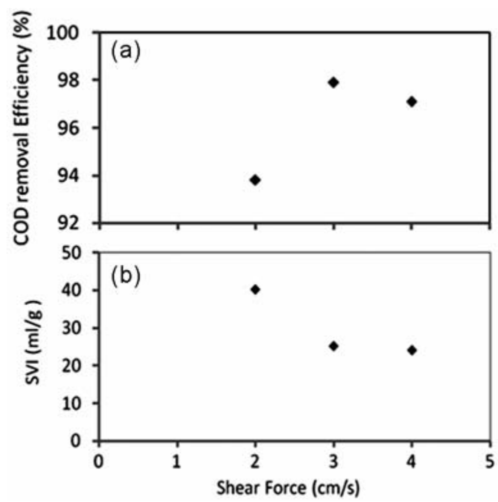

Fig. 4—(a) Variation of COD removal efficiency with shear force (b) variation of SVI with shear force

Table 2—Reactor performance under varying operating conditions

	Trial						
Operating conditions	1	2	3	4	5	6	7
Upflow air velocity, cm/s	2	3	4	3	3	3	3
Settling time, min	5	5	5	3	10	5	5
VER, %	50	50	50	50	50	25	75
Reactor Performance							
Granule appearance	day 21	day 17	day 14	day 19	day 28	day 21	day 14
Matured granules	day 28	day 23	day 22	day 28	---	day 28	day 25
Steady state	day 32	day 28	day 33	day 30	---	day 32	day 31
Size of granule, mm	2.6	2.4	1.5	2.1	1.1	0.9	2.0
Settling velocity, m/hr	38.4	55.2	63.6	72	31.2	34.8	66
MLSS, mg/L	5500	7900	9500	10000	3500	8200	5600
SVI, ml/g	40	25	24	23	75	40	28
Effluent COD, mg/L	125	46	60	45	195	102	30
COD removal efficiency, %	93.8	97.9	97.1	97.8	90.1	94.9	98.5

the sludge separation and pollutant removal efficiency. Moreover, it is evident from the results that the large size of the granule may not be considered as a target for granulation, if effluent quality is considered. The reason for the development of smaller granules at higher shear force may be attributed to the fact that high shear force causes more frequent collisions among granules, and as a result loosely packed cells are removed and formation of big granules is prevented[19]. It is evident from the experimental results that shear force in the form of upflow air velocity exerts a selection pressure for aerobic granulation process, but increased shear force is not the main strategy for pollutant removal efficiency.

Influence of settling time

The main feature of SBR process is its cyclic operation. Each cycle consists of filling, aeration, settling, and decanting. In SBR, settling time can act as a selection pressure by deciding the quantity and type of particles to be retained in the reactor. A short settling time can help to retain dense sludge particles with good settling ability and to wash out light particles with poor settling ability. Keeping upflow air velocity as 3 cm/s and VER as 50 %, the reactor was operated for 3 different settling times i.e., 3, 5 and 10 min (trial 4, 1 and 5). When the SBR was operated with settling time as 3 min, the MLSS was found to reduce considerably in the initial period of operation due to the wash out of poorly settleable sludge particles. The reactor could achieve a MLSS concentration above 2000 mg/L after two weeks only. The granules were first visible on day 19, and soon after that MLSS showed a marked increase. Steady state was achieved on day 30 and the MLSS reached around 10000 mg/L towards the end of operation. When the settling time was increased to 5 min, the MLSS was 7900 mg/L as explained in trial 2. Experiments with settling time as 10 min, formation and development of granules were delayed and they could not be get matured even after running up to 40 days. The MLSS also showed a drastic reduction towards the end of operation. The average size of the granules at three trials were 2.1, 2.4 and 1.1 mm (trial with settling time 3, 5 and 10 min) respectively. The ability of the sludge to settle was measured in terms of SVI and settling velocity and the values are given in Table 2. The trial with settling time as 10 min resulted in a higher value of SVI (75 mL/g) and lower value of settling velocity (31.2 m/hr) compared to those obtained in other two trials. It can be concluded that a lower settling time favours a higher settling velocity. The variations of COD removal efficiency and SVI with settling time were studied.

Qin *et al*., (2004a, b) suggested that very weak selection pressure in terms of long settling time did not favour aerobic granulation and the settling time required for successful aerobic granulation would not be longer than 15 min[13,28]. McSwain *et al*., (2004) and Adav *et al*., (2009) also concluded with experimental results that short settling times in SBR cycle select for fast settling granules and the initial mass wash-out and continual removal of flocs affects species selection during start-up and produces a less diverse but more stable population[17,20]. Various enhancement strategies were studied by Gao *et al*., (2011) and was concluded that stepwise reduction in settling time can result in efficient granulation in SBR[19].

The attempt of aerobic granulation with settling time as 10 min could result in a COD removal efficiency of 90.1% only. Trials with settling time 5 and 3 min did not make much difference in terms of COD removal efficiency (97.9 and 97.8% respectively), even though the MLSS concentrations were 7900 and 10000 mg/L respectively. It is evident from the results that same pollutant removal efficiency can be achieved at a lower settling time which can positively affect the overall economy of the treatment system. Investigations relating the settling time with COD removal are found scanty, hence the results of this study are expected to be useful for the development of aerobic granular treatment system for full-scale applications.

Influence of volume exchange ratio (VER)

It was reported that the volume exchange ratio of SBR would serve as an effective driving force of aerobic granulation and the minimum settling velocity of granules may serve as a selection pressure to drive aerobic granulation[21]. In the present study, keeping the settling time as 5 min and upflow air velocity as 3 cm/s, three different VERs were attempted as 25, 50 and 75% (trial 6, 2 and 7). When the VER was kept as 25%, it was observed that the MLSS concentration was increased, but fluffy flocs dominated in the reactor. Tiny granules were appeared on day 21, but the size of the granule reached a maximum size of 0.9 mm only, even towards the end of the trial run. In the next trial with VER as 50%, granules appeared on an earlier day (day 17) and they grew to a size of 2.4 mm with a fairly good settling velocity of 55.2 m/hr. When the VER was increased to 75%, the compactness and the settling velocity of the granules were improved a lot even though the MLSS was low compared to the previous two trials. In a column type SBR, VER has an important role because it determines the traveling distance above the discharging port (L - distance between water surface and discharging port). As VER increases, L is also increased. Keeping the settling time fixed, if VER is increased, only particles with higher settling velocity will reach below the discharge port, and hence remained in the reactor, and lighter particles with lower settling velocities will be discharged from the reactor. Thus VER can act as a selection pressure in aerobic granulation in SBR.

The steady state COD removal efficiencies and SVI for trials with VER were found to be 25, 50 and 75%. Wang *et al*., (2006) reported the % COD removal for 20% and 80% VER as 92±1 and 99±1 mg/L respectively, which are in consistent with the results of the present study.

The reactor performance under various operating conditions is summarized in Table 2.

Selection pressures on aerobic granulation

Out of the many influencing parameters, hydrodynamic shear force in terms of superficial upflow air velocity, settling time and volume exchange ratio are considered as the selection pressures in aerobic granulation. Each of these factors was studied with three different values. In the attempt to study shear force as a selection pressure, concrete evidences were obtained to support the concept. Higher the upflow air velocity, the smaller and more compact were the granules developed, and hence the lower was the SVI. But a proportional improvement in COD removal could not be achieved as shear force was increased. The ultimate aim of any treatment process is maximum pollutant removal efficiency at minimum cost. Under such circumstances, higher shear force in terms of more supply of air can not be entertained, but an optimal value of shear force should be arrived for rapid settleability and pollutant removal., In the present study, good reactor performance in terms of granule formation, biomass development, SVI and settleability and COD removal was obtained with upflow air velocity of 3 m/s, which an air needs supply of 6 L/min. It was also observed that there was no specific relation between size of the granule and COD removal., The results suggested that if effluent quality is considered, large sized granules may not be considered as a target.

When the influence of settling time as a selection pressure in aerobic granulation was studied, the results reinforced the fact that short settling times can enhance granulation in SBR while long settling times result in suspended sludge. McSwain *et al*., (2004) studied the influence of settling time on the formation of aerobic granules by operating two SBRs with settling time as 2 min and 10 min, and could develop granules in both cases, hence it was concluded that short settling times are only necessary to select for predominantly granular sludge[17]. Same COD removal efficiency (96±1%) was achieved in both cases, even though granules with more settleability and better SVI were achieved with settling time 2 min. In the present study also showed that same COD removal efficiency can be achieved at a lower settling time, which can save the time, space requirement and cost of treatment. Similar to the findings of Wang *et al*., (2006), the outcomes of the present study also give the strong influence of VER on aerobic granulation in SBR[21]. Granules with excellent settleability could be developed and high efficiency of COD removal could be achieved at high VER.

Conclusion

Aerobic granules are successfully developed in SBRs operated at different operating conditions. The following conclusions can be drawn from this study.

Hydraulic selection pressure in terms of shear force, settling time and VER is a decisive parameter in the formation of aerobic granules in SBR. Very weak selection pressures such as low shear force, long settling time and low VER did not favour aerobic granulation.

High shear force enhances the biomass growth, reduces the SVI and improves the sludge settleability. But optimum shear force has to be selected in terms of COD removal and in the economic point of view.

A long settling time of the order 10 min could not yield a fully developed granular sludge with good settling ability. A settling time of 3 min is sufficient to produce well developed granules with excellent settleability and to achieve a COD removal of 97.8%.

High VER of the order 75% could be an effective operating condition in terms of high COD removal and low SVI.

This study provides a new cultivation strategy of selecting optimal combination of various selection pressures for developing aerobic granules in SBRs.

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