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# Development and Utilization of Aerobic Granules for Soy Sauce Wastewater Treatment: Optimization by Response Surface Methodology

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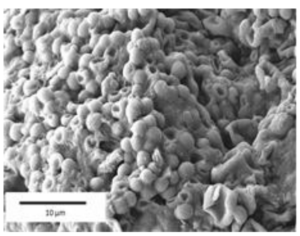
**Graphical abstract**




**Abstract**

This study examined some important factors for optimal aerobic granular sludge performance using soy sauce wastewater as a substrate in a lab-scale alternating anaerobic/aerobic sequencing batch reactor (SBR). The SBR was equipped with a circulation process to restrict the concentration gradient of granular biomass during the anaerobic phase. The influence of the circulation rate was investigated together with operation time on the SBR performances. Aerobic granular sludge (AGS) took 60 days to appear and the average diameter was 2.0 mm (with a maximum value of 2.5 mm). Response Surface Methodology (RSM) was used for experimental design, analysis and optimization. The results showed that the maximum COD removal (90%) and good SVI performance of 55.3 mL/g were obtained at the highest value of the operation time (60 d) and at moderate circulation rate (25.2 L/h). The maximum values of MLVSS/MLSS have been found to be 89% at the highest value of the factors (60 d and 36.0 L/h). At optimum point (33.62 L/h of circulation rate and 60 d of operation time), the amount of COD removed, MLVSS/MLSS and SVI were 86.5%, 88.8% and 58.6 mL/g, respectively.

*Keywords*: Aerobic granular sludge; circulation rate; SBR; soy sauce wastewater; RSM

**Abstrak**

Beberapa faktor penting yang mempengaruhi prestasi optimum enapcemar berbutir aerobic menggunakan air sisa kicap telah dikaji. Air sisa kicap merupakan substrat di dalam Reaktor Kelompok Berjujukan (SBR) anaerobik/aerobik yang berskala makmal. SBR dilengkapi dengan proses peredaran untuk mengelakkan berlakunya jurang kepekatan biomas berbutir semasa fasa anaerobik. Pengaruh kadar edaran dan masa operasi terhadap pencapaian SBR telah dikaji. Enapcemar Berbutir Aerobik (AGS) mengambil masa selama 60 hari untuk terbentuk dan mempunyai diameter berpurata 2.0 mm (dengan diameter maksimum 2.5 mm). *Response Surface Methodology* (RSM) telah digunakan untuk merekabentuk eksperimen, menganalisis dan pengoptimuman. Hasil kajian ini menunjukkan bahawa penyingkiran COD yang maksimum (90%) dan prestasi SVI yang baik sebanyak 55.3 mL/g telah diperolehi pada masa operasi yang maksimum (60 hari) dan pada kadar peredaran yang sederhana (25.2 L/h). Nilai maksimum MLVSS/MLSS sebanyak 89% telah diperolehi pada nilai faktor tertinggi (60 hari dan 36.0 L/h). Pada titik optimum (dengan 33.62 L/h kadar peredaran dan 60 hari masa operasi), jumlah COD yang dikeluarkan, MLVSS/MLSS dan SVI adalah 86.5%, 88.8% dan 58.6 mL/g.

*Kata kunci*: Enapcemar berbutir aerobic; kadar peredaran; SBR; air sisa kicap; RSM



## 1.0 INTRODUCTION

Aerobic granular sludge is a dense self-immobilized microbial consortium and it is one of the huge achievements in wastewater treatment in the twentieth century. The advantages of high biomass retention, effective sludge settlement and successful application in SBR technology for over a decade have attracted many researchers to focus in field of aerobic granulation technology. However, due to strict legislations on wastewater pollution discharge, significant attention has been focused on compact high-rate bioreactors which combined the aerobic granular sludge system with anaerobic processes in a single reactor. A combination process of aerobic and anaerobic in a single reactor is competent to enhance the simultaneous organic and nitrogen removal efficiency besides it is an efficient treatment known as straight forward system where there is no return sludge required, cost effective and has small footprints. Treating high organic strength industrial wastewaters with aerobic or anaerobic treatment system alone do not achieve effluents that comply with legislation [1]. Due to Vera *et al.* [2], the use of anaerobic/aerobic processes are more resistant to hydraulic and organic shock loading compared with aerobic or anaerobic reactors alone, while it is more efficient in organic removal, has a small surplus biomass production and no pH alteration required. The work of Filali *et al.* [3] showed that combination of anaerobic/aerobic conditions to be helpful for granulation and allow simultaneous aerobic nitrification, anaerobic denitrification and phosphorus removal to be more effective.

In anaerobic-aerobic bioreactors, the efficient collision between liquid flow and granules is a main factor that induced the development, mass transfer, extracellular polymeric substances (EPS) production, molecular biology, structure and stability of aerobic and anaerobic granules [4]. This is exposed to the significance of hydrodynamic conditions in cell aggregation and self-immobilization process. Although the understanding of hydrodynamic is still unclear except for hydrodynamic shear force, which is a key factor that influence granulation, as studied from Qin *et al.* [5], Wu *et al.* [6] and Liu and Tay [7] showed that compact granules were developed under high hydrodynamic shear force and it takes a long period for seed sludge to develop into granular under low hydrodynamic shear force whether in anaerobic or aerobic reactors.

Studies have shown that the circulation process for anaerobic phase in anaerobic-aerobic bioreactor can provide anaerobic condition and develop hydrodynamic conditions as well as provide simultaneous mixing between microbe and substrate [8-9]. The circulation rate of mixed liquor in the reactor is an essential operation parameter in the circulation processes. It has been applied to improve the contact between substrate and biomass within the treatment system by expending the hydrodynamic conditions either in aerobic phase by aeration intensity or anaerobic phase by circulation process which intensifies hydraulic mixing and results in better performance and stability.

An optimal choice of circulation rate was found to favor the formation of aerobic granular sludge (AGS) and enhance the overall performance of reactor to remove simultaneous carbon, nitrogen and phosphorus. Therefore, the aim of this study is to analyze granular sludge formation in an anaerobic-aerobic SBR in order to optimize the development of AGS with good ability to degrade substrate, stable structure and excellent settling properties. It is expected that this study will contribute to a better understanding on the role of circulation rate for anaerobic conditions in aerobic granulation. Thus far, the effect of circulation rate and operation time on the removal of COD, MLVSS/MLSS and SVI in SBR has not been reported in literature.

## ■2.0 MATERIALS AND METHODS

### 2.1 Soy Sauce Wastewater and Characterization

The soy sauce wastewater samples were collected from a local soy sauce processing company in Johor Bahru, Malaysia. The soy sauce wastewater treatment plant (WTP) receives about 50 tons of soy sauce processing wastewater daily. The WTP comprises preliminary treatment (oil trap slump and pH stabilizer), secondary treatment (two aeration tanks and a clarifier) to reduce COD, N, P and tertiary treatment (chemical reaction tank). The samples were taken and instantaneously transported to the laboratory, and were kept at a temperature of 2 – 4 °C to minimize microbial activities in the wastewater. The characteristic of soy sauce wastewater sample is given in Table 1.

### 2.2 Activated Sludge

The activated sludge used in the reactor was obtained from Taman Harmoni sewage treatment plant located in Johor Bahru, Malaysia. The MLSS concentration was 8100 mg/L and 99.27 mL/g of SVI at pH of 6.95.

### 2.3 Reactor set-up and Operation

3 L borosilicate glass column of SBR was used, with an internal diameter of 6.5 cm and a height of 100 cm. The SBR was operated to cultivate AGS in the anaerobic-aerobic sequencing batch mode at 8 h of successive cycles: 5 min feeding of 1.5 L soy sauce wastewater from the bottom of the reactor, 460 min reaction phase which consist of 120 min of anaerobic stage and 340 min of aerobic stage, 10 min settling, 5 min effluent discharge with 50% of volumetric exchange ratios and 5 min idle. In order to provide anaerobically, the liquid in the reactor was circulated over the reactor. The circulation process promoted an adequate mixing of the reactor content during the anaerobic stage. Aeration was supplied through an air bubble diffuser by an air pump at the reactor bottom, and the airflow rate was controlled by a gas-flow controller at a volumetric flow rate of 0.18 m/h (1.50 cm/s superficial air flow velocity). The experiments were conducted in a room temperature (25–30°C). The pH and temperature of the SBR were monitored throughout the experiment. The activated sludge collected from the aeration tank of the municipal sewage treatment plant was used as the seed sludge.

### 2.4 Circulation Phase Studies in Single Column

Process of circulation was introduced during anaerobic stages. The soy sauce wastewater in the upper reactor was channeled into the bottom of the reactor using the peristaltic pump (Cole-Parmer System Model, 6-600 rpm) for 120 min in every cycle at the desired flow rate of 36.0 L/h (R1), 25.2 L/h (R2) and 14.4 L/h (R3). This experiment was started after the formation of aerobic granules. All the experiments were conducted at room temperature. Samples were collected from the exit of the column.

### 2.5 Modeling using RSM

Design of Expert version 6.0.11 software (STAT-EASE Inc., Minneapolis, USA) was used for modeling and optimization purpose. The circulation rate was modeled and optimized by using the Response Surface Methodology (RSM). The RSM used in this work was coupled with central composite design (CCD). Two variables (circulation rate and operation time) were investigated using the RSM and the response was collected in terms of COD removal, MLVSS/MLSS and SVI. The 3D representation of the results and contours helped to identify the optimum region and eventually provides the optimum condition for the circulation rate during anaerobic stage. The experimental range and levels of independent variables are tabulated in Table 2. Meanwhile, ANOVA analysis was performed at 95% significance level.

**Table 1** Characteristics of soy sauce wastewater

| Parameter | Unit | Soy sauce wastewater |
|---|---|---|
| pH | pH/units | 6 |
| COD | mg/L | 5400 |
| $BOD_5$ | mg/L | 2620 |
| Colour | ADMI | > 600 |
| Suspended solid | mg/L | 480 |
| Turbidity | - | 304 |
| Total dissolved solid | mg/L | 4050 |
| Ammonia-N | mg/L $NH_3$-N Ness | 21 |
| Total nitrogen | mg/L N-TNT | 70 |
| Total phosphorus | mg/L $PO_4^{3-}$-TNT | 55 |

**Table 2** Experimental range and levels of variables

| Variables | Range and levels | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| (A) Circulation rate | 14.4 L/h | 25.2 L/h | 36.0 L/h |
| (B) Operation time | 1 d | 31 d | 60 d |

## 2.6 Analytical Methods

Analyses were conducted according to Standard Methods for the Examination of Water and Wastewater [10]: COD, MLSS, mixed liquor volatile suspended solids (MLVSS), total nitrogen (TN), nitrite, nitrate and ammonia. The SVI was determined according to de Kruek [11] and the settling velocity of an aerobic granule was determined as time taken for an individual granule to settle at a certain height in a glass column filled with tap water. Then, the obtained bed volume was divided with the dry weight of the biomass in the reactor. The morphological and structural images of the granules were observed periodically using a stereomicroscope equipped with digital image processing and analyzer (PAX-ITv6, ARC PAX-CAM). A scanning electronic microscope (FESEM-Zeiss Supra 35 VPFESEM) was used to examine the external morphology and microstructure composition within the granule.

# ■3.0 RESULTS

## 3.1 Granules Development in Soy Sauce Wastewater

Figure 1 displays the morphological transformation of the seed sludge from the 14th day to 60 day of inoculation. On 5th day of inoculation, there was excessive washout of the inoculated biomass from the reactor preferentially selects for the growth of good settling bacteria and resulting in the accumulation of aerobic granules in the reactor. The biomass present in the reactor during this period was fluffy and irregular shape but granules were not observed yet. After 14th day of inoculation, the seed sludge started to aggregate into small granules (0.1–0.5 mm). The morphology of these initial granules was in irregular-shaped with non-clear boundary as shown in Figure 1a. By 60th day, the non-clear boundary and irregular-shaped granular sludge slowly disappeared and was replaced by smooth, regular, non-fluffy and round shape granules with diameters from 2.0 and 2.5 mm (Figure 1b). Afterward, the quantities of the dense granules with smooth spherical surface and clearly defined boundary were increased towards the end of the experiment. Comparing the size of the granules with the earlier research on real wastewater [12, 13], the diameter of aerobic granules developed in this work using soy sauce wastewater is much larger for the similar time frame. This could be due to the different substrate characteristics supplied to the reactor, which has a profound impact on the microbial structure and the diversity of aerobic granules.

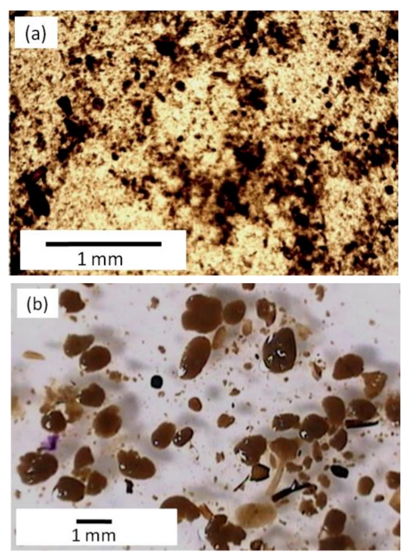



**Figure 1** Morphology of AGS in the reactor (a) Fluffy and filamentous aerobic granular sludge at 14th day of inoculation (b) Matured and dense aerobic granular sludge at day 60 of inoculation

The growths of the dense granules from seed sludge are associated with the ability of microbial interacts with each other and existence of extracellular polysaccharide substances (EPS) in the granule that acts as a real structural gel between microbial cells to strengthen the granules' structural integrity[14]. The structure of the 60th day of aerobic granule was examined by SEM. The SEM examination clearly shows the surface of round shaped granule is colonized with coccoid bacteria, which have a spherical or nearly spherical shape (Figure 2). The coccoid bacteria are tightly attached together by EPS. EPS was secreted by each microbial is important in maintaining membrane structure as it acts as shielding barrier.

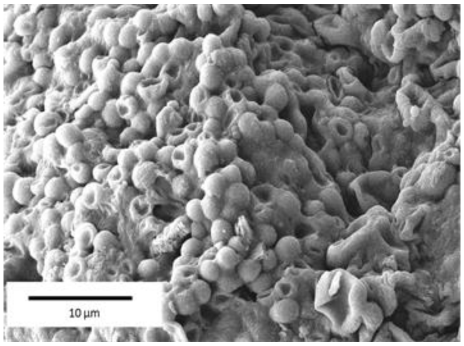



**Figure 2** SEM observation of surface microstructure of matured aerobic granules and coccoid bacteria densely packed at the outer layer of granule

The profile of SVI and biomass concentration (MLSS, MLVSS) throughout the experiment is represented in Figure 3. The SBR started up with a poor settling ability (99 mL/gSS of SVI) and the reactor experienced almost complete wash-out at 5th day of inoculation due to the poor settling ability of the seed sludge and short settling time (*i.e.* 5 min) applied. Resulting in, MLSS concentration sharply decreased to as low as 2.2 g/L from 7.4 g/L. The MLSS and MLVSS concentration was kept fluctuated until 21st day of inoculation. This is probably due to the microorganisms in the reactor were started to be acclimatized with the soy sauce wastewater. After about 21st day, the SVI fluctuated in range of 54 – 139 mL/gSS presumably severe biomass was wash-out together with effluent due to poor settling velocity of the seed sludge during the initial stage of granules inoculation. The remaining biomass in the reactor grows into micro colonies that transformed into dense and compact granules [12].

The SVI value was improved from 78 mL/gSS at the start-up to 28 mL/gSS at the end of the experiment. While the average of granular sludge settling velocity in the reactor improved from 15 m/hr at day 25 to 45 m/hr at the end of experiment. The average settling velocity of compact granules achieved in the present study was greater than reported by Tay [15], which was in the range of 30 – 35 m/h by feeding with synthetic wastewater. The result showed that the settling ability of biomass improved together with granulation process. While, a stable concentration of 9.9 g MLSS/L and 7.6 g MLVSS/L was reached at the end of experiment, which were slightly higher than those granules cultivated in raw soy protein wastewater reported by Wei [16] *i.e.* 7.02 g/L. As a result of settling ability improvement, MLSS kept increasing despite the excess sludge discharged.

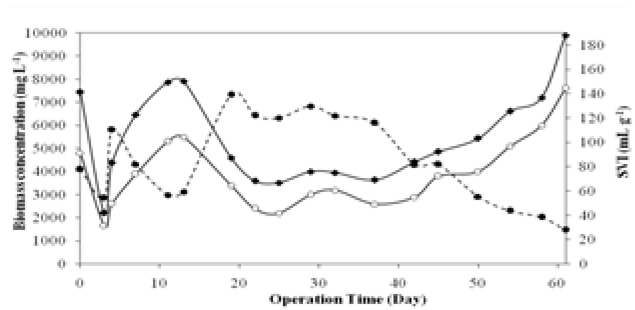



**Figure 3** The concentration of biomass and SVI during granulation process (♦) MLSS concentration; (○) MLVSS concentration; (●) SVI.

## 3.2 Treatment Efficiency

The biological treatment process using anaerobic–aerobic system is recommended as a feasible technology for simultaneous removal of organic and nitrogen. To further study the operation status of the SBR, an analysis was performed to assess nitrogen and oxygen profile in the anaerobic-aerobic cycle on 50 day of inoculation. Figure 4 shows the nitrogen and oxygen profiles in anaerobic-aerobic cycle at 50 day of aerobic granules inoculation in the reactor. During the anaerobic phase, the oxygen concentration reaches below than 0.3 mg$O_2$/L and ammonia concentration was rapidly decreased to 37 % from the initial concentration (15 mg/L) after 2 h in anaerobic phase, presumably due to the utilization of ammonia anaerobically by microorganism for nitrogen source and microbial growth. The TN concentration gradually depleted from 68 to 26 mg/L at the end of anaerobic phase. Both nitrite and nitrate concentration remained negligible as the concentrations were below than 0.5 mg/L since they were consumed via denitrification.

Basically, the denitrifying bacteria are facultative, which can consume both oxygen and nitrogen for cell growth and generate energy. During the aerobic phase, the oxygen profile was gradually increased to 3.7 mg$O_2$/L and reach plateau at about 6.5 mg$O_2$/L at the end of aerobic phase. The ammonia concentration was decreasing from 9.5 to 3.8 mg/L during aerobic phase due to the oxidation process by nitrifying bacteria, *Nitrosomanas* and *Nitrobacter* into nitrate and nitrate respectively. Since the main product of nitrification was nitrite and nitrate, their concentration were observed to be higher in aerobic phase 90% and 88% respectively, than in anaerobic phase. The TN concentration was decreasing until the end of aerobic phase indicating that the occurrence of simultaneous nitrification and denitrification in the SBR system.

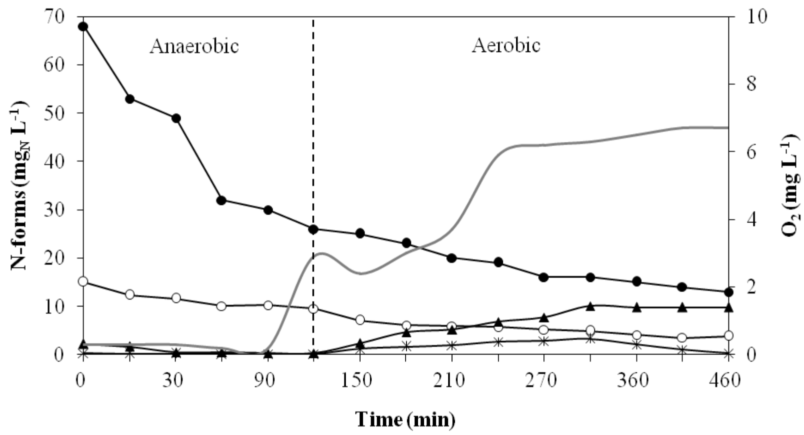



**Figure 4** Nitrogen and oxygen concentration profiles of SBR cycle after the development of mature granules in the reactor (▬) oxygen concentration (●) TN (▲) nitrate (ж) nitrite (○) ammonia

**Table 3** Experimental variables and results for the SBR

| Run | Factors | | Responses | | |
|---|---|---|---|---|---|
| | A: Circulation rate (L/h) | B: Operation time (day) | COD rem. (%) | MLVSS/MLSS (%) | SVI (mL/g) |
| 1 | 25.20 | 1 | 40.0 | 53.2 | 99.3 |
| 2 | 36.00 | 1 | 40.1 | 55.4 | 110 |
| 3 | 14.40 | 1 | 45.8 | 51.6 | 118 |
| 4 | 25.20 | 31 | 58.2 | 67.4 | 74.8 |
| 5 | 25.20 | 31 | 58.9 | 69.0 | 73.0 |
| 6 | 25.20 | 31 | 60.1 | 70.1 | 73.9 |
| 7 | 36.00 | 31 | 64.6 | 73.4 | 71.6 |
| 8 | 25.20 | 31 | 63.7 | 71.6 | 72.8 |
| 9 | 25.20 | 31 | 66.2 | 72.5 | 71.0 |
| 10 | 14.40 | 31 | 57.6 | 68.9 | 74.8 |
| 11 | 25.20 | 60 | 90.0 | 87.3 | 55.3 |
| 12 | 36.00 | 60 | 89.1 | 89.0 | 60.5 |
| 13 | 14.40 | 60 | 82.6 | 84.9 | 67.7 |

**Table 4** ANOVA results for response parameters

| Response | Prob. | $R^{2a}$ | Adj. $R^{2\,a}$ | Adeq. P | SD[b] | CV[c] | Press | Prob. LOF[d] |
|---|---|---|---|---|---|---|---|---|
| COD removal | <0.0001 | 0.9535 | 0.9443 | 25.707 | 3.88 | 6.17 | 294.84 | 0.3652 |
| MLVSS/MLSS | < 0.0001 | 0.9895 | 0.9873 | 58.018 | 1.36 | 1.93 | 22.71 | 0.9960 |
| SVI | < 0.0001 | 0.9714 | 0.9509 | 20.263 | 4.14 | 5.26 | 833.56 | 0.0083 |

[a] $R^2$ and adj. $R^2$= coefficient of determination
[b] SD = standard deviation
[c] CV = coefficient of variation
[d] Prob. LOF = probability lack of fit

## 3.3 Circulation system in anaerobic phase

The circulation process of mixed liquor between substrate and wastewater is the important process of operation in the anaerobic–aerobic SBR system in order to provide anaerobic condition and optimum hydraulic mixing could improve the substrate transfer at the granule surface and densified aggregates for efficient performance of treatment. The circulation rate can be varied by changing the rotation speed of the peristaltic pump. CCD method was used to generate a correlation between two different process variables (circulation rate (A) and operation time (B)) and three responses (% of COD removal, MLVSS/MLSS ratio and SVI). 13 runs of experiments were conducted as suggested by the software. Table 3 shows the experimental runs conducted and the response obtained. The response shows that the percentage of COD removal was in the range of 40–90%, while the percentage of MLVSS/MLSS ratio was in between 51.6 and 89.0%, while SVI value was 55.3 – 118 mL/g.

Table 4 demonstrates the analysis of variance (ANOVA) done for the percentage of COD removal, MLVSS/MLSS ratio and SVI value. Model terms were evaluated by the P-value (probability) with 95% confidence level. Moreover, the "Prob> F" for lack of fit shows the value more than 0.05 which indicates the experiment is highly reproducible. In order to choose the appropriate model, the model with highest polynomial in which the additional terms were significant and not aliased was chosen[19].

**COD removal:** Figure 5 obtained from the Design Expert 6.0.11 software shows the interaction between circulation rate and operation time to remove COD. The coded model for COD removal in linear model fitting is exhibited in Equation (1).

$$\% \text{ COD removal} = 62.84 + 1.30A + 22.63B \quad (1)$$

Where A is circulation rate in L/h and B is operation time in day. From Figure 5, it can be observed that the COD removal percentage reached its maximum at 25.2 L/h circulation rate on 60 days of operation time. The minimum COD removal happens at 25.2 L/h of circulation rate during first day of operation time. This could be due to the moderate circulation rate (25.2 L/h) has less mutual collisions among sludge granules, resulting in insufficient driving force for the COD to overcome the mass transfer resistance between the liquid and granules [13]. Besides, first day of operation time is basically for the adapting process to the new environment of the granular with soy sauce wastewater. Hence, the insufficient operation time reduce the COD removal efficiency. During the granular sludge treatment, the substrate in wastewater is diffused into granule's surface as external mass transfer, follow by intra-granule mass transfer and biochemical reaction within the granule [17]. Therefore, the low external mass transfer is due to lower circulation rate would limit the performance of the treatment, including microbial growth and biodegradation. In the present study, the mixed liquor circulation enhances the mass transfer between the substrate and granules during the anaerobic phase, thus accelerating the granulation process [17].

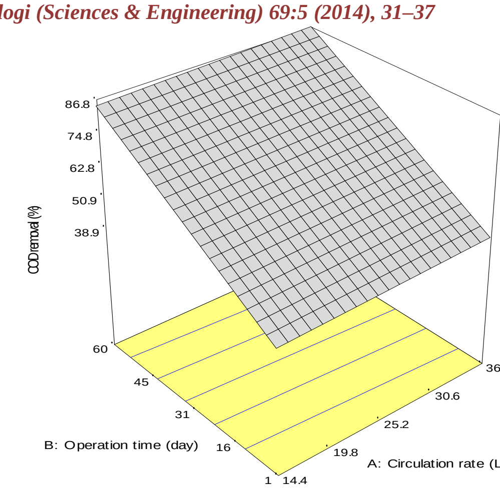



**Figure 5** Effect of circulation rate and operation time on COD removal

**MLVSS/MLSS ratio:** The MLVSS/MLSS ratio represents the concentration of active microorganisms in the reactor and it may also relate to the amount of the organic loading rate supplied to the reactor [18]. Regarding to Muda *et al.* [8], decreased in MLVSS/MLSS ratio may indicate an increase of inert particles within the granules due to the accumulation of more inert solids within the granules. The coded model for MLVSS/MLSS percentage in the linear model fitting is given in Equation (2).

**Table 5** Optimization criteria for COD removal, MLVSS/MLSS and SVI

| Criteria | Goal | Lower limit | Upper limit |
|---|---|---|---|
| Circulation rate (L/h) | In the range | 14.4 | 36.0 |
| Operation time (day) | In the range | 1 | 60 |
| COD removal (%) | Is maximize | 40 | 90 |
| MLVSS/MLSS (%) | Is maximize | 51.6 | 89.0 |
| SVI (mL/g) | Is minimize | 55.3 | 118.0 |

**Table 6** Optimized value of the process

| Solution | Circulation rate (L/h) | Operation time (day) | COD removal (%) | MLVSS/MLSS (%) | SVI (mL/g) | Desirability |
|---|---|---|---|---|---|---|
| 1 | 33.61 | 60 | 86.5 | 88.7736 | 58.6 | 0.952 |
| 2 | 33.74 | 60 | 86.5 | 88.7989 | 58.7 | 0.952 |
| 3 | 26.14 | 60 | 85.6 | 87.3435 | 57.1 | 0.939 |

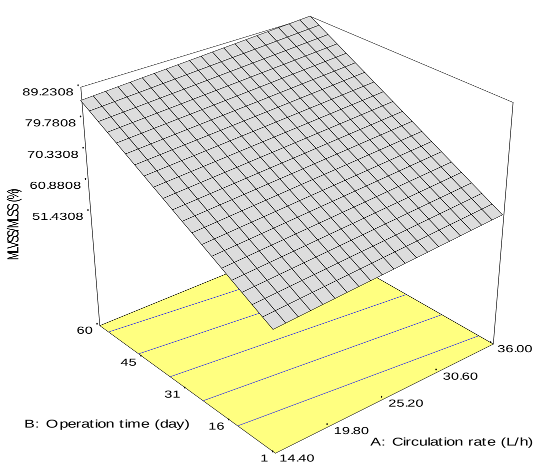



**Figure 6** Effect of circulation rate and operation time on MLVSS/MLSS

$$\% \text{ MLVSS/MLSS} = 70.33 + 2.07A + 16.83B \quad (2)$$

Where A is circulation rate in L/h and B is operation time in day. The Figure 6 shows the interaction between operation time and circulation rate on percentage of MLVSS/MLSS ratio as plotted by Design of Expert 6.0.11 software. The ANOVA values for MLVSS/MLSS are shown in Table 4. The observation reveals that the percentage of MLVSS/MLSS increased when the operation time was increased at circulation rate increased. The maximum MLVSS/MLSS (87.3%) was observed for a circulation rate 25.2 L/h and operation time of 60 d. Under constant operation time, a slight increase in MLVSS/MLSS was observed for higher circulation rate. This is confirmed by Equation (2), which shows that operation time has a greater affect than circulation rate on the response.

**SVI:** SVI is an indication of the sludge settles ability in the reactor which is a useful test that indicates changes in the sludge settling characteristics and quality. SVI was determined as a response in this study. The minimum SVI of 55.3 mL/g was obtained in the reactor at operational conditions of 25.2 L/h and day 60. The maximum SVI value was 118.0 mL/g at circulation rate of 14.4 L/min and operation time of 1 day. The quadratic model fitting for SVI in coded form is given in Equation (3).

$$SVI = 71.49 - (3.07A) - (23.97B) + (5.72A^2) + (9.82B^2) + (0.20AB) \quad (3)$$

Where A is circulation rate in L/h and B is operation time in day. Figure 7 shows the SVI contour plots. SVI is gradually decreased due to the formation of matured and dense granules which had excellent settling ability when the operation time increased. Therefore, operation time decreased SVI in higher circulation rate.

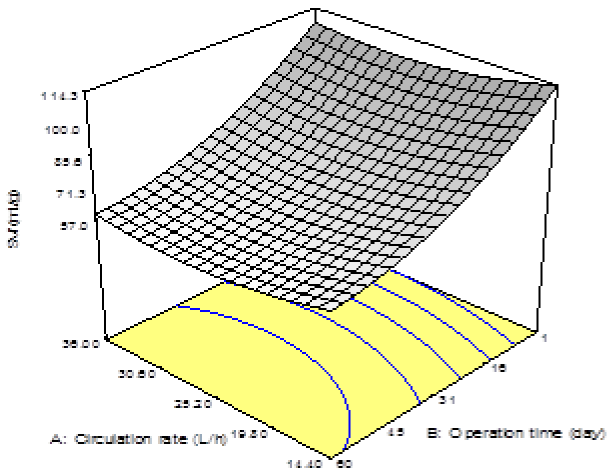



**Figure 7** Effect of circulation rate and operation time on SVI

## 3.4 Optimization

The models developed in Section 3.3 were further optimized using the RSM method. The models were optimized for the process variables (circulation rate and operation time) and the responses (% COD removal, % MLVSS/MLSS ratio and SVI). The optimization was done using the Design Expert 6.0.11 software based on the goals that are desired in this experiment. The list of criteria and its goal are listed in Table 5. The main objective of this experiment was to optimize the COD removal, MLVSS/MLSS and SVI of the SBR. Hence, the "% of COD removal", "MLVSS/MLSS" was set to maximum ("higher is better") and "SVI" was set to minimum ("smaller is better") within the range of process variables set in this experiment. The Design Expert 6.0.11 software searches for the ideal combination for the goals set for each of the criteria [19]. Solutions are sorted by desirability, based on how well the specified goals are met. The closer all goals are met, the higher the desirability number will be. Generally, there could be a number of solutions for the optimization process that has been done.

In this work, three solutions were given by the software for the given goals. Nevertheless, the desirability value was in range 0.939 to 0.952 which indicates near ideal achievement of the goals. Table 6 displays the value of optimized process of this work. Desirability is an objective function to determine a best combination of responses, which ranges from zero to one for least to most desirable respectively. The numerical optimization finds a point that maximizes the desirability function.

# ■4.0 CONCLUSION

In this study, for the first time, aerobic granules were successfully developed using soy sauce wastewater, without any dilution in 8 h cycle time of SBR system. The aerobic granules formation in the soy sauce wastewater took 60 days to achieve stable and clear round outer shape aerobic granular sludge with largest diameter of 2.5 mm. The developed AGS shows good results that indicated the feasibility of the anaerobic-aerobic phase in the reactor to perform simultaneous nitrification-denitrifiction for removing nitrogen from the wastewater. In the circulation studies, the AGS was successfully used to remove the COD of the SBR effluent. The interaction between the process variables (circulation rate and operation time) was studied. The responses (% COD removal, % MLVSS/MLSS ratio and SVI) showed that the highest COD removal achieved was 90% (at operation time of 60 days and circulation rate of 25.2 L/h). Meanwhile, the highest percentage of MLVSS/MLSS was 87.3%. It was found that the optimum values of the COD removal, MLVSS/MLSS and SVI were attained at operation time of 60 days and circulation rate of 30.61, 30.92 and 31.04 L/h.

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