ISSN : 0974 - 7451 Volume 11 Issue 6

Environmental Science
An Indian Journal
Critical Review

ESAIJ, 11(6), 2015 [179-186]

A mini-review onaerobic granular sludge cultivation

Moein Hedayati Moghaddam[1]*, Morteza Esfandyari[2], Amin Hedayati Moghaddam[3]
[1]Department of Mechanical Engineering, Semnan University, Semnan, (IRAN)
[2]Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, (IRAN)
[3]Department of Chemical Engineering, Ferdowsi University of Mashhad, Mashhad, (IRAN)
E-mail: moein_hedayati67@yahoo.com

ABSTRACT

To improveaerobic treatment of wastewater, cultivation of aerobic granular sludgeseems to be useful. Since excess nitrate and phosphate compounds promoteforming algal blooms and eutrophication in receiving water, in recent years many investigations have been conductedto improve the efficiency of COD, nitrogen and phosphate removal. Applying aerobic granular sludge is a novel and promising technique due to its advantages over conventional activated sludge process and sequencing batch reactor (SBR) such as improved settling ability and higher biomass retention as well as capable of toleration significantly higher organic loading rates because of their ability to retain biomass. In this study, a mini-review over papers and patents in this field was done.

KEYWORDS

Granulation;
Aerobic;
Sequencing batch reactor (SBR);
Hydrophobicity.

INTRODUCTION

In recent years many investigations have been conducted for improving the efficiency of activated sludge wastewater treatment systems in order to approach stringent discharge standards. On the other hand, nitrogen elimination is necessary to control eutrophication since excess nitrate stimulates theformation of algal blooms in receiving water[1]. A conventional activated sludge has several disadvantages such as relatively high energy consumption, high biomass production, sludge bulking, large reactor volume required, sensitivity to shock loading, high operation costs and problems associated to the disposal of large amount of sludge[2]. Wastewater treatment with sequencing batch reactor (SBR) is a better alternative, compared to the activated sludge process. SBRs offer various advantages, including minimal spacerequirements and ease of management[3]. Besides, SBRsand modified SBRs have been successfully applied to treat complex chemical wastewaters[4-6], and also they are able to remove nitrogen compounds[7-8].

SBR cycle consists of five phases (filling, aeration, settling, decanting and idle); but for nitrogen and phosphorous removal,maintaining anaerobic–aerobic condition (with anoxic and aeration phases in each cycle) is necessary.

Wastewater treatment using biofilm is another method that has obtained especial consideration due tosome drawbacks of activated sludge process. A biofilm is by definition a layer of prokaryotic or eukaryotic cells, attached to a substratum surface and placed in an organic matrix of biological body[9]. The main challenging termcorresponded to the application of aerobic biofilms is the three-phase separation of water, air, and biomass during imposing different hydraulic loading rate (OLR)[10, 11]. Aerobic and anaerobic granules are considered as one of the biofilm varieties.

The granulation is classified into two categories: anaerobic and aerobic. Strong and compact microbial structure, less sedimentation time, improved settling ability and higher biomass retention as well as capability of tolerating significantly higher OLRs because of granules ability to retain biomass are the main advantages of the aerobic granules over conventional activated sludge flocs[12]. Through aerobic granulation, access to the concentrated SBR is possible. Due to shorter settling time compared to conventional SBR, more cycled could be done. By granule formation, the concentration of sludge will increase, which is due to a decrease in sludge volume index[13].

Granules are able to treat the wastewater with high OLR because they have ability to preserve the biomass. Whiles activated sludge systems are able to treat wastewater with OLR of 0.5 to 2 $kgCOD \cdot m^{-3} \cdot d^{-1}$[14].

One of the weak points of aerobic granules is the diffusion barrier. Although diffusion barrier can be removed because of formation of channels within the granules[15]. The concentration of oxygen could immediately decrease to 0 at the high depth of granules. This depth depends on the oxygen utilization rate, porosity, and challenging of granules. The typical depth of aerobic region in microbial biofilm under aerobic condition is 50-200 μm. The bacterial existed in anaerobic region will produce gases and organic acids that can damage granules. So, for confidence about the stability of aerobic granules, their diameter must be less than two times of thickness of aerobic layer[16].

Jing et al. (2002) assessed the applicability of aerobic granular sludge in treating wastewater containing considerable amount of phenol by column-type sequential aerobic sludge blanket reactor maintained at a loading rate of 1.5 g $phenol \cdot L^{-1} \cdot d^{-1}$. They reported that at the phenol concentration of 2 $g \cdot L^{-1}$, the maximum specific phenol degradation rate was achieved[17].

Jang et al. (2003) applied aerobic granules to simultaneously remove nitrogen and COD in a SBR. The maximum nitrification efficiency of up to 97% and COD removal efficiency of up to 95% were achieved[18]. Tsuneda et al. applied an aerobic upflow fluidized bed (AUFB) reactor in a nitrification process for inorganic wastewater containing 500 $g.m^{-3}$of NH^{+4}-N through granulation of nitrifying bacteria. They reported that nitrifying granules were detected in the reactor after operation for 100 days, and their size increased to 346mm after 300 days[19]. If granules are large enough for the diffusion of phosphorous and nitrogen into core and inhibit reaction hold up, nitrite will advance simultaneous removal[20].

Moussavi et al. (2010) studied the degradation of phenol of a saline wastewater in aerobic granular SBR. They assessed the effect of inlet concentration of phenol, cycle time, filling cycle time, shock loading, and total dissolved solids (TDS) concentration on the performance of the reactor. They reported that the aerobic granular SBR could remove more than 99% of influent phenol at inlet phenol concentrations of up to 1000mg/L, total cycle time of 17h (15.5h aerating, 1h filling, and 30min settling, decanting and idle) and TDS concentrations up to 8%[21].

GRANULE FORMATION

Granulation can be stated as aggregation of cells through cell to cell immobilization for forming the contiguous multicellular community[22]. To form granular sludge in aerobic systems, the settling time should be decreased to values that only particles with the ability to form granules can stay in reactor[20]. Due to electrostatic forces and hydration interactions, bacteria naturally have no interest in aggregation and granulation[23].

As was stated before, there are two types of granulation (aerobic and anaerobic). Up-flow anaerobic sludge blanket (UASB) is a culture to grow anaerobic granules. Microorganisms in UASB have to grow in granular form, if it does not, they will be washed out by continuous up flow stream[13]. It is important to note that UASBs are able to treat wastewater with OLR of 40 kg COD $m^{-3}d^{-1}$[14]. Anaerobic granules have several disadvantages such as long start-up time, fairly high operative temperature,poor capability for nutrients (nitrogen and phosphorous) removalandthey are not useful for treating wastewater with low OLRs[24].

Since granulation has been investigated in the context of methanogenic systems, it is assumed that special syntrophic bacterial interactions have the fundamental role in this process, whereas granulation is not limited only to methanogenic bacteria. But rather, granulation of acidogenic bacteria, nitrifying bacteria, denitrifying bacteria, and aerobic heterotrophic bacteria have been reported[13].

Beun et al. (1999) described the aerobic granulation in a SBR as follows: During the start-up period, main part of biomass in the reactor is filamentous mycelium. Because of stress in the reactor, the filamentous bacteria would be detached from the surface of pellets and the pellets would be denser, consequently. The granules grow until their dimension approach to 5-6 mm and their inner part decompose due to lack of oxygen. It is seemed that the mycelium pellets act as a matrix in which colonies of bacteria grew on it. After collapsing of the mycelium pellets because of cellular decomposition, the bacterial colonies would save themselves because they are large enough for settling. These micro colonies grew and turned to granules and finally the bacterial population could be predominant[13].

In another study, Zheng et al. (2006) studied the aerobic granule formation in a SBR. They reported that during the first 14 days of start-up period, no significant change in sludge morphology was observed. The only considerable change was sludge color turn from grayish brown to yellow. On day 15 tiny yellow granules appeared. During the next 15 days (15-30 days), the aerobic granules grew in size gradually. On day 33, filamentous bacteria were observed on the granule surface led to weak settling ability. On day 40, the settling sludge ability was recovered. Thereafter, the black granules appeared and yellow granules vanished gradually. On day 55, only black granules existed in the reactor[25].

So far all the aerobic granulation only took place in SBR and continuous environments did not earn any successes in this field. The reason of this phenomenon has not been specified clearly, yet[22].

The effects of operative parameters

The operative parameters that would influence the mechanism and performance of granular sludge formation are composition of wastewater, organic loading rate, feast-famine condition of organic compound, reactor design, auxiliary agent, settlement time, volumetric exchange ratio, aeration rate (hydrodynamic shear force), and intermittent feeding that are explained in following:

Loading rate of substrate

Unlike anaerobic granulation, in aerobic granulation a wide range of organic loading rate was reported (2.5-15 kgCOD·m^{-3}.d^{-1}). Liu et al. (2005) concluded that the concentration of substrate had no effect on granulation but influenced the kinetic behavior of granules[22]. Studies on granular sludge revealed that aerobic granulation faced difficulty as OLR decreased. As an example, aerobic granulation for municipal wastewater with low OLR (less than 1 kgCOD·m^{-3}·d^{-1}) is very slow, which leads to much times to reach steady state[20].

Tay et al. (2004) reported that in laboratory-scale sequentialaerobic sludge blanket reactor (SASBR) for OLRs of 1 and 2 kg COD·m^{-3}·d^{-1}, aerobic granulation was not observed. For OLR of 4 kg COD·m^{-3}·d^{-1} aerobic granules appeared after 12 d and eventually grew and became stable. After 18 days of operation at the OLR of 8 kg COD·m^{-3}·d^{-1}unstable aerobic granules were appeared[26].

Moy et al. (2002) reported that at OLR of 6 kgCOD·m^{-3}·d^{-1} granules based on glucose substrate had fluffy structure, which SEM images revealed that filamentous bacteria are predominant. But at OLR of 9 kgCOD·m^{-3}·d^{-1}, the irregular morphology of granules are smoother while increasing the OLR to 12 and 15 kgCOD·m^{-3}·d^{-1} led to similar granules with larger diameter. Generally, an increase in OLR leads to better settling properties[14].

Liu et al. (2003) studied the effect of OLR on cell hydrophobicity and reported that cell hydrophobicity was not dependent on OLR. It seemed that the aerobic heterotrophic granulation isinsensitive to OLR in the range of 1.5-9 kgCOD·m^{-3}·d^{-1}[27].

Amount of OLR has no effect on formation of aerobic granules. But physical properties of granules depend on the amount of OLR. By increasing the OLR from 3 to 9 kg COD·m^{-3}, the granule size would raise from 1.6 to 1.9 mm. Similar phenomenon has been reported for anaerobic granules. Increasing the OLR would increase the growth rate of microorganisms and decrease the stability of granules. In aerobic granulation system, hydraulic retention time (HRT) must be short enough to suppress the growth of suspended particles and on the other hand should be long enough to prepare the possibility of microbial growth and accumulation[23].

Diversity of Nutrients: an increase in ammonium concentration leads to a decrease in the hydrophobicity property and an increase in polysaccharide production, which damage the aerobic granulation. Yang et al. (2004) reported that the presence of ammonium inhibited the energy metabolism of bacteria and therefore suppressed the aerobic granulation. By increasing the N/COD ratio, the activity of nitrifying and denitrifying bacteria would be increased. But the population of heterotrophic bacteria would decreases[23].

The COD/N greater than 90 leads to excessive growth of filamentous bacteria, which damage settling properties[20]. Yang et al. (2003) reported that for granular sludge-SBR at high substrate N/COD ratio, the nitrifying and denitrifying bacteria exhibit high activity but the activity of heterotrophic bacteria decreases as the N/COD was decreased[28]. Yang et al. (2004) said that free ammonium significantly decreased the cell hydrophobicity and cell polysaccharides content that are responsible for failure in aerobic granulation. Accordingly, theyconcluded that aerobic granules formed only when the concentration of free ammonia was less than 23.5 mg·L^{-1}[29].

Lin et al. (2003) reported that the size of P-accumulating granules decreased as P/COD in substrate ratio increased, while the structure of the granules became more compact and denser as P/COD ratio increased[30].

Hydrodynamic shear force

Hydrodynamic shear force could act as detachment force. High detachment force leads to a compact and dense biofilm[31]. Hydrodynamic shear force would stimulate the production of cellular polysaccharides, which has significant effect on the formation and stability of aerobic granules[32]. Liu et al. (2003) reported that high shear force increased the cell hydrophobicity, which would facilitate the aerobic granulation[27].

Feast-famine regime

Aeration phase in SBR consists of two parts. In the first part, organic compound decompose and be consumed by microorganisms. And in the second part, microorganisms face with a lack in substrate. During famine period, the bacteria will be hydrophobic and their aggregation will be feasible, consequently. The aggregation of bacteria is their natural reaction to sustain famine cycles[22]. High cell hydrophobicity was essential for microbial aggregation and sustaining the aerobic granulation process[33].

From thermodynamic point of view, the hydrophobicity of bacteria will decrease the Gibbs free energy of the surface that facilitates the relation between two bacteria and leads to more compact structure. On the other hand, the starveling clogs of bacteria connect to each other by fibrous links, which facilitate the cell to cell connections[34].

Type of bacteria

Selection the bacteria with slower growth rate leads to more stable granules. Accordingly, using the compounds with slow degradability properties produces bacteria with slow growth rate[35].

Feeding strategy

The results show that the high ratio of feast-famine or periodic feeding leads to the formation of compact and dense granules[22].

Dissolved oxygen (DO)

Results show that the concentration of dissolved oxygen has no drastic effect on granulation[22]. There are several reports that the aerobic granules would form at the DO level of 0.7 to 2 mg·L^{-1}. So, DO is not an effective parameter in the formation of aerobic granules[23].

Reactor structure

The air bubble traverses a spiral path in SBR that makes homogenous circular path and regional vortex. The circular stream will arrange the clogs in a manner that have the minimum surface free energy. In the SBR column, high ratio of height to diameter make perfect circular path that result in effective hydraulic attrition on microbial clogs. Furthermore, high ratio of H/D (the ratio of column height to column diameter) will make feasible the oxygen transfer and leads to a smaller reactor. Recent reports show that aerobic granulation takes place at various ratio of H/D[22]. Due to importance of settling velocity, high H/D ratio is useful. On the other hand, short settling time makes the reaction time longer[13]. High H/D ratio leads to complete circular path and more attrition. Also, bacteria would be separated from each other according to their settling velocity. If continuous stirred tank reactor (CSTR) is used, granules would be subjected to regional hydrodynamic force, raising stream, randomized collision[23]. In TABLE 1, the structures of several reactors applied for aerobic granulation have been explained.

Sludge retention time

In any research in recent 100 years, there was no report about the granulation in activated sludge with various SRT[22]. Accordingly, SRT will have no effects on aerobic granulation.

Auxiliary agent

Nancharaiah et al. (2008) studied the effect of Nitrilotriacetic acid as synthesized chelating agent on the granulation on the substrate of acetate. They reported that the diameter of aerobic granules would increase by using the chelating agent[35]. Jiang et al. (2003) investigated the effect of calcium ion on granulation and reported that its positive effect is due to establishment of a connection between positive ion and negative load of the bacteria surface[36].

Temperature and pH

Growth rate and the consequent competition of microorganisms would be influenced by temperature variation[37]. Temperature and pH are important parameters in anaerobic granulation, but considerable effect on aerobic granulation have not been reported, yet. The investigation on the long-term effect of temperature showed that at low temperature, the formation of granular sludge was hard and the resulted sludge would be washed out and removed. But if granular sludge forms at high temperature and thereafter temperature decreases, the structure of granular sludge would not being influenced dramatically and only the removal of a few compounds would be affected.[37].

Cycle time

Cycle time must be long to give the bacteria the enough time for growth and aggregation. It is important to note that if the cycle time is too long, the bacteria will be hydrolyzed and the granules will damage. Studies have shown that if the cycle time is optimized but the settling time is more than 15 min, the granulation will not take place[22].

Settling time

A short settling time leads to high cell hydrophobicity that contributes to aerobic granulation. Besides, cell polysaccharides content increases as settling time decreases. Cell polysaccharides have significant role in bio-film integrity, which facilitate aerobic granulation[38].

Volume exchange ratio

Volume exchange ratio is the decanted volume to the total volume of reactor. Higher exchange ratio leads to better aerobic granulation. Wang et al. (2006) studied the effect of volume exchange ratio on aerobic granulation by four SBR with different volume exchange ratio (20-80%). They reported that a fast granulation occurred in the reactor with highest volume exchange ratio[39]. It is found from Stock equation that the particles with high settling velocity are circular and weighted whiles small, light, and unformed particles have slow settling velocity. Accordingly, the only weighted bio-films stay in SBR as volume exchange ratio increases.

Liu et al. (2001) investigated the effect of type of substrate on aerobic granulation. They reported that aerobic granules formed in reactor after two weeks. They reported that substrate composed of

TABLE 1 : The structural properties of reactors used for aerobic granulation

References	Working volume (L)	Internal diameter (cm)	Height (cm)	Volume exchange ratio
[21]	4	15	40	0.5
[28]	2.4	6	80	0.5
[17]	2	5	-	0.5
[40]	2.4	5	120	0.5
[18]	8	10	150	0.67
[19]	6.3	5	3.2	-
[30]	2.5	5	120	0.5
[26]	1.7	6	60	0.5
[25]	4	7	110	0.55
[22]	-	5	127	0.5
[39]	1.96	5	-	0.2-0.8
[41]	2.4	5	150	0.5

glucose and maltose stimulated the filamentous growth. They reported that the type of substrate has no effect on the pattern of granulation. The glucose based granules have fluffy surface that is possibly due to the presence of filamentous bacteria[34]. Tay et al. (2002) reported that the type of substrate (glucose and acetate)has no considerable effect on aerobic granulation, but their characteristics such as settleability, granule size, physical strength, roundness, hydrophobicity, and activity are comparable[40].

MORPHOLOGY OF AEROBIC GRANULES

Aerobic granules have multi-layer structure: outer layer is aerobic and is a mixture of autotrophic and heterotrophic bacteria, and anaerobic or anoxic core, which consists of anaerobic or nitrifying microorganisms[37]. Aerobic ammonium oxidizing bacteria are present at the depth of 70-100 μm. Aerobic granules consist of pores that are prolonged to the depth of 900 μm and transfer oxygen and nutrient. The maximum porosity was observed at the depth of 300-500 μm. Compact structure of polysaccharides forms at the depth of 400 μm. Anaerobic bacteria have been observed at the depth of 800-900 μm from the granule surface. And a layer of dead bacteria were detected at the depth of 800-900 μm[23]. Wantic and Catler(2009) said that in a microbial population, bacteria would be distributed in a manner that each of them would have the best condition in environment[42].

CONCLUSION

The granulation is classified into two categories: anaerobic and aerobic. Strong and compact microbial structure, less sedimentation time, improved settling ability and higher biomass retention as well as capability of tolerating significantly pretty high organic loading rates (OLRs) because of granules ability to retain biomass are the main advantages of the aerobic granules compared to conventional activated sludge flocs. Aerobic granular sludge make feasible the simultaneous COD and nutrients removal.

REFERENCES

[1] R.Bernardes, H.Spanjers, A.Klapwijk; Modelling respiration rate and nitrate removal in a nitrifying-denitrifying SBR treating domestic wastewater, Bioresource technology, **67**, 177-189 **(1999)**.

[2] M.Singh, R.Srivastava; Sequencing batch reactor technology for biological wastewater treatment: a review, Asia Pacific Journal of Chemical Engineering, **6**, 3-13 **(2011)**.

[3] D.Kulikowska, E.Klimiuk, A.Drzewicki; $BOD_5$and COD removal and sludge production in SBR working with or without anoxic phase, Bioresource technology, **98**, 1426-1432 **(2007)**.

[4] M.Casellas, C.Dagot, M.Baudu; Set up and assessment of a control strategy in a SBR in order to enhance nitrogen and phosphorus removal, Process biochemistry, **41**, 1994-2001 **(2006)**.

[5] S.Sirianuntapiboon, O.Ungkaprasatcha; Removal of Pb^{2+} and $Ni^{2+}$by bio-sludge in sequencing batch reactor (SBR) and granular activated carbon-SBR (GAC-SBR) systems, Bioresource technology, **98**, 2749-2757 **(2007)**.

[6] S.Sirianuntapiboon, J.Sansak; Treatability studies with granular activated carbon (GAC) and sequencing batch reactor (SBR) system for textile wastewater containing direct dyes, Journal of hazardous materials, **159**, 404-411 **(2008)**.

[7] S.Morling; Nitrogen removal and heavy metals in leachate treatment using SBR technology, Journal of hazardous materials, **174**, 679-686 **(2010)**.

[8] J.Y.Jung, Y.C.Chung, H.S.Shin, D.H.Son; Enhanced ammonia nitrogen removal using consistent biological regeneration and ammonium exchange of zeolite in modified SBR process, Water Research, **38**, 347-354 **(2004)**.

[9] R.Bos, H.C.Mei, H.J.Busscher; Physico chemistry of initial microbial adhesive interactions–its mechanisms and methods for study, FEMS microbiology reviews, **23**, 179-230 **(1999)**.

[10] M.K.De Kreuk; Aerobic granular sludge: scaling up a new technology, **(2006)**.

[11] Y.Liu, S.F.Yang, S.F.Tan, Y.M.Lin, J.H.Tay; Aerobic granules: a novel zinc biosorbent, Letters in Applied Microbiology, **35**, 548-551 **(2002)**.

[12] H.Linlin, W.Jianlong, W.Xianghua, Q.Yi; The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules, Process biochemistry, **40**, 5-11 **(2005)**.

[13] J.Beun, A.Hendriks, M.Van Loosdrecht, E.Morgenroth, P.Wilderer, J.Heijnen; Aerobic granulation in a sequencing batch reactor, Water Research, **33**, 2283-2290 **(1999)**.

[14] B.Y.P.Moy, J.H.Tay, S.K.Toh, Y.Liu, S.T.L.Tay; High organic loading influences the physical characteristics of aerobic sludge granules, Letters in Applied Microbiology, **34**, 407-412 **(2002)**.

[15] S.L.Tay, V.Ivanov, S.Yi, W.Q.Zhuang, J.H.Tay; Presence of anaerobic Bacteroides in aerobically grown microbial granules, Microbial ecology, **44**, 278-285 **(2002)**.

[16] J.H.Tay, V.Ivanov, S.Pan, S.T.L.Tay; Specific layers in aerobically grown microbial granules, Letters in Applied Microbiology, **34**, 254-257 **(2002)**.

[17] H.L.Jiang, J.H.Tay, S.T.L.Tay; Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol, Letters in Applied Microbiology, **35**, 439-445 **(2002)**.

[18] A.Jang, Y.H.Yoon, I.S.Kim, K.S.Kim, P.L.Bishop; Characterization and evaluation of aerobic granules in sequencing batch reactor, Journal of biotechnology, **105**, 71-82 **(2003)**.

[19] S.Tsuneda, T.Nagano, T.Hoshino, Y.Ejiri, N.Noda, A.Hirata; Characterization of nitrifying granules produced in an aerobic upflow fluidized bed reactor, Water Research, **37**, 4965-4973 **(2003)**.

[20] M.Coma Bech; Biological nutrient removal in SBR technology: from floccular to granular sludge, **(2011)**.

[21] G.Moussavi, B.Barikbin, M.Mahmoudi; The removal of high concentrations of phenol from saline wastewater using aerobic granular SBR, Chemical Engineering Journal, **158**, 498-504 **(2010)**.

[22] Y.Liu, Z.W.Wang, L.Qin, Y.Q.Liu, J.H.Tay; Selection pressure-driven aerobic granulation in a sequencing batch reactor, Applied microbiology and biotechnology, **67**, 26-32 **(2005)**.

[23] Y.Liu, J.H.Tay; State of the art of biogranulation technology for wastewater treatment, Biotechnology Advances, **22**, 533-563 **(2004)**.

[24] Y.Liu, S.F.Yang, L.Qin, J.H.Tay; A thermodynamic interpretation of cell hydrophobicity in aerobic granulation, Applied microbiology and biotechnology, **64**, 410-415 **(2004)**.

[25] Y.M.Zheng, H.Q.Yu, S.J.Liu, X.Z.Liu; Formation and instability of aerobic granules under high organic loading conditions, Chemosphere, **63**, 1791-1800 **(2006)**.

[26] J.H.Tay, S.Pan, Y.He, S.T.L.Tay; Effect of organic loading rate on aerobic granulation.I: Reactor performance, Journal of environmental engineering, **130**, 1094-1101 **(2004)**.

[27] Y.Liu, S.F.Yang, Q.S.Liu, J.H.Tay; The role of cell hydrophobicity in the formation of aerobic granules, Current Microbiology, **46**, 270-274 **(2003)**.

[28] S.F.Yang, J.H.Tay, Y.Liu; A novel granular sludge sequencing batch reactor for removal of organic and nitrogen from wastewater, Journal of biotechnology, **106**, 77-86 **(2003)**.

[29] S.F.Yang, J.H.Tay, Y.Liu; Inhibition of free ammonia to the formation of aerobic granules, Biochemical Engineering Journal, **17**, 41-48 **(2004)**.

[30] Y.M.Lin, Y.Liu, J.H.Tay; Development and characteristics of phosphorus-accumulating microbial granules in sequencing batch reactors, Applied microbiology and biotechnology, **62**, 430-435 **(2003)**.

[31] Y.Liu, Y.M.Lin, S.F.Yang, J.H.Tay; A balanced model for biofilms developed at different growth and detachment forces, Process Biochemistry, **38**, 1761-1765 **(2003)**.

[32] J.H.Tay, Q.S.Liu, Y.Liu; The role of cellular polysaccharides in the formation and stability of aerobic granules, Letters in Applied Microbiology, **33**, 222-226 **(2001)**.

[33] J.H.Tay, S.Pan, Y.He, S.T.L.Tay; Effect of organic loading rate on aerobic granulation.II: Characteristics of aerobic granules, Journal of environmental engineering, **130**, 1102-1109 **(2004)**.

[34] J.H.Tay, Q.S.Liu, Y.Liu; Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor, Journal of Applied Microbiology, **91**, 168-175 **(2001)**.

[35] Y.V.Nancharaiah, H.M.Joshi, T.V.Krishna Mohan, V.P.Venugopalan, S.V.Narasimhan; Formation of aerobic granules in the presence of a synthetic chelating agent, Environmental Pollution, **153**, 37-43 **(2008)**.

[36] H.L.Jiang, J.H.Tay, Y.Liu, S.Tiong-Lee Tay; Ca 2+ augmentation for enhancement of aerobically grown microbial granules in sludge blanket reactors, Biotechnology letters, **25**, 95-99 **(2003)**.

[37] M.De Kreuk, M.Pronk, M.Van Loosdrecht; Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures, Water Research, **39**, 4476-4484 **(2005)**.

[38] L.Qin, J.H.Tay, Y.Liu; Selection pressure is a driving force of aerobic granulation in sequencing batch reactors, Process Biochemistry, **39**, 579-584 **(2004)**.

[39] Z.W.Wang, Y.Liu, J.H.Tay; The role of SBR mixed liquor volume exchange ratio in aerobic granulation, Chemosphere, **62**, 767-771 **(2006)**.

[40] J.Tay, Q.Liu, Y.Liu; Characteristics of aerobic granules grown on glucose and acetate in sequential aerobic sludge blanket reactors, Environmental technology, **23**, 931-936 **(2002)**.

[41] S.Toh, J.Tay, B.Moy, V.Ivanov, S.Tay; Size-effect on the physical characteristics of the aerobic granule in a SBR, Applied microbiology and biotechnology, **60**, 687-695 **(2003)**.

[42] P.Watnick, R.Kolter; Biofilm, city of microbes, Journal of Bacteriology, **182**, 2675-2679 **(2000)**.