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Research paper

Performance of Aerobic Granular Sludge in Treating Soy Sauce Wastewater at Different Hydraulic Retention Time

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Abstract

Aerobic granular sludge had shown its capability in treating soy sauce wastewater, but its reactor performance, granules properties and biokinetics in different hydraulic retention times (HRT) is still unknown. To ensure the reactor is performed in optimum condition, a judicially selection of HRT is important. The study was conducted in a high and slender column operated according to a sequential batch reactor (SBR) with a sequence of aerobic and anaerobic/anoxic reaction phases. Three different HRTs (8, 16, 24 h) and different anaerobic and aerobic reaction time were evaluated. In the study demonstrated the increase in HRT could reduce the organic loading rate (OLR) as well as biomass yield (Y_{obs}, Y), endogenous decay rate (k_d) and overall specific biomass growth rate ($\mu_{overall}$). It was observed a slight increase in the mixed liquor suspended solid (MLSS) and the granules mean size as the OLR decreased. Meanwhile, in the lowest HRT reactor, a narrow diameter range of aerobic granule from 3 to 100 µm was observed due to the development of small and dense granules. The HRT of 24h with aerobic and anaerobic/anoxic reaction time of 3.88 and 7.77h respectively is the SBR's best performances due to the improvement of the aerobic granular physical properties.

Keywords: *Aerobic granular sludge, Biokinetics, Hydraulic retention time, SBR, Soy sauce wastewater*

1. Introduction

In general, soy sauce wastewater has high COD, BOD, total suspended solid (TSS), protein and has deeper colour due to the fermentation process, that contribute to the strong odours during wastewater treatment [1]. In the effort of designing an effective treatment system for soy sauce wastewater, many researches have conducted their studies on the topic, which include using the sequencing batch reactor (SBR) system. As such, a compact bioreactor of aerobic granular sludge technology based on SBR system is recommended to reduce the reactor volume and shorten the retention time by providing a good solid-liquid separation packed with microbial communities in a single reactor [2].

Besides, a literature has reported the ability of aerobic granular sludge for treating soy sauce wastewaters, which involves both anaerobic and aerobic processes in SBR [3]. To meet a complete degradation process in treating recalcitrant compounds especially, the combination process of anaerobic and aerobic condition is necessary [4]. However, as the wastewater treatment system develop, a need to develop an efficient nutrient removals system especially in treating high strength organic wastewater to provide good sanitation is crucial.

Significantly, HRT was found to be an important key parameter, which can improve the removal rates of all targeted substances especially in SBR [5]. As identified by Pan *et al.* [6], short HRT and relatively high OLR in a treatment system is favourable for the aerobic granular sludge formation. This is because short HRT in a system causes wash out of the poor settling biomass, while high OLR ensures the growth of retained biomass in the system. Basically, the microorganisms in the SBR system experienced a hydraulic selection pressure due to the reactor configuration such as HRT, which required the biomass either being washed out or retained in the system to be bound together to form good settleability granules [7].

However, as the HRT is known to have remarkable influent on the aerobic granular sludge development, the present study aimed to determine the HRT relationship with the aerobic granular sludge's performances for treating soy sauce wastewater, besides monitoring the capability of soy sauce wastewater as the substrate media for cultivating the granular sludge under certain HRT. As the studies on the aerobic granulation kinetic behaviour in utilizing substrate at different HRTs would show different behaviour of aerobic granulation kinetics [8], the granules properties, reactor performance, and biokinetics changes are examined.

2. Materials and Methods

Fig.1 is a flowchart illustrating the overall outline of the experimental work for this study.

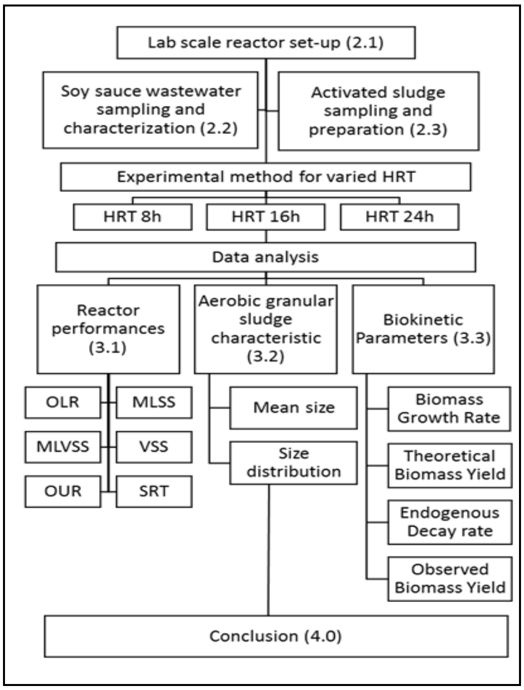

Fig.1: Flowchart of the experimental work.

2.1. Reactor Set-Up

A SBR (100 cm in height, 6.5 cm in diameter) with working volume of 3L was used in this study. The influent was supply at the bottom of the reactor and the effluent was discharged via an outlet port positioned at mid-height of the reactor column, yielding a 50% of volumetric exchange ratio. A timer box was installed to control the pumps; influent, effluent, circulator and aerator in the reactor. An air bubble diffuser was placed at the bottom of the reactor. Fig.2 shows the schematic drawing of the reactor set-up.

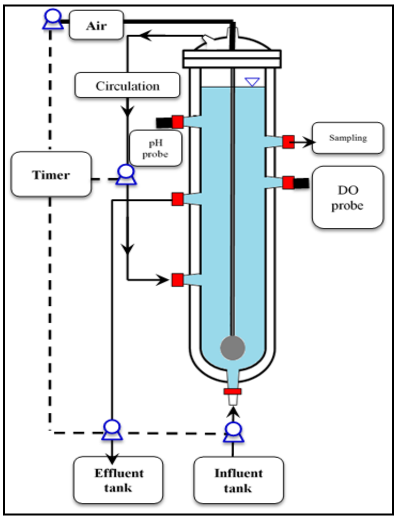

Fig. 2: Schematic drawing of the a laboratory scale SBR

2.2. Wastewater Characteristics

The soy sauce wastewater sample was taken from a soy sauce factory located at Johor Bahru, Malaysia. It was sampled weekly and stored in cold storage room at a temperature of 4 °C to prevent the wastewater to biodegrade by the microbial action. The soy sauce wastewater contains a small amount of chili flakes, uncrushed soybean, inorganic and organic salts and also grey water from washings activities. These substances are readily biodegradable and it will result in high oxygen consumption upon discharge of wastewater in receiving surface water which which contributed to high level of COD, BOD and suspended solid (SS). The characteristics of the soy sauce wastewater was as follows: COD, 7620 ± 80.4 mg/L; BOD, 4967 ± 566 mg/L; total suspended solids (TSS), 4230 mg/L; total solid (TS), 13900 mg/L and pH, 6.3 – 7.5.

2.3. Seed Sludge Preparation

The reactor was inoculated with 1.5L of freshly activated sludge, collected from an aeration tank of Municipal Wastewater Treatments Plants, Johor Bahru, Malaysia. The sludge was sieved to pass 1.0 mm mesh to remove debris and small particles. The sludge was acclimatized with mixed wastewater of domestic and soy sauce for a week. The activated sludge was loose and fluffy with mixed liquor suspended solid (MLSS) is 23.9 ± 2.9 g/L and the sludge volume index (SVI) is 120-150 ml/g.

2.4. Analytical Methods

Sample analysis included MLSS, mixed liquor volatile suspended solid (MLVSS), COD, BOD, SS and TS, all according to Standard Methods [9]. MLVSS was determined by ashing the dry sample at 550°C in a furnace for 15 min. The pH and DO were continuously monitored with the electro probe sensors inserted in the reactor and recorded by a pH/DO meter (Orion 3-Star Benchtop pH/DO Meter). The granules developed in the reactor were analyzed for their physical characteristics: the granules diameter and the granules size distribution. The morphological and structural observations of granular sludge were conducted periodically by using a stereo microscope equipped with digital image analyzer (PAX- ITv6, ARC PAX-CAM). The particular size distribution of the granules was measured using the laser particle size analysis system (Mastersizer 3000, Malvern instruments). The formulas and calculations for kinetic parameters included the theoretical biomass yield (Y), observed biomass yield (Y_{obs}), endogenous decay rate (k_d), and overall specific biomass growth rate ($\mu_{overall}$), and were referred Muda *et al*. [10] and Rojas *et al.* [11].

2.5. Experimental Procedures

The reactor operation mode was based on SBR system. The system cycle time consists of fil (5 min), anaerobic/anoxic (146 – 466 min), aerate (73 – 233 min), settle (10 min), discharge (5 min) and idle (1 min) stages. Air was supplied by an air pump at 2 L/min volumetric flow rate throughout the aeration stage. The wastewater in the reactor was allowed to circulate using peristaltic pump (ColeeParmer System Model, 6–600 rpm) at 18 L/h flow rate during the anaerobic/anoxic stage. The details of the experimental conditions are shown in Table 1. The HRT was varied between 8 and 24 h in three reactors namely R1, R2 and R3. The OLR was varied based on the operated HRT. The reactor was operated at room temperature (27±1 °C) while the pH throughout the experiment was between 7.0 and 8.0.

Table 1: Detailed experiment conditions for the reactor system.

Reactor	Cycle time (h)	Aeration (h)	Anaerobic/ anoxic (h)	HRT (h)	OLR (kgCOD/m^3/day)
R1	4	1.22	2.43	8	14. 7 – 40.8
R2	8	2.00	5.68	16	1.7 – 26.3
R3	12	3.88	7.77	24	4.9 – 13.7

3. Result and Discussion

3.1 Physical Profile of the Reactor System

During the operation, monitoring the concentration of biomass is important to ensure the active biomass in the reactor is adequate for completing the degradation process, especially for a system deals with a wide range of OLR [12]. The solid retention time

(SRT) of a system represents the average detention time of the biomass and controlled operationally by the biomass concentration. The SRT of R1, R2 and R3 was determined at the end of every experiments in this study, as shown in Table 2. In the reactor that applied 24 h of HRT had 8.53 ± 1.8 days of SRT which is the longest, followed by 3.06 ± 1.1 days in R2 and shortest SRT showed in R1 (1.67 ± 0.6 days). It was found that the SRT in the reactor system was proportionally related to the HRT applied.

According to Liu and Liu [13], the SRT in a reactor system influences the growth rate of microorganisms, which is important in designing an aerobic granular sludge system. The longer SRT applied in a system would lower the specific bacteria growth rate. Therefore, the operation strategy of short SRT was applied in the aerobic granular sludge technology to fasten the bacteria growth rate to enhance the granulation process. Furthermore, the performances of a reactor system in utilized nitrification and COD are also influenced by the SRT [14]. This is because the optimum SRT allowed the bacteria to grow faster and a stable population would developed for the biochemical transformation process [15].

Table 2: The physical properties of the reactors at different HRTs.

Reactor	**R3**	**R2**	**R1**
HRT (h)	**24**	**16**	**8**
OLR (kgCOD/m^3/day)	4.9 – 13.7	1.7 – 26.3	14. 7 – 40.8
MLSS (g/L)	12.22 ± 0.87	9.97 ± 0.25	11.23 ± 0.99
MLVSS (g/L)	10.9 ± 1.2	8.53 ± 0.7	10.6 ± 0.9
VSS$_{effluent}$ (g/L)	3.93 ± 0.1	1.67 ± 0.3	1.8 ± 0.1
OUR (mgO$_2$/L/h)	4.87 ± 0.14	10.47 ± 1.66	17.17 ± 1.54
SRT (d)	8.53 ± 1.8	3.06 ± 1.1	1.67 ± 0.6

OUR in the reactors were determined at the last 10 minutes before the aeration stage was stopped. The OUR represents the growth activities of heterotrophic and autotrophic [16]. From the result, the OUR value increased when the HRT decreased due to the increased OLR. According to Obaja *et al.* [17], the OUR is higher as the substrate is higher in the system because of the higher viable biomass activity. This proven in this study when R1 with 8 h of HRT was supplied with the highest OLR (14.7 – 40.8 kgCOD/m^3/day) achieved the highest OUR (17.17 ± 1.54 mgO$_2$/L/h). The lower OUR value in the system indicated that the degradation of external substrate. As the substrate was completely consumed and degraded, the biomass was in a starvation condition and the endogenous respiration occurs [10]. According to Ni and Yu [16], the maximum OUR of 350 mg O$_2$/L/h is the combined oxygen uptake capacity from both heterotrophs and autotrophs. 75 mgO$_2$/L/h is the maximum OURs for the autotrophs and approximately 275 mg O$_2$/L/h are heterotrophs. From the Ni *et al.* [18] study, the total oxygen was consumed by the heterotrophs is 61%, while the autotrophs consume only 39% in the reactor.

3.2 Physical Characteristics of Aerobic Granular Sludge

The physical characteristics of aerobic granular sludge had affected by HRT and OLR of a reactor [19]. All the three reactors were successful in developing aerobic granular sludge. However, the structural features and morphology of aerobic granular sludge produced were different at different HRTs. R1 was operated with 8 h of HRT developed smaller aerobic granular sludge with mean diameter of 0.8 ± 0.2 mm and become more compact than granules in R2 and R3. The aerobic granular sludge in R3 had the highest mean diameter of 2.2 ± 0.1 mm, while aerobic granular sludge grew into 1.3 ± 0.7 mm in R2 on day 63 operation. The mean diameter of granules appeared to be correlated with the HRT. The result indicates that HRT is one of the effective operating parameter that control the granules size in a reactor.

In the lowest HRT, the mean size of aerobic granular sludge in R1 was the smallest compared to other reactors. According to Rosman *et al.* [19], lower HRT would enhance the extracellular polymeric substances (EPS) production. A higher hydraulic selection pressure is due to the shortest cycle time applied to the system. The EPS production could facilitate both cohesion and adhesion of cells and play a crucial role in develop and maintaining structure integrity in an immobilized cells community. Meanwhile, the size of aerobic granular sludge in R3 was the largest compared to other reactors. The growth of the largest aerobic granular sludge size in 24h of HRT was due to the loosely linked microbes within the granules and existed of cavities become the granules less dense.

In addition, 24 h of HRT in R3 resulted the system encounter the longest cycle time. Therefore, a weak hydraulic selection pressure on the biomass was served in the system. This is because the frequency of dispersed and filamentous particles being discharged through the effluent withdrawal is lesser. Therefore, the excessive filamentous microorganisms was retained and survived in the system, resulting in sloughing detachment of the particles and producing big aggregates [20]. Hence, the filamentous organism’s entanglement might increase the size of granule rapidly but loosely. This reason might also account for the largest aerobic granular sludge size developed in R3 for only 63 days operation time compared to other reactors. The development of large granules were not stable, which became loose and then gradually disintegrated [21].

Interestingly, the particle size distribution measurements show a clear impact of the HRTs on the granule size. Aerobic granular sludge size distribution at different HRTs of 8 h, 16 h and 24 h shown in Fig.3. In R1, a narrow diameter range from 3 to 100 µm was observed under the lowest HRT (i.e. 8 h). This is due to the development of small and dense aerobic granular sludge. Moreover, the granules size followed the dynamic population balance, which is the simultaneous breakage and granulation mechanisms, where higher granulation ability and strength integrity of aerobic granules will benefit in developing the larger granules size [22]. There are two peaks were observed in the particle size distribution under 16 h of HRT. One peak at mean size of 100µm and another peak at mean size of 1300 µm, which indicated that small granules still coexisted with the large granules in the R2 even after the mean granules size grew bigger.

Therefore, a wide diameter range of aerobic granular sludge existed in R2 with 4 to 3080 µm. Meanwhile, in R3 with the highest HRT of 24 h, almost 10% of aerobic granular sludge volume was from size of 1500 µm. The reactor also shows a wide range of granules size which is from 5 to 3080 µm, which mean granulation is a gradual process of small size of seed sludge developed into stables and large granules [23]. In addition, too long HRT caused more flocculent sludge retained in the system with slow growth rate of the microorganisms, which became a disadvantage to the biomass aggregation. As explained by Pan *et al.* [6], an optimum HRT operated in a reactor might lead to a rapid granulation process and maintained the stabilization of granular sludge with enhanced high microbial activities.

3.3 Biokinetics Parameter

Muda *et al.,* [10] proposed that the substrate degradation rate and biomass production process that influenced the biomass concentration in a system is known as the kinetic parameters. In this study, the kinetic study was performed at OLR of 4.9–13.7, 1.7–26.3 and 14.7–40.8 kg COD/m^3/day with 24, 16, and 8 h of HRT, respectively. The applied parameters were obtained at the end of experiment for each HRT. SRT is the one of the most critical parameters to evaluate the reactor performance and to determine the kinetic value [24]. The value of SRT for 24, 16, and 8 h of HRT was 8.53, 3.06 and 1.67 days respectively. Table 3 showed the results of the kinetic parameters for every HRT.

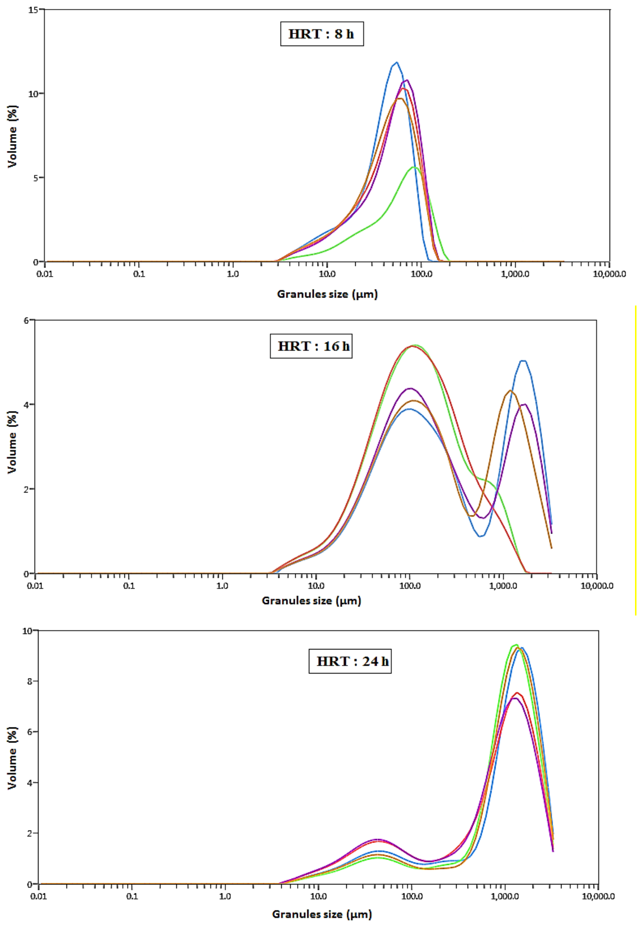

Fig. 3: Aerobic granular sludge size distribution at different HRTs of 8 h, 16 h and 24 h.

The increased of SRT demonstrated the decrease of $\mu_{overall}$ in the reactor system. It explains that the longest period of HRT led to the slowest $\mu_{overall}$ since the biomass was exposed to the longest period of endogenous respiration or decaying [25]. As reported by Moy *et al.* [26] and Zheng *et al.* [27], lower $\mu_{overall}$ and longer SRT allowed the aerobic granular sludge to withstand high OLR and maintain structural stability. The biomass decay rate (k_d) is the biomass loss rate during the endogenous respiration [25]. 0.203 d^{-1} is the highest value of k_d in R3 with the highest HRT (i.e. 24 h). The highest value of k_d represent the highest decay rate of biomass. Since the OLR in R3 with 24 h of HRT was low, the biomass activity become limited as the OUR value was low (13.75 ± 0.37 $mgO_2/L/h$) and lead to the high biomass loss. Compared to these results, Gao *et al.* [28] reported lower k_d with 0.006–0.027 d^{-1} was achieved.

According to Soltani *et al.* [24], observed biomass yield (Y_{obs}) is an important parameter that represents the ratio of biomass production to the mass of substrate consumed. Y_{obs} explains the sludge productivity in the reactor system. From the observation, $\mu_{overall}$ and Y_{obs} were closely related to the OLR of the reactor, while the OLR relied on the operated HRT. The theoretical biomass yield (Y) is derived from Y_{obs} and the difference is due to the variation of mechanisms, such as biomass endogenous metabolism, predation, death and decay rate, which are represented by k_d [29]. From the result, increased of the SRT from 1.67 to 8.53 d shows the decreasing of k_d value from 0.046 to 0.013 d^{-1}, respectively. As the highest OLR (14.7–40.8 $kgCOD/m^3/day$) supplied in the system, the k_d value increased. Muda *et al.* [10] also experienced the same trend but the k_d value achieved was lower than this study. From the result, the biomass loss rate in the 24 h of HRT was 0.013 d^{-1}, followed by 16h of HRT with 0.035 d^{-1} and 0.046 d^{-1} in 8h of HRT. The biomass loss rates reported by Muda *et al.* [10] were lower than achieved in this study by with 0.0096 to 0.0060 d^{-1} of the k_d value.

Table 3: Kinetic coefficients of aerobic granular sludge at different HRTs.

Kinetic parameters	**HRT**		
	24h	**16h**	**8h**
OLR ($kgCOD/m^3/day$)	4.9–13.7	1.7–26.3	14.7–40.8
SRT (d)	8.53	3.06	1.67
OUR ($mgO_2/L/h$)	4.87 ± 0.14	10.47 ± 1.66	17.17 ± 1.54
Observed specific biomass growth rate, $\mu_{overall}$ (d^{-1})	0.117	0.277	0.599
Biomass decay rate, k_d (d^{-1})	0.013	0.035	0.046
Observed biomass yield, Y_{obs} (mgVSS/mgCOD)	0.338	0.371	0.865
Theoretical biomass yield, Y (mgVSS/mgCOD)	0.374	0.417	0.932

The sludge production in the R1 (i.e. 8 h HRT) was the highest compared to other reactors. This can be seen by the highest Y_{obs} value obtained in the system with 8 h of HRT and can be related to the highest biomass in the effluent (2.27 MLVSSg/L) was being washout from the R1 and less amount of substrate was being utilized. Therefore, there are less sludge production from the R3 with 24 h of HRT and R2 (16 h of HRT) as the Y_{obs} value was lower.

In a mean time, high utilization rate of the substrate (i.e. soy sauce wastewater) in R3 and R1 was achieved. In addition, the Y_{obs} value was increased as the SRT was decreased. This is referred as the sludge production in the wastewater treatment plant increased with decrease of the sludge age [29]. Y value in 24 h of HRT was the lowest compared to other reactors. The result demonstrated that low biomass was produced in the reactor but higher in rate of substrate removal as the Y_{obs} value was low with the lowest $\mu_{overall}$ and k_d was achieved. In summary, the kinetic parameters could provide good prediction on the aerobic granular sludge system performances and system design of SBR in treating soy sauce wastewater.

4. Conclusion

This results demonstrated that HRT could affected the physical properties of the reactor, physical characteristic of aerobic granular sludge and the biokinetics. Highest HRT (24h) promoted the formation of the highest mean size of aerobic granular sludge with diameter of 2.2 ± 0.1 mm. The granular biomass concentration in the reactor improved which is mainly due to the increase in the OLR. Moreover, longest SRT was observed (8.53 ± 1.8 days), which would lower the specific bacteria growth rate. As the aerobic granules developed, the small granules still coexisted with the large granules in all reactors. Meanwhile, the biomass decay rate in the system (k_d) and biomass yield (Y) was low. From the study, sludge age, $\mu_{overall}$ and granules sludge production of were greatly influenced by the HRT. The highest HRT resulted in the highest of sludge age with the lowest of biomass growth rate and sludge production in the system. These findings represent important information about the best HRT and the reaction time of aerobic and anaerobic/anoxic that is crucial to improve the performances of aerobic granulation in treating soy sauce wastewater.

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