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Cultivation of Aerobic Granular Sludge Using Chitosan as A Nucleating Agent for Wastewater Treatment

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Abstract: Two identical high and slender cylindrical laboratory-scale reactors with a 2 L working volume was set up in this study. Each reactor was inoculated with 1 L of activated sludge from a local food industry and operated continuously with a cycle time of 24 hours. In every cycle, 1L of synthetic wastewater was supplied with 1120 mg/L of COD concentration to each reactor. In order to investigate the effect of the addition of chitosan on the granulation process of aerobic granular sludge (AGS), 10g of chitosan was added to a reactor, while another reactor was operated as a control reactor.

After 16 days of operation, the reactor with the addition of chitosan showed higher removal efficiency of COD which is 40% compared to the control reactor, which is 42%. Meanwhile, MLSS and MLVSS in the reactor with chitosan increased up to 4780 mg/L of MLSS and 3560 mg/L MLVSS, higher than the control reactor with 3850 mg/L of MLSS and 2560 mg/L of MLVSS. Furthermore, AGS round in shape, compact and brown in color with an average size of 5.62mm was successfully cultivated in reactors with the addition of chitosan after 16 days of operation. Meanwhile, AGS in the control reactor was developed into an average size of 4.13mm.

In a conclusion, the AGS system with supplemented chitosan is proven to accelerate granule cultivation without compromising the effluent quality. Therefore, AGS can be an advantageous treatment system to be implemented in the wastewater industry as it has good performance in degrading organic and nutrient in wastewater besides it is environmentally friendly, low cost, and can improve the performance of the conventional wastewater treatment sector.

Keywords: Wastewater, Aerobic Granular Sludge, Chitosan, Food Industry Sludge

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1. Introduction

Several processes are used in treatment facilities to achieve the required water quality goals. When compared to standard wastewater treatment methods, biological-based systems have various benefits. However, advancements in this sector must be properly considered. Besides, the sequencing batch reactor (SBR) is one of the biological treatments to process activated sludge systems for wastewater treatment. SBR can handle equalization, primary clarification, biological treatment, and secondary clarification all in one reactor vessel. Time is perhaps the most crucial part of any wastewater treatment procedure. Any treatment plan requires time to complete, and some treatments take longer than others. AGS is one of the processes that can take several days to achieve appropriate levels of treatment results, depending on the ratio of sewage to sludge and how the process occurs.

However, traditional activated sludge systems that use anaerobic granulation require a long start-up period and are not suited for the removal of certain nutrients and contaminants from wastewater. Sometimes the solid material at the bottom is not compacted effectively, and the sludge contains a lot of water. To overcome this disadvantage, this study has focused on the development of aerobic granulation technology. However, the granulation process of aerobic sludge in the presence of a carrier has rarely been studied.

Chitosan, a non-toxic and biodegradable biopolymer, is gradually becoming recognized for its application in wastewater treatment. It binds to tiny, suspended particles, pollutants, bacteria, heavy metals, and other contaminants. In this study, it will identify the effect of chitosan as a nucleating agent of aerobic granular sludge. Then, analyses the performance of the developed aerobic granular with chitosan. The objectives of this study are to investigate the effect of chitosan as a nucleating agent of aerobic granular sludge in enhancing the granulation and to analyses the performance of the developed aerobic granular with chitosan in the SBR system.

2. Methodology

Laboratory experiments presented in this chapter are carried out to investigate the development of Aerobic Granular Sludge (AGS) with the presence of chitosan in treating wastewater. This experiment is conducted in the Water and Wastewater Laboratory, Faculty of Engineering Technology, University Tun Hussein Onn Malaysia, Campus Pagoh, Johor.

2.1 Flowchart

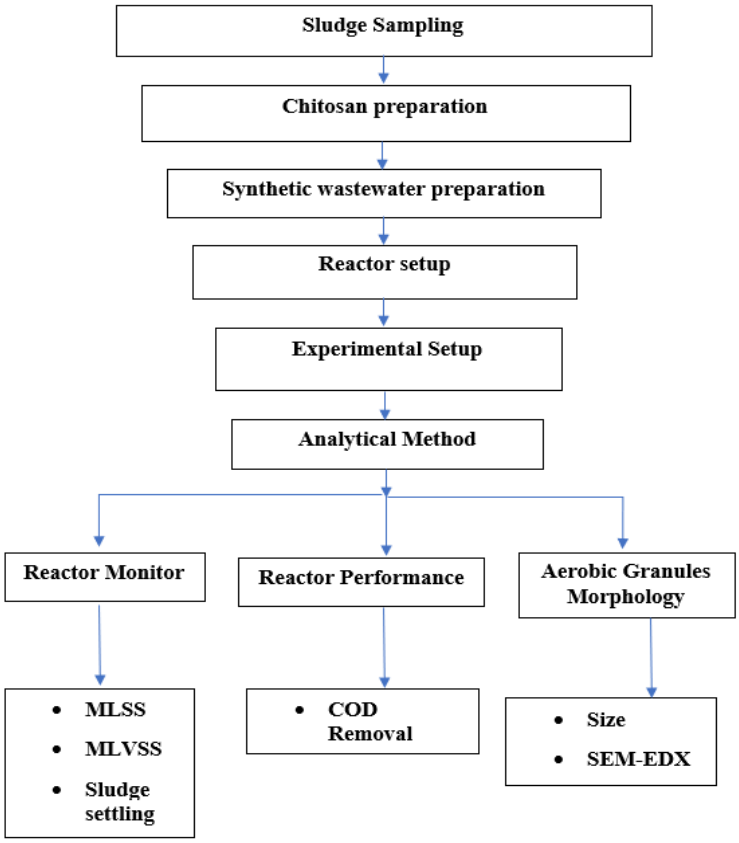

Figure 2.1: Flowchart of the methodology studies

2.2 Sludge sampling

The activated sludge is collected from New Star Food Sd. Bhd., a food processing industry located in Batu Pahat, Johor. The location is shown in figure 3.2. The activated sludge is collected from an aeration tank and stored in the ice cooler during transport to the Water and Wastewater Laboratory. Then, the sludge is chilled at 4oC in the storage room to minimize the microbiological decomposition of solids for further use.

2.3 Chitosan preparation

Chitosan was bought from a local supplier. It is 100% organic and environmentally friendly. One packet of chitosan is around 1000g, and it comes in powder form. The chitosan was light brown in color and had an unpleasant smell. The supplier helped to dry the chitosan completely before sending it to delivery. The chitosan then was then kept in a tight container for further use as a nucleating agent.

2.4 Synthetic wastewater

The synthetic wastewater is prepared by proportionate dilution with distilled water and fed into the reactor along with nutrients for optimal microbial growth 1ml of each was added to the feed solution. The influent wastewater pH is adjusted to 7 by adding NaHCO3. Bicarbonate of soda.

2.5 Reactor setup

A laboratory-scale reactor using a high and slender cylindrical column with a diameter of 9 cm, a height of 45.5 cm, and a working capacity of 2 liters is used to cultivate the aerobic granules and treat wastewater. To study the effect of chitosan in granulation, two identical lab-scale reactors are used. The reactor is operated 24 hours for a full cycle at room temperature (25 ± 2 ° C) based on the SBR system. During the aeration process, the air was supplied by an air pump from the bottom of the reactor through a bubble diffuser.

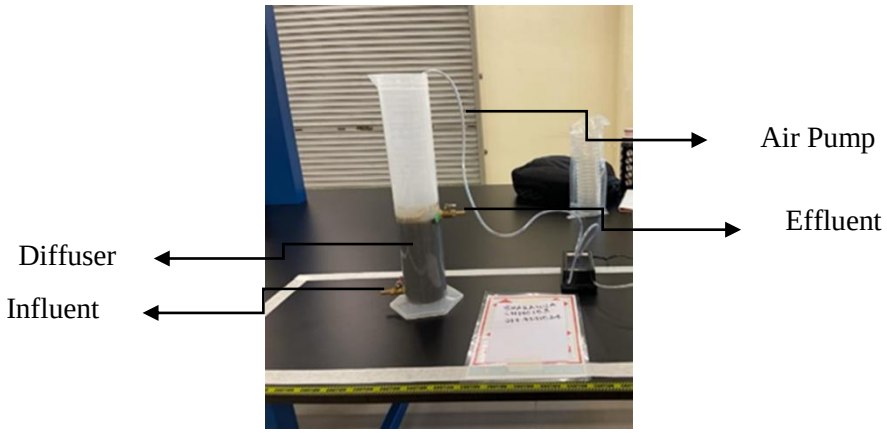

Figure 2.2: Schematic diagram of sequencing batch reactor (SBR)

2.6 Experimental setup

Two identical reactors containing 1l of activated sludge where its concentration of mixed liquor suspended solid is between the range of 2000mg/L to 4000mg/L and 1l synthetic wastewater labeled as R1, and R2. R1 is a control reactor where it is operated without any chitosan meanwhile R2 is operated with 10g of chitosan. Each reactor is supplied with 1120 mg/L of COD concentration.

2.7 Reactor monitor

- MLSS (Mixed Liquor Suspended Solids)

The MLSS were analysed using the Standard Method for the Examination of Water and Wastewater (APHA, 2007). A well-mixed sample must be filtered through a weighted standard glass-fiber filter to calculate the MLSS. The residue left on the filter is dried in an oven at temperatures ranging from 103 to 105 degrees Celsius, and the rise in weight of the filter indicates the total suspended particles in the mixed liquor sample.

$$\text{MLSS (g/L)} = \frac{(A-B)}{Volume\ of\ sample\ in\ lites}$$

Equation 2.1

where,

A = weight of filter + dried residue, g

B = weight of the dry empty filter, g

- MLVSS (Mixed Liquor Volatile Suspended Solids)

MLVSS data is critical in determining the system's operating behaviour and biological content. In the chamber furnace PLF Series 140-160, the filter used for MLSS testing is ignited at 550 °C for 30 minutes (PROTHERM, Turkey). The volatile solids in the sample are represented by the weight loss of the solids during the igniting process.

$$\text{MLSS (g/L)} = \frac{(A-B)}{Volume\ of\ sample\ in\ lites}$$

Equation 2.2

where,

A = Weight of filter + solids from (MLSS) test, g

B = weight of dry empty filter + solids after ignition, g

- Sludge Volume Index (SVI)

Each time the aerator was off to undergo the settling process, the volume of settled sludge in the reactor is measured after allowing it to settle for 15 min. SVI15 was determined from the settled volume after 30 min and the MLSS concentration.

$$\text{SVI (mL/g)} = \frac{\text{Settled Sludge Volume (mL/L)}}{\text{Mixed Liquor Suspended Solids (g/L)}} \times 1{,}000$$

Equation 2.3

- Settling Velocity (SV)

Each time the aerator was off to undergo the settling process, the volume of settled sludge in the reactor is measured after allowing it to settle for 15 min. SVI15 was determined from the settled volume after 30 min and the MLSS concentration

$$\text{Settling velocity (m/h)} = \text{distance/time}$$

Equation 2.4

2.8 Reactor performance

- Chemical Oxygen Demand (COD)

The COD analysed using the method described in the Standard Method for the Examination of Water and Wastewater (APHA, 2007). COD was determined using Hach high-range digestion vials 500-1500 mg/l and a Hach COD reactor model DR200. The digested vials were analysed for transmittance using DR6000 Spectrophotometers (Hach USA).

$$\text{COD removal efficiency (\%)} = \frac{COD_{in} - COD_{eff}}{COD_{in}} \times 100$$

Equation 2.5

Where,

CODin = influent concentration of COD

CODeff = effluent concentration of COD

- Granule size measurement

An electronic digital caliper was used to measure the size of the granules which were drained off and harvested at the steady state.

- Analytical measurement for aerobic granules morphologies

At the end of the experiment, the granular sludge was drained off and harvested at the steady state. Then, scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy used to analyses granule shape.

3. Results and Discussion

3.1 MLSS AND MLVSS

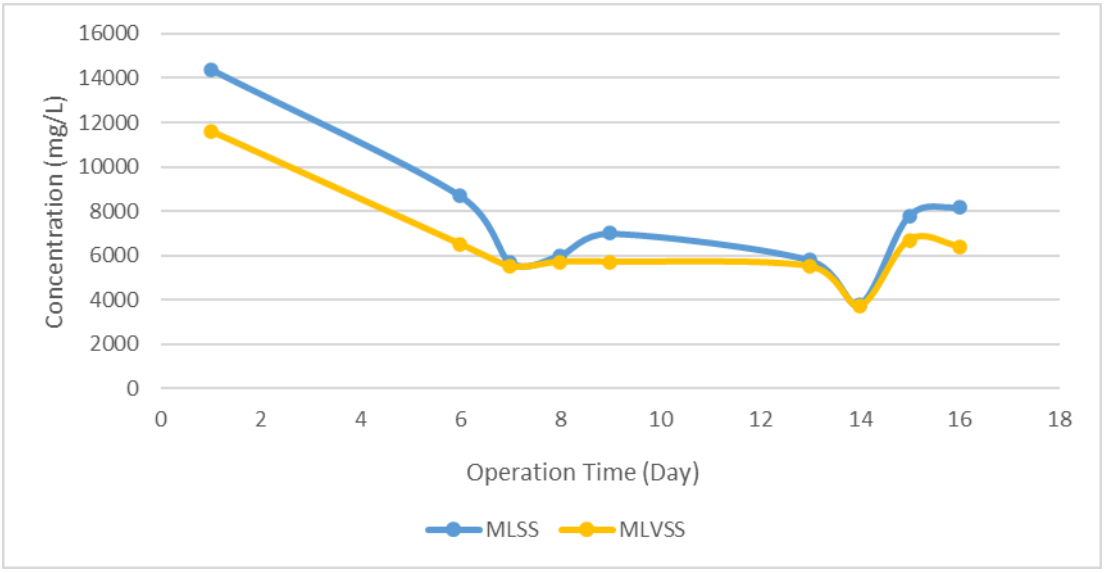

Figure 3.1: Concentration of reactor 1 during the granulation process

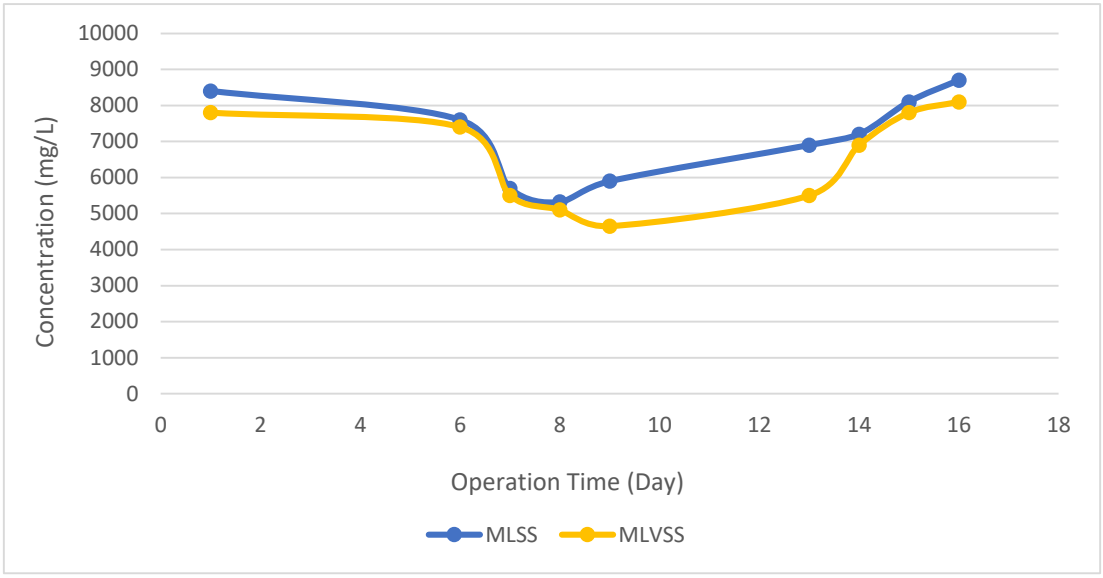

Figure 3.2: Concentration of reactor 2 during the granulation process

Based on Figures 3.1 and 3.2, MLSS concentration in the reactors was unstable over 8 days of the operation as well as the MLVSS concentration in the reactor due to the reactor encountering biomass wash-out caused by the poor settling ability of the activated sludge. The MLSS concentration in Reactor 1 decreased from 7000 mg/L to 3800 mg/L and the concentration in Reactor 2 decreased from 7600 mg/L to 5320mg/L. The MLVSS fluctuated throughout the 10 days of the operation. This occurs probably due to the microbial starting to acclimatize with wastewater in the reactor (Harun et al., 2014) During 2 weeks of operation, the concentration of MLSS and MLVSS started to increase. Where, Reactor 1 increased from 3800mg/L to 8200mg/L and reactor 2 6900mg/L to 8700mg/L. It can conclude that the biomass concentration will increase in trend over the operation time as the number of organic matter and active biomass increase due to the development of granules. Thus, MLSS and MLVSS in Reactor 2 with chitosan has higher reading than the constant Reactor 1.

3.2 Sludge settling

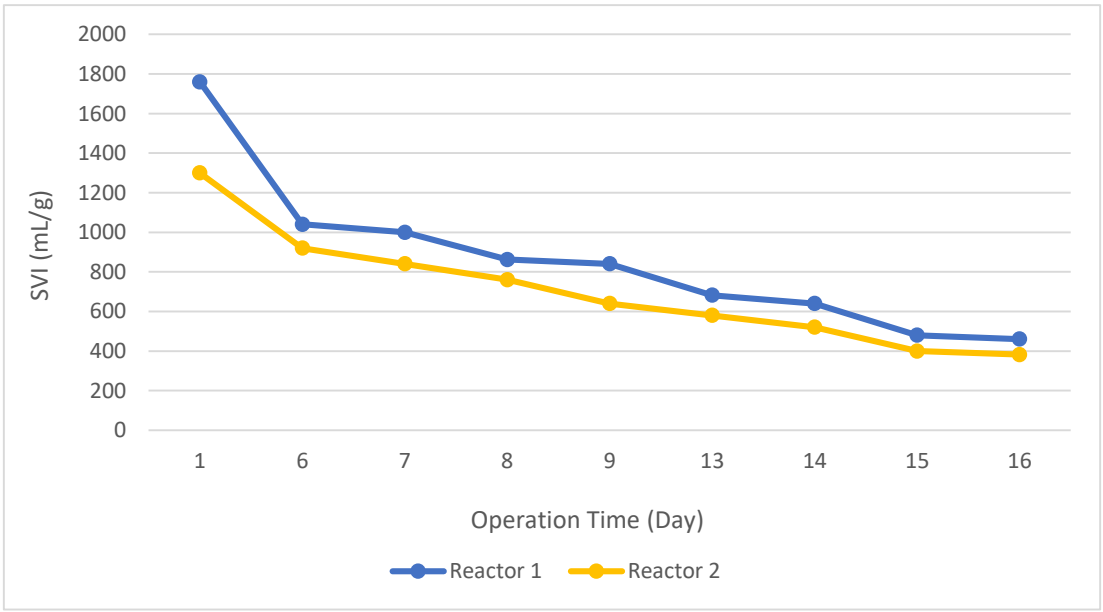

Figure 3.3: Sludge Volume Index (SVI) during the granulation

Throughout the reactor operation, the value of SVI for Reactor 1 which consists of only sludge and synthetic wastewater was improved from 1760 mL/g at the beginning to 460 mL/g at the end of the reactor operation. Besides, the value of SVI for Reactor 2 which consists of sludge, synthetic wastewater, and chitosan was improved from 1300 at the beginning to 382 mL/g at the end of the reactor operation.

The low SVI reading at the end of the operation days indicates that the sludge has good settling ability and compaction characteristics. Thus, comparing both reactors it is proven that Reactor 2 with chitosan has a better settling ability than reactor 1 without chitosan. Results corroborate the enhancement of granule formation when chitosan is used. This is because chitosan enhances surface hydrophobicity thus promoting cell aggregation. Because of the aggregation of microorganisms in the reactor, the particles settled due to an increase in particle density.

3.3 Settling Velocity

Table 3.1: Settling Velocity at the end of the operation on Day 16

Reactor 1 (m/h)	Reactor 2 (m/h)
1.09	1.36
1.33	1.42
1.36	1.45

The settling time used for this testing is 2 minutes. In reactor 1 most of the solid settled within two minutes, with a velocity of 1.36 m/h. Besides, in reactor 2, the settling velocity was 1.45 m/h in two minutes. Reactor 2 with chitosan has the highest settling velocity compared to constant reactor 1. This is proven that chitosan helps to form bigger particles in reactor 2 and shorten the time for the sludge to settle down. Chitosan as a natural coagulant aid/polymer helped in improving the settling velocity.

3.4 Chemical Oxygen Demand (COD) removal

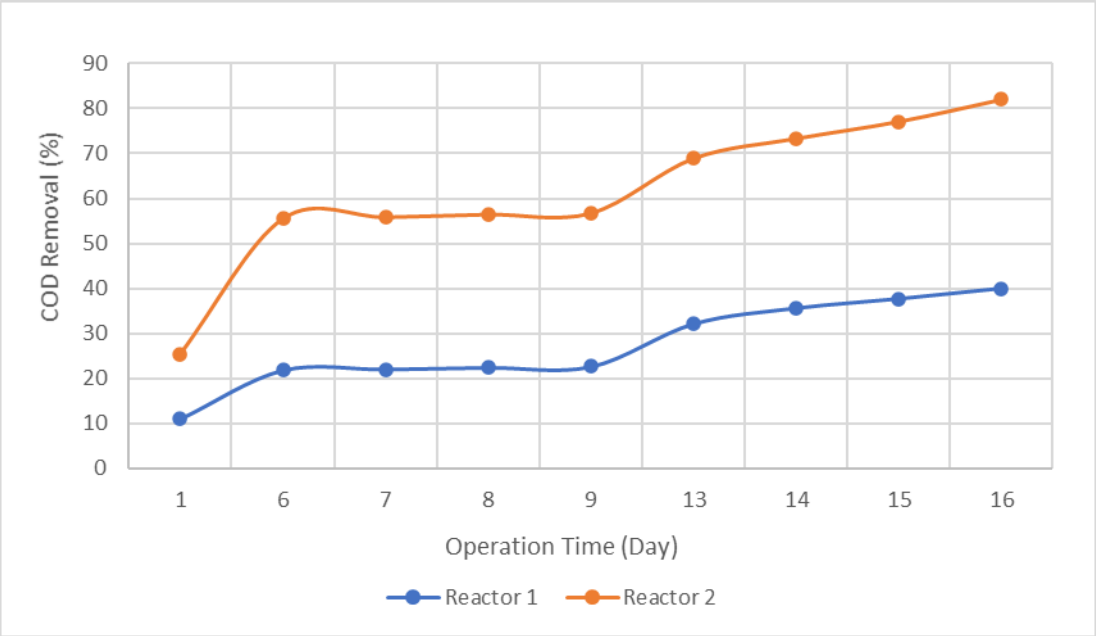

Figure 3.4: Profile removal performance of COD

During the first seven days, the COD removal of reactor 1 was 10% to 22% and reactor 2 was 14% to 34% during the start-up of the operation. Later after a few days, the high COD removal rate in the reactor was caused by the AGS beginning to form compact and dense granules, where reactor 1 had 37% of removal and reactor 2 had 39% of removal. The capability of microorganisms to develop and maintain well-settling granules played a significant role in improving the reactor's overall ability to remove COD (Tay et al., 2004). On the final day of testing, the COD removal of both reactors increased where reactor 1 had 40% removal and reactor 2 had 42% of removal. From the result, it can conclude that the removal of COD in reactor 2 is faster than the reactor 1. Hence, it is once again proven that chitosan played an important role in treating wastewater faster.

3.5 Aerobic granule morphology

The granule size is obviously an important parameter for an efficient AGS process because it is linked to function and microbial activity. Aside from that, the color of the aerobic granules was discovered to be dependent on the chemical composition and microbial population.

3.5.1 The granule size

The small granules form at the start of the operation in the reactors as shown in Figures 3.5 and 3.6, most likely because the microorganisms begin to adapt to the wastewater. After one week, in the reactors, a smooth structure and regular shape of granules started to form. Thus, figure 3.5 shows the granules in reactor 1 without chitosan with an average size of 4.13, and figure 3.6 shows the granule in reactor 2 with chitosan with an average size of 5.62. The granules in reactor 2 with chitosan was keep on increasing in size faster than in control reactor 1 to the end of the operation.

Figure 3.5: Granules from reactor 1 on Day 16

Figure 3.6: Granules from reactor 2 on Day 16

3.5.2 The granules found in reactor 1 and reactor 2.

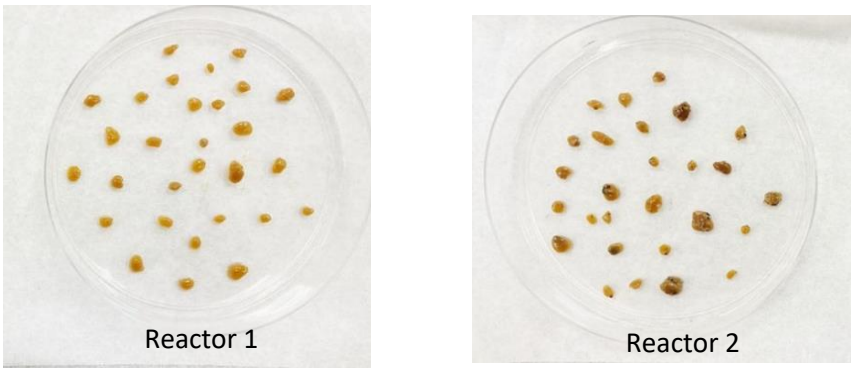

Figure 3.7: Tiny granules collect from reactors 1 and 2 after 16 days

At the end of the operation time that granules were collected from the reactor to observe the difference between its structures and colors. From the observations, the granules in Reactor 1 are primarily round, yellow in color, and very tiny. Besides, the granules in Reactor 2 were round and yellow but also black in color and quite compact. This shows that the development of granules was related to the microbial ability of chitosan It can conclude that the development of granules is better with the presence of a granulation carrier which is chitosan as it promptly initiates the formation of granules during the start-up of the operation.

3.5 SEM Analysis

A sample of granules from Reactors 1 and 2 was collected to analyze its shape using scanning electron microscopy (SEM). The aerobic granules which had a compact structure have different shapes and structures shown in Figure 3.8 and Figure 3.9.

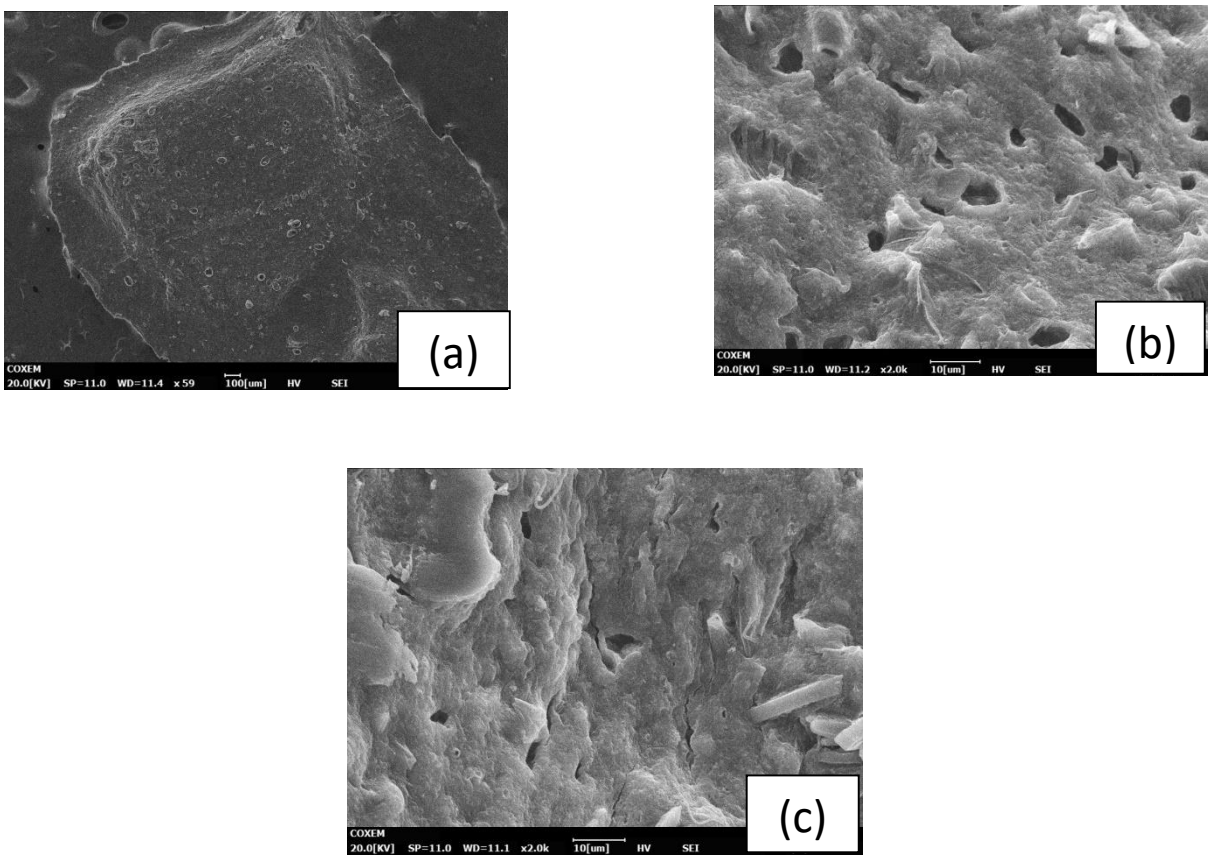

Figure 3.8: Shape and structure of the granule from reactor 1

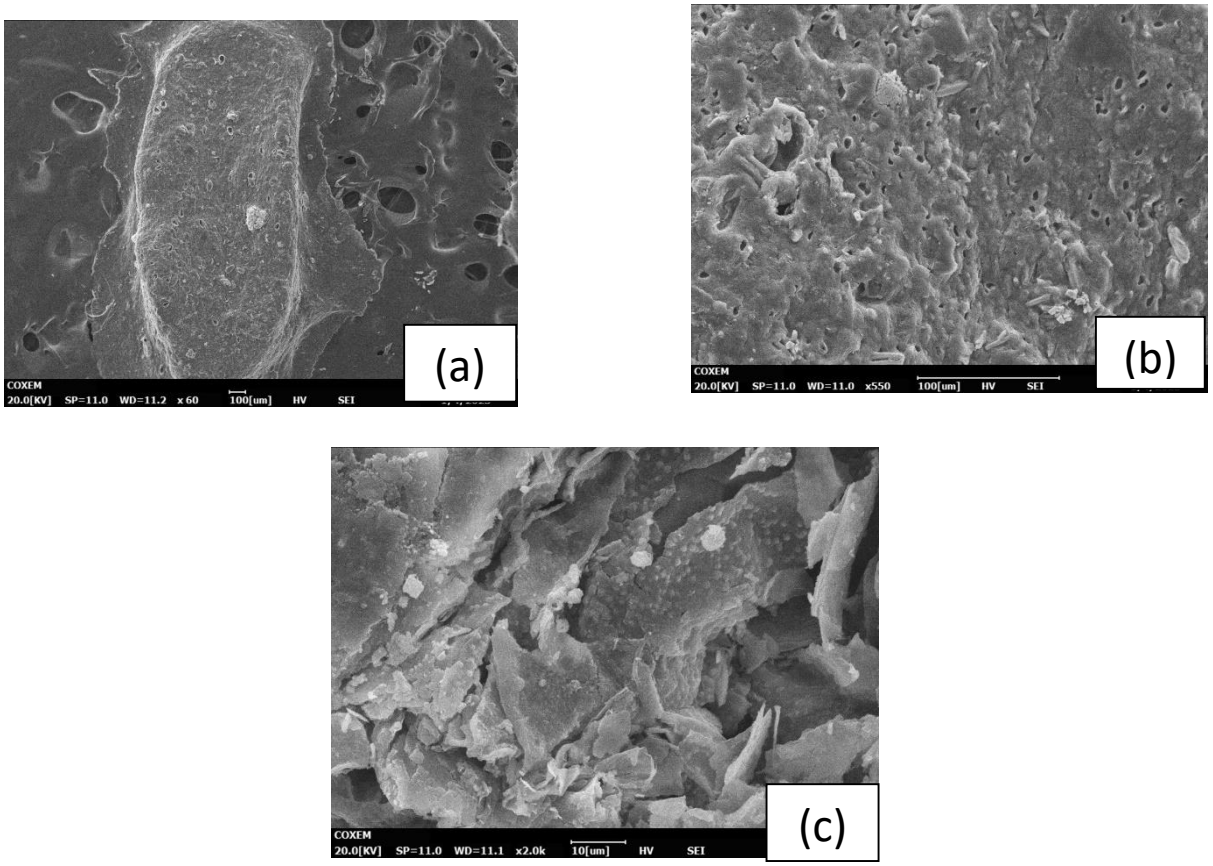

Figure 3.9: Shape and structure of the granule from reactor 2

The granule from the chitosan reactor in figure 4.9 clearly showed that the granule is more complex compared to the granule from the constant Reactor in figure 4.8. From this, we can know that the influent has relatively abundant calcium and magnesium, which would contribute to the formation of crystals. The crystals can be used as nuclei during the formation of spherical granular sludge. However, a large number of microorganisms mainly gathered on the outer surface of the chitosan granule in figure 4.9 (a) and (c) due to its easier acquisition of nutrients compared to the granule in the constant reactor. Figure 4.9 (c) compartment had various microorganisms, predominated by short rod-shaped and filamentous bacteria. In figure 4.8 (c) compartment had abundant filamentous bacteria. In figure 4.9 (c) compartment of the reactor, the dominant bacteria were short rod-shaped bacteria characterized by Metanobacterium. These results indicated that different distributions of microbes thrived at the inside of the 1st to 16th days compartment of the SBR system.

4. Conclusion

In conclusion, results from these AGS studies indicate the potential usage of chitosan for the development of aerobic granules. This will prove that the presence of a granulation carrier improves the development of granules. Aerobic Granular Sludge (AGS) can successfully develop in all reactors from R0 to R2. But the development of AGS in a Reactor with chitosan is rapid compared to the reactor without chitosan. At the end of the results, it is proved that chitosan is a good nucleating agent for accelerating the formation of aerobic granules in the AGS system. This research has the potential to be used in the wastewater industry since it is environmentally benign, inexpensive in cost, and improves the performance of the traditional wastewater treatment sector by shortening the time required to activate AGS development.

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