Efficiency of Aerobic Granulation Technology in Treating High Strength Soy Sauce Wastewater
(Kecekapan Teknologi Enapcemar Granul Aerobik dalam Merawat Air Sisa Kicap)

HASNIDA HARUN, AZNAH NOR-ANUAR, ZAINI UIANG, INAWATI OTHMAN & NOR HASTYIMAH ROSMAN

ABSTRACT
The present study investigated the efficiency of aerobic granular sludge (AGS) technology in treating effluent from soy sauce industry which is categorized as a high strength wastewater. The combination of anaerobic and aerobic granulation technology in SBR system was used in this study which was efficiently treated COD from the soy sauce wastewater where 87% of removal was achieved. Ammonia and colour was removed at a maximum of 87 and 76%, respectively, in the SBR system. Matured, dense and compact granules with 2.5 mm in diameters were developed with a good settling velocity (45 m/h) and 28 mL/gSS of sludge volume index (SVI). Hence, AGS technology was proven as an excellent treatment for soy sauce wastewater for being discharge into the environment, as the effluent was treated in one biological reactor with high hydraulic and organic loadings besides less production of sludge. In this study, the capabilities of AGS technology in treating relatively higher concentration of organic impurities present in the soy sauce wastewater were demonstrated.

Keywords: Aerobic granular sludge; aerobic processes; nitrification; soy sauce wastewater; SBR

INTRODUCTION
Soy sauce is one of the oriental products that was used as a condiment or colouring agent in foods preparation and widely consumed in Asian countries such as China, Japan, Thailand, Korea, Indonesia and Malaysia. Basically, there are two types of processes in the production of soy sauce: fermentation and chemical process. In fermentation process, soy sauce experienced a slow hydrolysis process which is up to six months under moderate temperature i.e. 30°C while for chemical process; soy sauce experienced a quick hydrolysis process by hydrochloric acid at 80°C for 8-10 h (Fukushima 1981; Luh 1995). In year 2010, at least 80 thousand tonnes of the condiments was produced in Malaysia and the number is expected to increase by 5% in subsequent year. However, the production of 1 tonne of soy sauce generated a large amount of high load wastewater which about 7-9 tonnes and the volume is expected to increase with the soy sauce production. The effluent typically contain high chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solid (TSS) and colour, as well as protein due to the fermentation process which contributed strong odors in the treatment works. Thus, the effluent needs an efficient treatment before being discharge to the environment in order to meet the stringent discharge standards.

Biological treatment using aerobic granular sludge in SBR has been practice for well since 1990. SBR technology is known as an efficient biological treatment in treating wastewater and capable to remove nutrient and organic compounds in one single reactor (Cirik et al. 2013). In recent years, the successful cultivation of aerobic granular sludge (AGS) in SBR for treating municipal wastewater has been widely reported in the literature. Dense and compact AGS structure has a good settling ability which allows high biomass retention in the reactor for degradation process of organic matter and it was one of the main factors for the
granulation field received attention by many researchers in the wastewater treatment. Combination of aerobic and anaerobic granulation technology in SBR has promised an optimum configuration especially in industrial wastewater treatment such as dairy (Schwarzenbeck et al. 2005), textile (Muda et al. 2010), livestock (Kishida et al. 2009), abattoir (Pijuan et al. 2009) and synthetic wastewater (Filali et al. 2012; Wu et al. 2012). However, aerobic granulation technology in the treatment of high strength and real wastewaters is still relatively under-research. Due to the advantages of the combination anaerobic and aerobic granulation technology in treating wastewater, it is a wise option for treating high strength soy sauce wastewater biologically. To date, there is no reported study on the use of anaerobic and aerobic granulation technology in treating wastewater from soy sauce industry.

Therefore, this study was conducted to investigate the possibility of developing aerobic granular sludge under anaerobic and aerobic technology based on SBR system in one bioreactor besides to determine the treatability performance in treating wastewater from the soy sauce industry. Moreover, this study observed as the reactor performance in nitrification-denitrification process with the presence of dense and mature AGS and it is expected to contribute further understanding of the aerobic granulation mechanism as well as its application in the treatment of high strength industrial wastewaters.

MATERIALS AND METHODS

EXPERIMENTAL DETAILS

A SBR column-type (100 cm high; 6.5 cm diameter) with a working volume of 3000 mL was used in the experiments. Figure 1 shows the schematic diagram of the reactor setup. For the start-up of reactor, activated sludge taken from municipal sewage-treatment plant was added, resulting in 5900 mg/L of initial mixed liquor suspended solid (MLSS) in the reactor. The reactor was attached to a timer box that connected to influent, circulation, aeration and effluent pump to control and to keep continuity of the system. The reactor was operated sequentially in 8 h for one cycle: 5 min feeding period of 1500 mL fermented soy sauce wastewater from the bottom of the reactor followed by 460 min of reaction phase which consist of 120 min of anaerobic and 340 min of aerobic conditions, 5 min of settling, 5 min of effluent withdrawal with 50% of volumetric exchange ratios (VER) and 5 min idle. In order to provide anaerobic condition, the liquid in the reactor was circulated over the reactor by a peristaltic pump (Cole-Parmer System Model, 6-600 rpm). 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 m³/h (1.50 cm/s superficial air flow velocity). The reactor was operated at room temperature (25-30°C) with pH and DO continuously monitored.

FIGURE 1. Schematic diagram of aerobic granulation system

RESULTS AND DISCUSSION

SOY SAUCE WASTEWATER CHARACTERISTICS AND SEED SLUDGE

The raw soy sauce fermentation wastewater was collected from a wastewater treatment plant (WTP) of a local soy sauce processing company in Johor Bahru, Malaysia and was kept at a temperature of 2-4°C to prevent any microbial activities in the wastewater. The existing 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 characteristic analysis of the soy sauce fermentation wastewater is given in Table 1. The seed sludge used in the reactor was taken from an aerated tank in domestic wastewater treatment plant. The MLSS concentration is 8100 mg/L and 99.27 mL/g of SVI.

ANALYTICAL METHODS

The parameters of wastewater samples were analyzed for COD, MLSS, mixed liquor volatile suspended solid (MLVSS), NH₄⁺, NO₂⁻, NO₃⁻, total nitrogen (TN), colour and SVI in accordance with the standard APHA methods (APHA 2005). The pH and DO in the reactor was monitored using pH/DO probe meter (Orion 3-Star Benchtop pH/DO meter). The aerobic granular sludge development in the reactor was analyzed for the structural and morphological of granule, settling velocity and SVI continuously. The morphological and structural images of the granules were observed periodically using a stereo microscope equipped with digital image processing and analyzer (PAX-IT v6, ARC PAX-CAM). A scanning electronic microscope (FESEM-Zeiss Supra 35 VPFESEM) was used to examine the external morphology and microstructure composition of the granule. 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.

STRUCTURAL AND MORPHOLOGICAL OF GRANULES

Excessive washout of biomass from the reactor on day 5 operation resulted in only well-settled sludge remained and the retained biomass in the reactor during this period
was fluffy and irregular shape. After an initial wash out of the biomass, the fluffy sludge was slowly transformed into small granules (0.1–0.5 mm) as shown in Figure 2(a). The small granules developed at early stage of the operation are due to the short settling time i.e. 5 min that preferentially selects for the growth of good settling bacteria and resulted in the accumulation of aerobic granules in the reactor. After four weeks of operation, the non-clear boundary and irregular-shaped granular sludge slowly disappeared and was replaced by a smooth structured and regular shaped of dense granules with diameters from 1.0 to 1.5 mm, but the developed granules were easily crushed when a high shear force was directly applied on them. According to Seviour et al. (2012), the growths of the granules are associated with the ability of microbial interacts with each other and the existence of extracellular polysaccharide substances (EPS) in the granule that acts as a real structural gel between the microbial cells to strengthen the granules’ structural integrity.

During the seven weeks of operation, dense and round-shaped AGS with a diameter between 2.0 and 2.5 mm was observed while the reactor performance reached a steady-state condition. A larger and dense granule (2.0 – 5.0 mm) was reported by Abdullah et al. (2011) after nine weeks operation of palm oil mill effluent (POME) treatment in SBR system. The selections of granules were obtained when the dense granules showed an excellent settling ability. Afterward, the quantities of the dense granules with smooth spherical surface and clearly defined boundary were increased towards the end of the operation as shown in Figure 2(b). The seed sludge, aeration rate, settling time, SBR cycle operation and substrate composition have been found to affect the granular development.

The SEM examination clearly shows the surface of round shaped granule is colonized with coccoid bacteria, which have a spherical or nearly spherical shape. The coccoid bacteria are tightly attached together by EPS. EPS which was secreted by each microbiol is important in maintaining membrane structure as it acts as shielding barrier. Moreover, SEM was clearly observed these bacteria formed cluster arrangement surrounding the opening of cavity as shown in Figure 2c. The cavities are the transmission channels for nutrients and metabolites matter for microbial needs in the inner part of the granule (Hu et al. 2012).

### SLUDGE SETTLING AND BIOMASS PROFILE

The profile of SVI and biomass concentration (MLSS, MLVSS) throughout the operation is shown in Figure 3. In the present study, the reactor was started up with a poor settling ability (99 mL/gSS of SVI) of seed sludge and filamentous suspended growth was predominant in the reactor. Over the three weeks operation, the SVI fluctuated in range of 54 - 139 mL/gSS due to severe biomass wash-out with effluent since the seed sludge had a poor settling velocity. The remaining biomass in the reactor grows into micro colonies that transformed into dense and compact granules (Abdullah et al. 2011). Six weeks of operation, the SVI decreased to 82 mL/gSS and compact granules were observed in the reactor. Throughout the operation, the SVI value was improved from 78 mL/gSS at the start-up to 28 mL/gSS at the end of the operation. Whereas the average of granular sludge settling velocity in the reactor improved from 15 m/h at day 25 to 45 m/h at the end of operation. The average settling velocity of compact granules achieved in the present study was greater than reported by Tay et al. (2001), which was in the range of 30–35 m/hr by feeding with synthetic wastewater. The result showed that the settling ability of AGS was improved together with the granulation process.

Characteristic of the developed granules reflect the efficiency of settling properties and biomass concentration (MLSS, MLVSS) in the reactor. The MLSS and MLVSS concentration in the reactor gradually keep increasing after the start-up. During the first week operation, the reactor experienced almost complete wash-out due to the poor settling ability of the seed sludge and short settling time (i.e. 5 min) applied. After that, MLSS concentration in the reactor was sharply decreased as low as 2.2 from 7.4 g/L and the biomass concentration was fluctuated until week three of operation. This is probably due to the microorganisms in the reactor were started to acclimatize with the soy sauce fermentation wastewater. After 8 weeks

**TABLE 1. Characteristics of fermented soy sauce wastewater**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Fermented soy sauce wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physico-chemical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>pH/units</td>
<td>6</td>
</tr>
<tr>
<td>Chemical oxygen demand</td>
<td>mg/L</td>
<td>5400</td>
</tr>
<tr>
<td>Biological oxygen demand</td>
<td>mg/L</td>
<td>2620</td>
</tr>
<tr>
<td>Colour</td>
<td>ADMI</td>
<td>&gt; 600</td>
</tr>
<tr>
<td>Suspended solid (SS)</td>
<td>mg/L</td>
<td>480</td>
</tr>
<tr>
<td>Turbidity</td>
<td></td>
<td>304</td>
</tr>
<tr>
<td>Total dissolved solid</td>
<td>mg/L</td>
<td>4050</td>
</tr>
<tr>
<td>Inorganic composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammoniacal nitrogen (AN)</td>
<td>mg/L</td>
<td>21</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>mg/L</td>
<td>70</td>
</tr>
<tr>
<td>Total phosphorus (TP)</td>
<td>mg/L</td>
<td>55</td>
</tr>
</tbody>
</table>
operation, a stable concentration of 9.9 g MLSS/L and 7.6 g MLVSS/L was reached and slightly higher than those granules cultivated in raw soy protein wastewater reported by Wei et al. (2013) i.e. 7.02 g/L. As a result of settling ability improvement, the MLSS kept increasing despite the excess sludge was discharged.

NITROGEN PERFORMANCE

The biological treatment process using anaerobic–aerobic system is recommended as a feasible technology for simultaneous removal of organic and nitrogen (Qin & Liu 2006; Wu et al. 2012). To further the study on operation status of the reactor, an analysis was performed to assess nitrogen and oxygen profile in the anaerobic-aerobic cycle on day 50 of operation. Figure 4 shows the nitrogen and oxygen profiles in anaerobic-aerobic cycle after the matured granules developed in the reactor. During the anaerobic phase, the oxygen concentration reaches below than 0.3 mg O₂/L and the ammonia concentration was rapidly decreased after 2 h in anaerobic phase until 37% from the initial concentration (15 mg/L). This is presumably due to the utilization of ammonia in anaerobic condition 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. 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 microorganism which can consume both oxygen and nitrogen for cell growth and generating energy (Gao et al. 2011).

During the aerobic phase, the oxygen profile was gradually increased to 3.7 mg/L and reach plateau at about 6.5 mg/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, *Nitrosomonas* and *Nitrobacter* into nitrate and nitrite, respectively (Fernandes et al. 2013). Since the main outcome products of nitrification were nitrite and nitrate, their concentration were observed to be higher in aerobic phase compared in anaerobic phase which is 90 and 88%, respectively. The TN concentration was decreasing until the end of aerobic phase indicated that simultaneous nitrification and denitrification process in the SBR system was occurred.

REMOVAL EFFICIENCIES OF GRANULES

Figure 5 shows the COD, ammonia and colour concentration in the influent, effluent and percentage removal during the operation. Throughout the third week of inoculation, the COD effluent concentration fluctuated around 620 to 920 mg/L and then increased up to 78% of COD removal at day 22. The fluctuation of COD removal was aggravated by foaming and excessive sludge wash-out during the early stage of operation. As the granular sludge was slowly developed, the COD removal percentage increased steadily and maintained up to 87% at the end of experiment. The high percentage of COD removal achieved significantly due to the existence of a potential microorganism in the granular sludge, which effectively degrades the high-loaded fermented soy sauce wastewater.

At the initial stage operation, the percentage removal for ammonia and colour was 37 and 10%, respectively. As the granular sludge was developed during the third week, a satisfactory result of ammonia removal was obtained where the ammonia concentration was decreased by 10.0 mg/L and reached a plateau stage at about 3.0 mg/L at the end of operation. The colour of effluent also improved from dark brown to clear brown. The percentage of colour removal significantly increased due to the microbial degradation activity of melanoids, a main coloured compounds in the wastewater that form due to the chemical reaction between amino compound and carbohydrates known during the soy sauce fermentation (Kim & Lee 2007). The removal efficiency increased at maximum of 76% for

---

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

**FIGURE 5.** Profile of removal performances in SBR system for (a) COD, (b) ammonia and (c) colour of fermented soy sauce wastewater (●) Influent (●) effluent (○) percentage removal
ammonia and 58% of colour at the end of the operation. The results indicated that the ammonia and colour removal mainly relied on microbial growth itself during the granules development and the sufficient removal efficiency confirmed that there was an existence of active microbial activities in the reactor (Muda et al. 2010).

CONCLUSION
Compact and matured aerobic granular sludge was successfully cultivated in a single SBR system fed with soy sauce fermentation wastewater at 7 weeks operation with a diameter of 2.5 mm on average and 28 m/h of SVI was achieved. Despite the great variability in composition, stable performances were observed for all the examined parameters. An 8 h cycle time operated in SBR system demonstrated that the removal efficiency achieved for COD, ammonia and colour was at maximum of 87, 76 and 58%, respectively, in treating the wastewater. Therefore, the possibility of anaerobic-aerobic phase in aerobic granulation application was verified in our study and it is an attractive nitrogen removal technology for the soy sauce fermentation wastewater.

ACKNOWLEDGEMENTS
The authors would like to thank the Ministry of Education (MOE) and Universiti Teknologi Malaysia (UTM) for providing necessary facilities to conduct this experiment and granting us the Research University Grant (RUG-Grant Q.J130000.2501.01H54) for this research.

REFERENCES

Hasnida Harun, Aznah Nor-Anuar*, Zaini Ujang, Inawati Othman & Nor Hasyimah Rosman Department of Environmental Engineering Faculty of Civil Engineering Universiti Teknologi Malaysia 81310 Skudai, Johor Malaysia
Aznah Nor-Anuar* & Zaini Ujang Institute of Environment and Water Resource Management WATER Research Alliance Universiti Teknologi Malaysia 81310 Skudai, Johor Malaysia

*Corresponding author; email: aznah@utm.my

Received: 5 December 2013
Accepted: 9 February 2014