



Effect of feeding regime, batch and continuous, on aerobic granulation process treating industrial soft drink wastewater

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ABSTRACT

Aerobic granular sludge process, as an effective biotechnological process, was increasingly drawing the interest of researchers in the area of biological wastewater treatment. In this research, laboratory experiments were conducted to investigate the effect of feeding regime on the granulation process in a sequencing batch reactor (SBR) and continuous feed and intermittent discharge (CFID) bioreactor treating soft drink wastewater. To have a proper comparison between batch and CFID regimes, both bioreactors were operated under the same conditions with the initial chemical oxygen demand (COD) concentrations of 300 and 500 mg/L and cycle time of 4 h. From the results, contrasting to CFID regime, the batch regime was capable to form granular sludge at the applied conditions. To investigate the aerobic granulation process in the CFID bioreactor, hydraulic retention time (HRT) values of 8 and 12 h with idling times of 60 and 90 min, respectively, were examined. In the CFID bioreactor, at HRT of 8 h (COD_{in} of 500 mg/L), the aerobic granules started to be appeared. By increasing HRT to 12 h (COD_{in} of 1,000 mg/L), the aerobic granules were enlarged with more stability. As a conclusion, the higher values of HRT favored the granulation process in the CFID bioreactor. In addition, from the scanning electron microscopic images, the granules formed in the SBR and CFID bioreactor showed different structures.

Keywords: Aerobic granulation; Feeding regime; Soft drink wastewater

1. Introduction

Water pollution has been a challenge in recent years throughout the world as a result of industrial development and rapid urbanization [1]. Therefore, wastewater treatment is one of the most vital matters, nowadays. Different physical, chemical, and biological methods have been investigated to treat various wastewaters. However, biological treatment systems are more interesting regarding easy operation and economic aspects. Activated sludge is a well-known biological system applied in the most wastewater treatment plants throughout the world [1].

Some of the main disadvantages of the activated sludge systems are related to the floccular nature of the sludge, as large area is required for aeration and sedimentation tank [2]. Granular

sludge is a suggested methodology to provide high microorganism concentration in smaller bioreactors and decrease the economic cost. The structure of granular sludge is more compact rather than flocculating sludge, which provides higher microbial population in less volume as well as higher rate of treatment.

Aerobic and anaerobic granular sludge has been assessed over the years. The performance of aerobic granular sludge systems has been widely reported in sequencing batch reactors (SBRs) to treat synthetic wastewater (mainly acetate and glucose as carbon source), and real wastewater such as dairy [3], abattoir [4], domestic [5], soybean-processing [6], brewery [7], and paper-making wastewaters [8,9].

Hanmin Zhang et al. [10] reported granule formation in sodium acetate and sodium propionate after 50 d of operation with the cycle time of 9 h and initial chemical oxygen demand (COD) concentration of 200 ± 20 mg/L. In another study, the aerobic granules were obtained over 80 d at the cycle time of 6 h and initial COD concentration of 1,000 mg/L fed by acetate

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[11]. In addition, a research group reported aerobic granule formation after 36 d by using nitrobenzene with the initial COD concentration up to 600 mg/L at a cycle time of 22 h [12].

From reactor design point of view, the compact high-rate bioreactors have been more investigated for wastewater treatment due to small space required and less production of odor and sludge [13]. SBR is considered as a suitable example for integrated bioreactors with smaller footprints, so operation and maintenance costs are lower than the systems with continuous regime.

One category of SBR system that operates with continuous feed and intermittent discharge (CFID) has attracted much interests nowadays. The CFID bioreactors collect wastewater in all stages of the treatment cycle while the effluent is discharged intermittently. Therefore, CFID bioreactor could present the both features of SBR and continuous systems. In our previous work, 91%, 60%, and 32% were reported, respectively, for COD, total nitrogen (TN), and total phosphorous (TP) removal in the optimum condition (at 12 h of hydraulic retention time [HRT] and 40 min/h of aeration time) treating an industrial wastewater in a CFID SBR [14].

In another study, the capability of CFID reactor and SBR in removing carbon and nutrients were compared and according to the results obtained, CFID showed higher carbon and nitrogen removal efficiencies [15]. In addition, airlift bioreactor based on CFID regime was operated for treating soft drink and milk processing wastewaters [16]. Moreover, in order to provide more mixed liquor suspended solid (MLSS) concentration in CFID bioreactor, the effect of high-frequency ultrasonic irradiation on the sludge settle ability and bioreactor performance were investigated [17].

Another approach to make the CFID regime more efficient is to provide aerobic granular sludge in the system. The aerobic granulation process in the CFID bioreactor has not been investigated up till now. The possibility of producing granular sludge in the CFID bioreactor is assessed in this study. In addition, the conditions to form granular sludge in both SBR and CFID bioreactors are presented and the structure of granular sludge is compared.

2. Materials and methods

2.1. Seed sludge and wastewater

The activated sludge was obtained from the aeration tank of Faraman's industrial wastewater treatment plant, Kermanshah, Iran. The soft drink wastewater, as a substrate, was collected from a working industrial wastewater treatment plant, Zam Zam's industrial plant, Kermanshah, Iran. The samples were stored in a cold room at 4°C. This storage technique had no noticeable effect on its composition. The wastewater characteristics are shown in Table 1. COD:N:P ratio was set about 100:15:5. The sludge was cultivated in an SBR reactor over a 20-d period to adapt the biomass to the soft drink wastewater and then both bioreactors were started-up with 2 L of adapted sludge (with MLSS concentration of 5,000 mg/L).

2.2. Bioreactor configuration

The schematic diagram of the SBR and CFID bioreactors is shown in Fig. 1. The Plexiglas bioreactor columns were

fabricated with an internal diameter of 7 cm and a liquid height of 54 cm with a total volume of 2 L. An automatic control valve was mounted on the reactor column at the height of 27 cm (50% of the total volume) for providing an intermittent discharge.

In the continuous feeding regime (CFID bioreactor), the industrial wastewater was continuously introduced into the reactor from the bottom, and the treated effluent was intermittently removed as supernatant at the end of each run. Hence, HRT was calculated based on 1 L as working volume (the volume which is filled and discharged).

2.3. Bioreactor operation

2.3.1. Batch feeding

MLSSs content of the reactor was set about 5 g/L by occasional sludge discharge. In the batch feeding regime, the industrial wastewater introduced at the bottom of the reactor and the sludge in SBR was allowed to settle for 20 min before discharging. In each cycle, about 1 L of the supernatant was removed, and the same volume of the fresh wastewater was fed. In the first stage (bioreactor start-up), after adding the prepared inoculums (2 L of adapted sludge with MLSS concentration of 5 g/L), the bioreactor was operated under 240 min of cycle time, 30 min feeding, 190 min aeration conditions, and 20 min of settling time. In this stage, Zam Zam's industrial wastewater was used as feed with initial COD concentration of 300 mg/L and COD:N:P ratio of 100:15:5. This was continued for 20 d when the stable granules were produced.

Table 1
Characteristics of soft drink wastewater

Parameters	Amount
COD (mg/L)	1,500–5,000
BOD ₅ (mg/L)	1,200–4,000
Settable solids (mg/L)	3–30
TP (mg/L)	2–5
TKN (mg/L)	30–80
TSS (mg/L)	40–50
pH	6.8–7.2

Note: BOD = biochemical oxygen demand; TKN = total kjeldahl nitrogen; and TSS = total suspended solids.

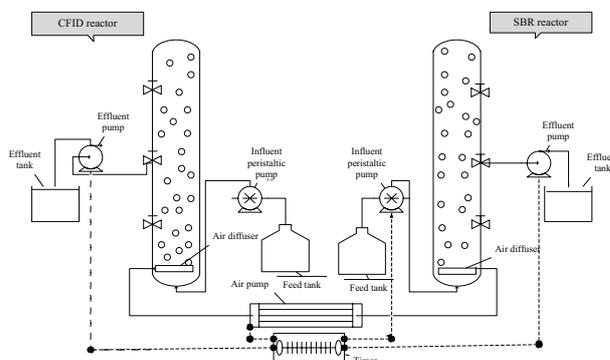


Fig. 1. Schematic diagram of the experimental setup (CFID and SBR bioreactors).

In the second stage, the operating conditions were similar to the first stage except for the initial COD concentration was 500 mg/L. It should be mentioned that in the second stage, SBR was re-inoculated in order to investigate the effect of increase in the COD concentration on the granulation process. Fresh biomass obtained from Faraman's industrial wastewater treatment plant was used for re-inoculation.

2.3.2. Continuous feeding

The soft drink wastewater was continuously introduced into the reactor from the bottom, and the treated effluent was intermittently removed as supernatant at the end of the each run. The CFID bioreactor was operated under room temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$). MLSSs content of the reactor was set about 5 g/L by occasional sludge discharge.

In the first stage, after adding 2 L inoculums with MLSS of 5,000 mg/L, the CFID bioreactor was operated at HRT, idling time, settling time, and influent COD concentration of 8 h, 60 min, 20 min, and 500 mg/L, respectively. This was continued until the core of the granules was produced in the reactor. Then, the operating condition was changed to HRT of 12 h, idling time of 90 min, settling time of 20 min and influent COD concentration of 1,000 mg/L. This condition was continued until mature granules were appeared. It should be mentioned that COD:N:P ratio was set around 100:15:5 at all experimental conditions. Similar to SBR, 200 mg/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 10 mg/L of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were added to the feed to promote granular sludge cultivation.

2.4. Analytical procedure

The concentrations of COD, TN, MLSS, sludge volume index (SVI), nitrate, and nitrite were determined by using standard methods [18]. For COD, a closed reflux colorimetric method was developed. Spectrophotometer (DR 5000, Hatch, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. TKN and N-NH_4 were determined by TKN meter Gerhardt model (Vapodest 10, Germany). Turbidity was measured by a turbidity meter model 2100 P (Hach Co., USA).

3. Results and discussion

3.1. COD concentration profile in SBR and CFID bioreactor

As feast/famine strategy is a key point to cultivate granular sludge in the SBR, in this study, the changes in COD concentration during the cycle time of 4 h at different initial COD concentrations (300, 500, and 700 mg/L) were measured (Fig. 2). At initial COD concentrations of 300 and 500 mg/L, the feast and famine conditions were provided in the bioreactor, while the phases were separated strictly in initial COD concentration of 500 mg/L. From the figure, at 700 mg/L of initial COD concentration, the famine phase which is required to produce granular sludge could not be achieved. Therefore, initial COD concentrations of 300 and 500 mg/L were selected to form aerobic granular sludge. It should be mentioned that each point in the curve is the average of three data as three cycles were monitored for each initial COD concentration.

In addition, similar to SBR, a typical COD concentration profile in the CFID bioreactor over a HRT of 4 h was

monitored as illustrated in Fig. 3. From Fig. 3, the feast and famine phases in the CFID bioreactor are not distinct about SBR, and the COD concentration is approximately constant around 150 mg/L after 50 min. Hence, to provide similar circumstances (feast/famine regime), idling time was applied in the CFID bioreactor at the beginning of each run. Over idling time, the bioreactor was not aerated, though the influent is entered to the bioreactor. As a fact, idling time provided a feast phase at the beginning of each run. The effect of idling time on the feast/starvation condition (HRT of 4 h) was examined in three levels including 30, 60, and 75 min (Fig. 3).

As can be seen in Fig. 3, idling times of 60 and 75 min provided longer feast phase in the bioreactor rather than 30 min, whereas famine period was problematic as COD concentration was not decreased to lower than 50 mg/L. Therefore, HRTs of 8 and 12 h were also examined with idling time of 60 and 90 min, respectively, as shown in Fig. 4. As can be seen in Fig. 4, a good famine phase is provided in the bioreactor around 400 and 500 min at HRTs of 8 and 12 h, respectively. Thus, HRTs of 8 and 12 h were selected as optimum conditions for the aerobic granulation process.

3.2. Aerobic granulation process

The SBR was operated for 60 d and the effect of the initial COD concentration on the sludge granulation was investigated. The aerobic granulation process at the initial COD concentrations of 300 and 500 mg/L was studied. As mentioned earlier, the feast and famine phases could be provided

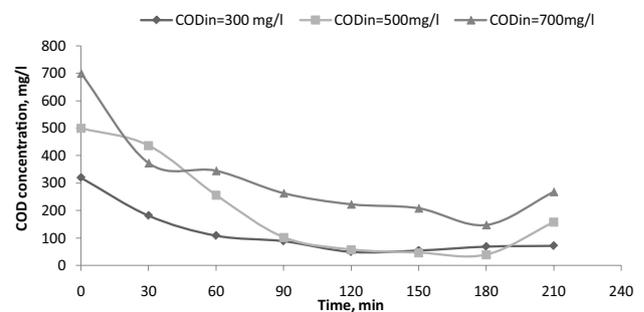


Fig. 2. The changing trend of COD concentration at different initial COD concentrations in SBR.

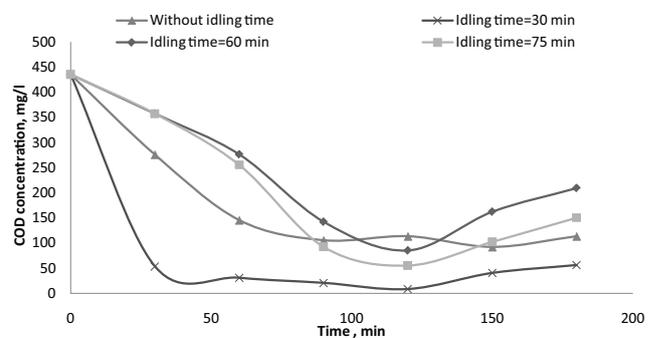


Fig. 3. COD profile in effluent of the CFID bioreactor at HRT of 4 h and influent COD concentration of 500 mg/L at different levels of idling time: without idling and with idling time (30, 60, and 75 min).

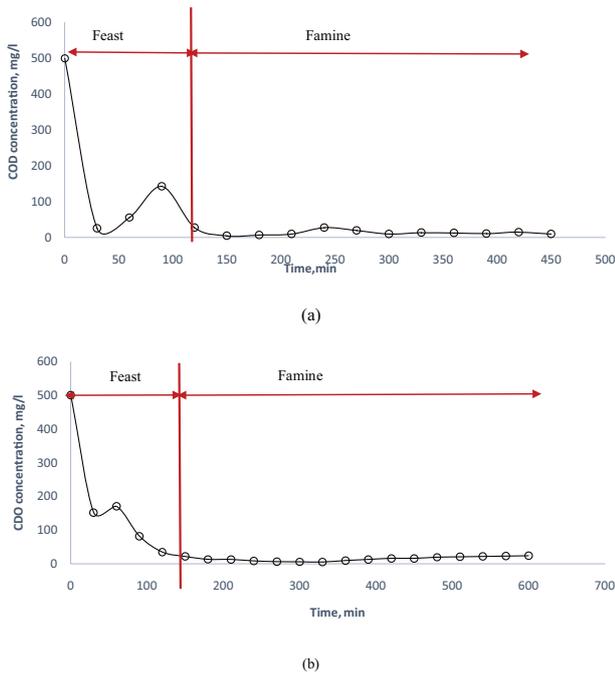


Fig. 4. COD profile in effluent of the CFID bioreactor at (a) HRT of 8 h and idling time of 60 min, and (b) HRT of 12 h and idling time of 90 min in CFID bioreactor.

at the bioreactor under the initial COD concentrations of 300 and 500 mg/L, respectively. The regular and scanning electron microscopic (SEM) images of the aerobic granules formed under batchwise feeding condition are presented in Figs. 5 and 6(a).

Moreover, the possibility of the aerobic granule cultivation in the CFID regime was investigated. In this mean, the CFID bioreactor was operated at three levels of HRT (4, 8, and 12 h) with different idling times. In the first part, to have an acceptable comparison between SBR and CFID bioreactors, the CFID bioreactor was operated with the influent COD concentration of 300 mg/L and HRT of 4 h (OLR = 1.8 kg/m³ d). Over 35 d of operation, there was no sign of granulation in the sludge of the CFID bioreactor; however, a stable granular sludge was obtained in SBR after 20 d. It should be mentioned that MLSS concentration in the CFID bioreactor was reduced gradually over 1 month of operation and the main sludge washed out from the bioreactor.

This result encouraged the author to examine higher organic loading rate by increasing influent COD concentration. Therefore, at the second stage, the CFID bioreactor was operated at the influent COD concentration of 500 mg/L and HRT of 4 h (OLR = 3 kg/m³ d). In addition, idling times of 30, 60, and 75 min were applied to provide a proper feast and famine phases. From the observation, MLSS concentration was reduced similar to the first stage, and the granular sludge was not obtained in HRT of 4 h. The measured

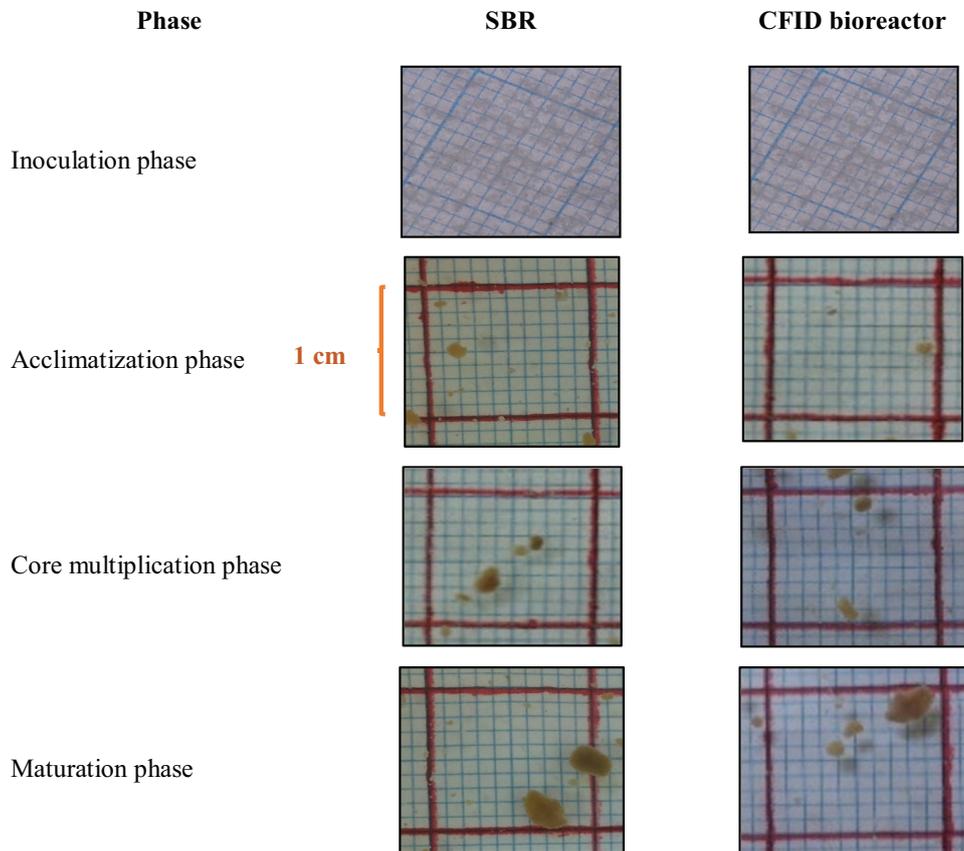


Fig. 5. Development of granular sludge at different phases.

responses during each condition are presented in the following sections.

Overall, HRT of 4 h was not suitable for granulation process in the CFID bioreactor. Hence, the HRT values of 8 and 12 h were examined, providing more time for famine phase. In the first step, HRT of 8 h and the influent COD concentration of 500 mg/L (OLR = 1.5 kg/m³ d) was applied in the bioreactor. In this condition, the signs of aerobic granulation via appearing the first cores were observed. Over the next step, the system was operated at HRT of 12 h and the initial COD concentration of 1,000 mg/L (OLR = 2 kg/m³ d). By increasing HRT to 12 h (initial COD concentration of 1,000 mg/L), the cores grew, and tiny granules appeared which was continued to get grown granules.

3.3. Physical properties of the granules

3.3.1. Granule morphology

Bioparticle density and diameter have been recommended as suitable factors to quantitatively describe the granulation process [19]. Hence, some pictures of the sludge during the granulation process in both bioreactors are presented in

Fig. 5. It should be mentioned that the pictures for the SBR refer to HRT of 4 h, whereas HRT was 8 h for the CFID bioreactor. As shown in Fig. 5, seed sludge had fluffy, irregular, and loose-structure morphology recognized as a flocculated sludge at first. The integrity of sludge was improved over 4 and 8 d at the SBR and CFID bioreactor, respectively (Fig. 5). After acclimatization phase, aerobic granules started to be recognized from activated sludge and then grown up in core multiplication step on days 7 and 15 at the SBR and CFID bioreactors, respectively. Over core multiplication step, the granules qualitatively multiplied and also the structure of granules changed. In this phase, the loose-structure granules were gradually changed to more robust and dense structure. The similar observation was reported by Wang et al. [20].

Maturation phase was marked by the appearance of mature granules as illustrated in Fig. 5. In the maturation phase, the structure of granules varied to more perfect and stronger. The diameter of mature granules was 0.5 mm in both bioreactors.

The SEM images of the aerobic granules formed at both bioreactors are presented in Figs. 6(a) and (b). The aerobic granules in the SBR were almost round with a clear

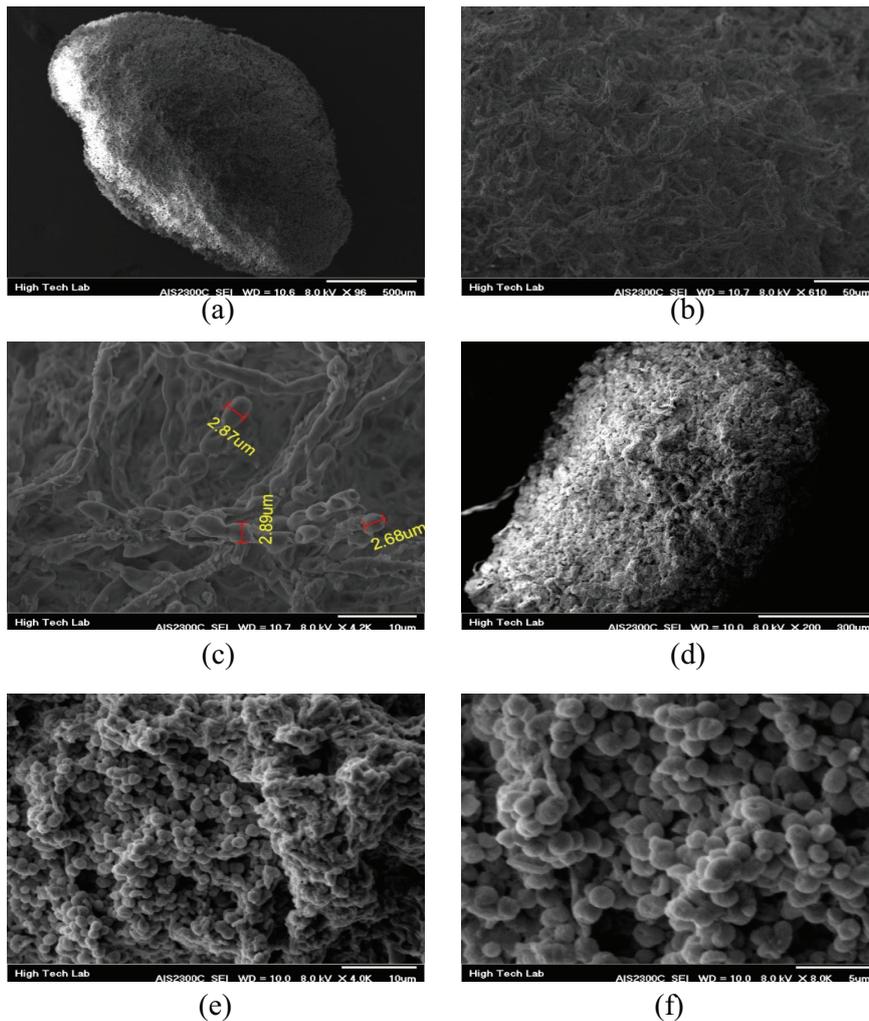


Fig. 6. The SEM images with different magnification for matured granules in (a)–(c) SBR and (d)–(f) CFID with HRT of 12 h.

boundary, recognized from the loose and irregular flocs of the seed sludge (Fig. 6(a)). The mature granules showed a very dense microstructure on the outer layer in which various rod-shaped bacteria were predominant, and the cells were tightly packed. In the literature, the similar compact structure of aerobic granules dominated by rod-shaped bacteria was reported in glucose-fed [21] and acetate-fed [22]. Besides, the chain structure of bacteria observed in the figure, indicating a good synergistic relationship among bacteria.

The SEM images of a matured granule taken from the working CFID bioreactor under HRT of 12 h are shown in Fig. 6(b). The SEM observations indicated that the granules were mainly made up of numerous bacterial microcolonies. As it is observed from the figure, the structure of granule was porous with many channels and cavities at the surface and interior regions of the granule.

Moreover, the microbial population of the granule was homogenous comprising coccus with a spherical shape, whereas rod-shape bacteria were dominant in the granules obtained from the SBR.

As an interesting finding from the SEM images, SBR generated granules with more integrity than what obtained for the CFID bioreactor, mainly because of the nature of the SBR to provide desired feast–famine strategy, which enables it to produce more integrated granules [23]. Besides, the cells were arranged in a chain structure in granules obtained from the SBR. Whereas an irregular structure of cell arrangement illustrated in the granules gained from the CFID bioreactor. As a result, the granulation process was occurred in the SBR and CFID bioreactor at different conditions.

3.4. Sludge volume index of the granular sludge

Settle ability of sludge is a critical factor in establishing well-settling particles in the reactors. The data of SVI over the granulation process in the SBR with the initial COD concentrations of 300 and 500 mg/L are displayed in Fig. 7(a). The SVI of primary seed sludge was 43 mL/g, and the range of floc size was 0.01–0.08 mm. From the observation, both initial COD concentrations indicated a slightly increasing trend of SVI at the beginning of granulation. The maximum values of SVI were reported at the days of 9 and 7, respectively, for initial concentrations of 300 and 500 mg/L, implying the initiation of granulation process. After that, SVI of sludge was reduced step by step as the first granular cores were observed. SVI is an important indicator to monitor the formation of aerobic granules. A reduction in SVI is a sign of developing granulation process [7].

After 20 d of operation, the seed sludge in the reactor was almost granulated and the SVI was around 20 mL/g. To have a comparison with other researches, in a study where glucose and acetate were used as feed, the minimum amount of SVI was reported about 33 mL/g after 40 d [24]. In addition, in another study done by Amini et al. [25], the minimum SVI of 68 mL/g was reported for aerobic granular sludge obtained with dairy wastewater. Consequently, from the data, it could be found that the aerobic granulation in SBR fed by soft drink wastewater at both initial concentrations of 300 and 500 mg/L is a prosperous process to synthesize an acceptable granular sludge with low SVI value in a short time of operation (20 d).

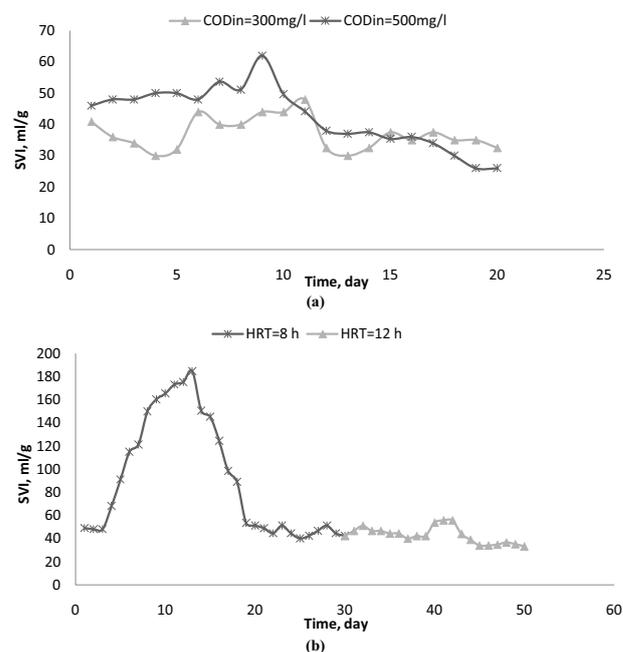


Fig. 7. Changes in SVI over the aerobic granule cultivation in (a) SBR and (b) CFID bioreactor at HRTs of 8 and 12 h.

Fig. 7(b) shows the SVI profile of the granulation process in the CFID bioreactor at HRTs of 8 and 12 h. The decreasing trend of SVI during the experiments after core formation (after 20 d) indicated a good settling ability of the sludge due to the granulation and discharging the flocs with poor settling.

The primary seed sludge of the CFID bioreactor had 50 mL/g of SVI and the median floc size of 0.01–0.08 mm. At HRT of 8 h, SVI increased to 180 mL/g during core formation. From the observations, the aerobic granules started to be formed when sludge showed better settling ability. After the appearance of first granular cores, SVI reduced step by step. At the end of acclimatization phase, SVI of the sludge was 130 mg/L in the CFID bioreactor. Over the second week, a significant change in SVI value was observed when some of the pinpoints appeared in the bioreactor. After 15 d, SVI sharply decreased and reached 40 mL/g during core multiplication at HRT of 8 h. At HRT of 12 h, after 2 weeks, the granular cores were nearly matured, and SVI was decreased to less than 40 mL/g. After 45 d, small and dense aerobic granules were fully obtained at the HRT of 12 h.

3.5. Process performance during granulation

3.5.1. COD removal efficiency

Fig. 8(a) shows the COD removal efficiency at the initial COD concentrations of 300 and 500 mg/L over granulation process in the SBR. From the figure, at the beginning of the process, the COD removal efficiency for the initial COD concentration of 300 mg/L was higher than that of 500 mg/L (92% at initial COD concentration of 300 mg/L against 65% at initial COD concentration of 500 mg/L). The range of COD removal efficiency over the granulation process was 90%–95% and 65%–88% at the initial COD concentration of

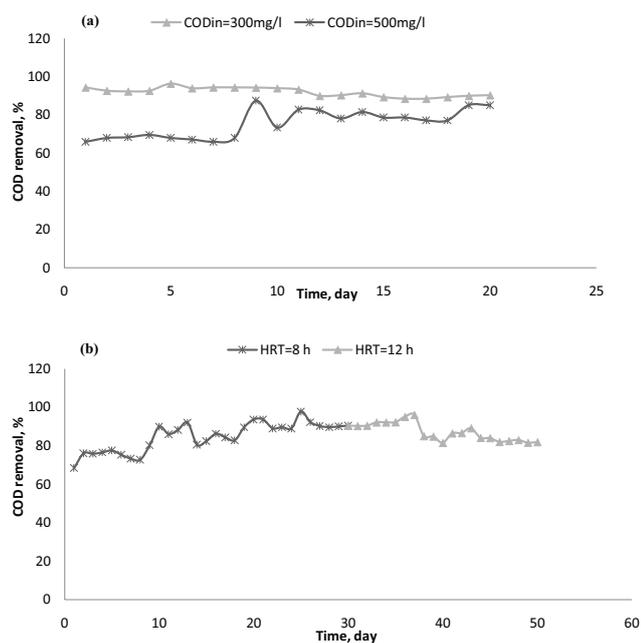


Fig. 8. Changes in COD removal efficiency during aerobic granule cultivation in (a) SBR and (b) CFID bioreactor at HRTs of 8 and 12 h.

300 and 500 mg/L, respectively. The response was approximately constant over the granulation process at the initial COD concentration of 300 mg/L, while the COD removal efficiency showed a slightly increasing trend at the initial COD concentration of 500 mg/L after the core multiplication phase.

In a previous study, the aerobic granular sludge was obtained in an SBR fed using initial COD concentration of 300 mg/L by milk processing wastewater which the maximum COD removal efficiency at a cycle time of 4 h was reported as 78% [17]. As both milk processing and soft drink wastewater are mainly biodegradable, this comparison indicates a good performance of obtained granular sludge to treat wastewater.

Fig. 8(b) illustrates COD removal efficiency in the CFID bioreactor at HRTs of 8 and 12 h for 55 d. At the beginning of the operation, the COD removal efficiency was about 70% at HRT of 8 h, but during acclimatization and core formation phases, the response was increased gradually. From the figure, after 20 d of operation, the COD removal efficiency was nearly constant (around 90%). The effect of increasing HRT from 8 to 12 h on the response was negligible.

3.5.2. Effluent turbidity

Effluent turbidity is a physical parameter to assess the process performance in biological treatment systems, so it was considered as a quality parameter to monitor the granule formation. The variation of the effluent turbidity at different initial COD concentrations for the SBR is shown in Fig. 9(a). As it is observed from the figure, the effluent turbidity of the bioreactor was lower than 20 NTU for the first week. At the core multiplication phase, the response increased to 80 and 45 NTU at the initial COD concentrations of 300 and 500, respectively. It was related to wash out of fluffy seed sludge

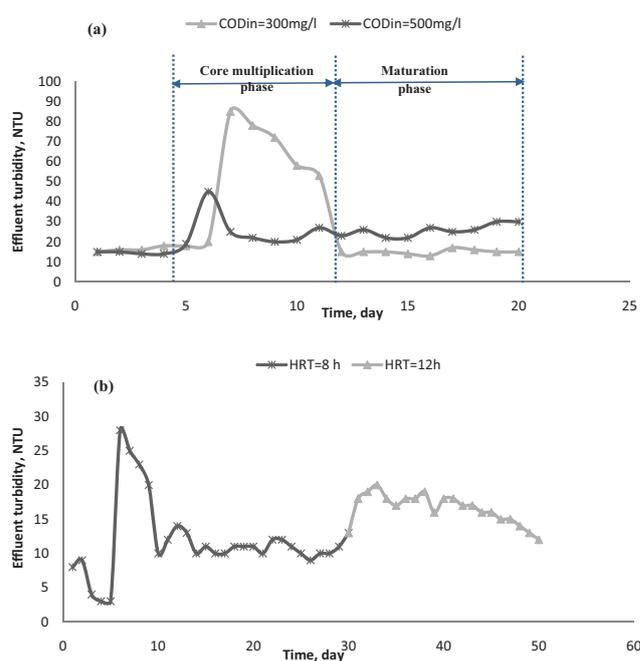


Fig. 9. The effluent turbidity over aerobic granular sludge for (a) SBR and (b) CFID bioreactor with HRTs of 8 and 12 h.

over the granulation process. As a result, over core multiplication, pinpoint particles appeared with higher settling velocity rather than fluffy flocs. Consequently, the flocs with low settling velocity washed out from the bioreactor. Nevertheless, over maturation phase, the effluent turbidity was decreased and remained almost stable at both initial COD concentration (15 and 25 NTU for initial COD concentrations of 300 and 500, respectively), representing granular sludge.

In the CFID bioreactor, the variation of the effluent turbidity over HRT of 8 and 12 h is depicted in Fig. 9(b). At first, the effluent turbidity of the bioreactor was lower than 5 NTU. At acclimatization phase, the effluent turbidity increased to 27 NTU because of discharging fluffy seed sludge from the reactor during the wash out process, verifying the existence of granular cores. After day of 10, the effluent turbidity was decreased to less than 15 NTU over core multiplication. By increasing HRT from 8 to 12 h, the effluent turbidity showed an increasing trend, while after maturation phase (day 45), the response decreased to 10 NTU. The low level of effluent turbidity confirms that the structure of the granular sludge was stable and resistant in conditions with shear forces. As a conclusion, the effluent turbidity of the granular sludge in the CFID bioreactor is lower than SBR (10 NTU in compare to 20 NTU), indicating an excellent performance of the aerobic granules in the CFID bioreactor to treat wastewater.

4. Conclusion

In this study, the effect of feeding regime, batch and continuous, on the aerobic granulation process was assessed. As a conclusion, the feeding strategy could be a significant factor in granulation process in addition of the other effective factors reported in the literature. To form aerobic granular sludge in different feeding regimes (batch and continuous), different

operating conditions are also required. The best condition to generate the aerobic granular sludge in SBR, used in this research (with considering the type of wastewater, reactor configuration, and operating conditions), was cycle time of 4 h and the initial COD concentration of 500 mg/L, whereas HRT of 12 h and influent COD concentration of 1,000 mg/L with idling time of 90 min was the desired condition in the CFID bioreactor. Besides, the morphology and structure of obtained aerobic granules in the SBR and CFID were different regarding shape, integrity, and the arrangement of microorganisms.

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