# THE INFLUENCE OF CA$^{2+}$ ION ADDITION ON PHYSICAL CHARACTERISTICS OF AEROBIC GRANULAR SLUDGE IN SEQUENCING BATCH REACTOR

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## Abstract

Aerobic Granular Sludge is a collection of microbes that allows for the simultaneous removal of carbon, nitrogen, and phosphorus in one reactor system. The formation of Aerobic Granular Sludge is influenced by many factors, such as the type of carbon source, hydrodynamic shear forces, amount of EPS and up to the presence of diavalent cations. In this study $Ca^{2+}$ ion was added to the reactor as an additional chemical with the aim of investigating the effect of $Ca^{2+}$ ion dosage on the physical characteristics of Aerobic Granular Sludge. The objective of this research is to find the influence of $Ca^{2+}$ ions addition to physical characteristics of aerobic granular sludge. There are 3 (three) reactors as a means to compare, with variations in the addition of $Ca^{2+}$ ions as much as 20 mg/l, 45 mg/l and 100 mg/l which are entered into artificial domestic wastewater. The results obtained from this study are the physical characteristics of granular in reactor 1 has an average diameter ranging between 1.12 mm - 1.72 mm, with an aspect ratio of 0.71, then in reactor 2 has an average diameter ranging between 1.63 mm - 2.23 mm, with an aspect ratio of 0.8, and Reactor 3 has an average ranging 1.78 mm - 3.14 mm with an aspect ratio of 0.81. SEM analysis conducted showed that reactor 3 showed the presence of more $Ca^{2+}$ ions than the other reactors. Physical characteristics get better as $Ca^{2+}$ ions increase.

**Keywords**: *aerobic granular sludge, physics, sequencing batch reactor, wastewater*

## Introduction

Aerobic Granular Sludge (AGS) is a collection of microbes that enables the simultaneous removal of carbon, nitrogen, phosphorus, and other pollutants in a single sludge system. AGS differs from activated sludge in terms of physical, chemical, and microbiological properties and offers a compact and cost-effective treatment for the removal of oxidized and reduced contaminants from wastewater. Aerobic Granular Sludge can be cultivated in a Sequencing Batch Reactor, with several influencing factors. Chen et al., 2022 recap the factors that influence the formation of aerobic granular sludge, including Organic Loading Rate, Feast Famine, Settling Speed, pH, Divalent Cations, hydrodynamic shear forces, molecular signals and EPS. Research conducted by Nancharaiah, 2019 and Guo et al., 2022 explained that Ca and HAP ions can increase the formation of Aerobic Granular Sludge. Another study conducted by Wang et al., 2022 explained that $Ca^{2+}$ has a big impact on physical properties, in this case providing better physical characteristics compared to those added with Magnesium Ion. Based on the history of these studies (Wang et al., 2022; Guo et al., 2022; Nancharaiah, 2019), $Ca^{2+}$ ions can support the formation of Aerobic Granular Sludge, but the range of doses in the reactor varies greatly, so in this study the significance of variations in the

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addition of $Ca^{2+}$ ions on the formation and physical characteristics of Aerobic Granular Sludge was examined. The physical characteristics that can be used to assess the formation of aerobic granular sludge are granular diameter size, aspect ratio, and aerobic granular physical structure. In this study will investigate the influence and differences in granular formed in terms of physically based on variations in the addition of $Ca^{2+}$ ions.

## Research Methodology

The research was conducted in the form of an experimental study by conducting a literature study on Sequencing Batch Reactor and Aerobic Granular Sludge, then preparing the reactor with all the components needed. It was followed by the seeding and acclimatization stage to get stable sludge to proceed to the main research stage, namely variations in the addition of $Ca^{2+}$ ions. When running the reactor with a predetermined variation, the process of taking and testing samples is carried out and after that data processing, analysis, and discussion are carried out to the conclusion.

*Instruments and Materials*

The instrument used in this research is a Sequencing Batch Reactor with dimensions of 10 cm in diameter and 80 cm in height with a working water volume of 3 liters. Then to support the operation of the Sequencing Batch Reactor, other supporting tools such as pumps and blower aerators are used to add dissolved air and set the Superficial Air Velocitier at 1.25 cm/second (Figure 1).

The material used in this study is activated sludge made by Degra Simba brand bacterial culture to a minimum MLSS concentration of 2500 mg/l, Degra Simba Brand was used because it has the same bacterial culture characteristics as activated sludge. Then artificial wastewater refers to research conducted by Yulianto, A et al, 2019 and Melati, 2014, namely artificial domestic wastewater with the Organic Loading Rate used is 2.5 kg COD/m3.day which consists of sodium acetate $CH_3COONa$ as the main carbon source with a concentration of 3473.43 mg/l, $NH_4Cl$ as a source of Nitrogen as much as 853.39 mg/l, $K_2HPO_4$ and $KH_2PO_4$ as a source of Phosphorus with a concentration of 31.38 mg/l and 24.56 mg/l, respectively, and MgSO4. $7H_2O$ with a concentration of 34.95 mg/l. Then another research material is $CaCO_3$ which is used as a source of Ca ions in water as a variation.

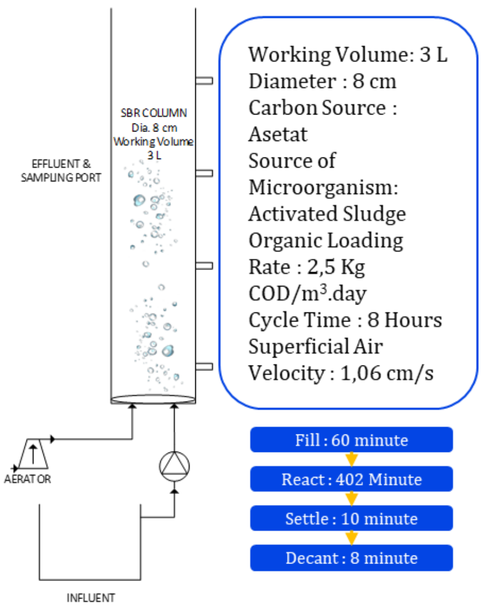



**Figure 1.** Reactor Configuration

*Seeding and Acclimatization*

Bacterial culture was inoculated in wastewater with gradual concentrations ranging from 500 mg/l, 1000 mg/l, 1500 mg/l and 2500 mg/l. During the seeding and acclimatization process, the pH value was kept in the neutral range of 7 - 8 to support bacterial growth, if there is an increase in pH value from the range, acid is added using 5% $H_2SO_4$, then if there is a decrease from the pH range, 5% NaOH will be added, until the pH returns to the neutral range. Then aeration was carried out for 24 hours and the seeding and acclimatization process was stopped when the MLSS value had reached a minimum value of 2400 mg/l.

*Reactor Operations*

The reactor operation on 4 weeks running with superficial air velocities of 1 cm/s was used, referring to research conducted by Devlin et al. 2017 which states that good superficial air velocities for the formation of aerobic granular sludge range from 1 - 3.2 cm/s. The reactor cycle time used is 8 hours, with reference to the results of research conducted by Melati, 2014 where the optimal cycle time for formation with acetate substrate is 8 hours with the Fill Phase for 60 minutes, React Phase for 402 minutes, Settle Phase for 10 minutes, and decant 8 minutes. The 8-hour cycle time means there are 3 cycles in 1 day. Then from the aspect of organic loading rate, 2.5 kg COD/$m^3$ day was used with a concentration in 1 cycle of 833.3 mg/l. The initial F/M ratio used was 1.04 mg COD/mg MLSS.

*Variation*

The dose variations used were $Ca^{2+}$ ion as a trace element of 20 mg/l (R1), $Ca^{2+}$ ion of 45 mg/l (R2), $Ca^{2+}$ ion as an excess dose of 100 mg/l (R3). To obtain $Ca^{2+}$ ions, technical specification $CaCO_3$ chemicals were used.

*Sample Collection and Analysis*

The sampling method used in this study is grab samples. The volume taken for each sampling is adjusted to the purpose of sampling. The reactor runs continuously and automatically so that the granulation process continues for 24 hours. Samples taken were tested on physical characteristic parameters, namely particle size, Aspect Ratio and structure.

Measurement of particle size was carried out through the photo with the help of Autocad 2021 software with a photo scale to the original distance of 1: 1. Diameter measurement is carried out by taking a minimum number of 10 granules and then determining the average. Aspect Ratio determination is based on particle size, and structure is tested using SEM-EDS instrument.

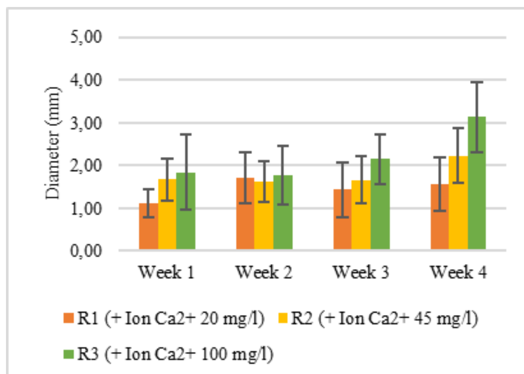
*Statistical analysis*

Statistical analysis was performed with One Way Anova using Microsoft Excel Data Analysis Toolpack to see the significance of the influence of $Ca^{2+}$ ion addition on the physical characteristics of aerobic granular sludge. In this one way anova test will use an Alpha value of 0.05.

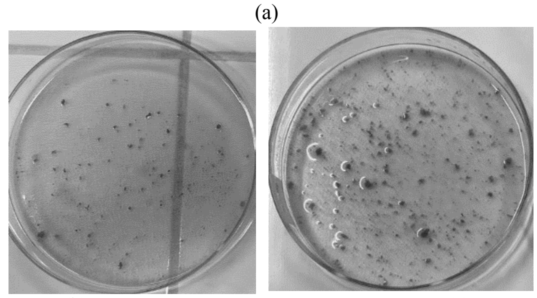
## Result and Discussion

*Diameter Size*

The diameter size of aerobic granular sludge has a variety of different ranges, but the smallest range value of the others is 0.2 - 0.6 mm based on research conducted by Azlina Mat Saad et al., 2018. Then other studies conducted by Beun et al., 1998, Toh et al., 2003 explained that the diameter of AGS can reach 16 mm in diameter. Another range summarized from the research of Andik Y et al., 2019, Thomas Johann, 2006, is 2 - 4.5 mm.




(a)



(b) (c)

**Figure 2.** (a) Average Particle Diameter Distribution; (b) Physical View of AGS from 20 mg $Ca^{2+}$ Ion /l Addition Reactor; (c) Physical View of AGS from 100 mg $Ca^{2+}$ Ion /l Addition Reactor

Figure 2 shows the results of weekly diameter measurements and the physical appearance of aerobic granular sludge at week 4. Reactor 1 has an average diameter of 1.12 mm - 1.72 mm; Reactor 2 has an average diameter of 1.63 mm - 2.23 mm, and Reactor 3 has an average diameter of 1.78 mm - 3.14 mm (Figure 2.a). Figure 2.b is the result of photographing using a camera against aerobic granular sludge samples taken from reactor 1 week 4, while Figure 1.c is the result of photographing aerobic granular sludge samples from reactor 3 week 4, from the picture it can be seen physically that aerobic granular sludge formed in reactor 3 has a larger diameter size. The smallest diameter distribution value in Reactor 1 in each week is 0.57 - 1.04 mm, then in Reactor 2 is 0.79 mm - 1.04 mm, and in Reactor 3 is 0,82 mm - 2.23 mm. The percentage of particle diameter <1 mm in reactors R1, R2, and R3 were 11.8%, 6.3%, and 1.2%, while on diameter 1-2 mm in reactors 1, 2 and 3 were 73.7%, 68.8% and 54.7%, while on diameter 2-3 mm were 10.5%, 21.3%, 33.7% respectively, while on diameter >3 mm in were 3.9%, 3.8%, and 10.5%. Research conducted by Ekholm, 2022 explains that in full scale granules that are > 2 mm in size have a distribution of 50 - 80%.

*Aspect Ratio*

Aspect ratio according to Beun et al., 1998 and Tay et al., 2001, can be calculated by dividing the minimum ferret diameter value by the maximum ferret diameter. Overall, the granule shape represented by the aspect ratio in all reactors did not have a significant difference when viewed from a visual perspective, which on average had an aspect ratio with a value of 0.8. The aspect ratio value is highly dependent on the shape of the granule formed. In the explanation given by Thomas, 2006, it is explained that the shape of the granule can be influenced by the substrate loading rate and by the shear force.

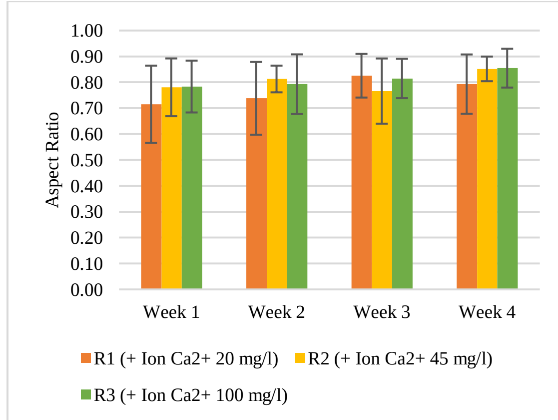



**Figure 3.** Particle's Aspect Ratio

Figure 3 shows a graph of the distribution of Aspect Ratio values from each reactor in each week, in this case the reactor with the addition of 100 mg/l $Ca^{2+}$ ions more than the other reactors shows that the aspect ratio owned by the particles has an average value close to 1, which is 0.81 while in the reactor with the addition of 20 mg $Ca^{2+}$ ions /l has a value of 0.77.

*Structure*

In this study, SEM observations were carried out, to evaluate morphology and confirm the concept of the role of $Ca^{2+}$ ions in the formation of aerobic granular sludge. Three (3) samples with the code Reactor Sample 1 - 20 mg/L $Ca^{2+}$, Reactor Sample 2 - 45 mg/L $Ca^{2+}$, Reactor Sample 3 - 100 mg/L $Ca^{2+}$ were SEM-EDS tested with various magnifications each with a minimum magnification of 30× and a maximum of 2500× and the elements tested were Ca, N, and P. The magnification with the back code (_1) indicates that the image was taken from the same grain.

Figure 4 shows the results of mapping and SEM analysis of each aerobic granular sludge reactor sample taken at week 4 of reactor operation. Based on the mapping results, it is known that the granule formers are N, P, and there is also Ca.

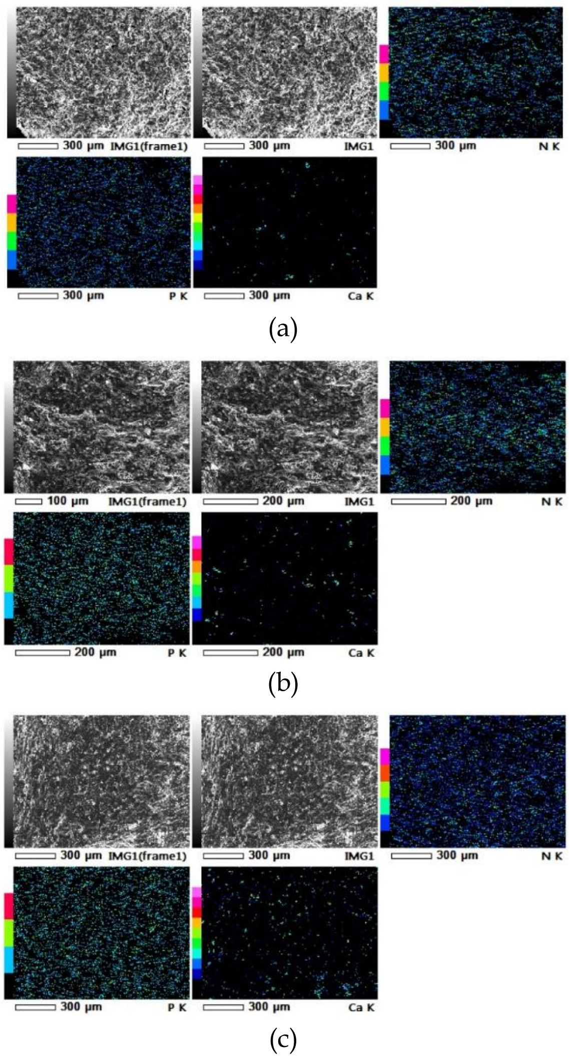



**Figure 4.** (a) SEM Analysis of Aerobic Granular Sludge Reactor 1 Particles; (b) SEM Analysis of Aerobic Granular Sludge Reactor 2 Particles; (c) SEM Analysis of Aerobic Granular Sludge Reactor 3 Particles

The ZAF Method used by the SEM-EDS machine provides the results of the analysis of aerobic granular sludge particles taken from reactor 1 (Figure 4.a) explaining that the atoms that play a large role in a particular area (attached to Figure 4) are N atoms with a percentage of 91.26%, P atoms 4.53% and Ca atoms 4.21%. Then in Figure 4.b where the variation of $Ca^{2+}$ ions added to the reactor as much as 45 mg/l. It is known based on the same mapping results the sample obtained the value of Atom N 90.81%, Atom P 4.31%, and Atom Ca 4.88%. As for the variation of $Ca^{2+}$ ions with the addition of 100 mg/l (Figure 4.c), in a certain area the value of N atoms is 89.83%, P atoms 2.98%, and Ca atoms 7.19%.

Based on this, it can be concluded that the presence of $Ca^{2+}$ ions in a granular is in line with the amount of $Ca^{2+}$ ions added to the reactor. In addition, this can confirm the concept of $Ca^{2+}$ as a bridge presented by Sobeck & Higgins in 2002.

## Conclusions

Based on the observations that have been made in this study, it is concluded that with the addition of $Ca^{2+}$ ions during reactor operation, the results obtained are the physical characteristics of granular in reactor 1 having an average diameter of 1.12 mm, 1.72 mm, 1.43 mm, and 1.57 mm, with an aspect ratio of 0.71, then in reactor 2 has an average diameter every week of 1.67 mm, 1.63 mm, 1.67 mm, 2.23 mm, with an aspect ratio of 0.8, and Reactor 3 has an average diameter every week of 1.85 mm, 1.78 mm, 2.15 mm, 3.14 mm with an aspect ratio of 0.81. The dominance of the percentage of particle diameter measuring 1-2 mm in reactors 1, 2 and 3 was 73.7%, 68.8% and 54.7%, the percentage of particle diameter measuring 2-3 mm in reactors 1, 2 and 3 was 10.5%, 21.3%, 33.7%. Particles formed from reactor 3 have a better aspect ratio value (0.81) than other reactors, and SEM-EDS analysis shows the presence of $Ca^{2+}$ ions in aerobic granular sludge particles from reactor 3 more than from other reactors. Based on the results of statistical analysis using one way anova the higher the addition of $Ca^{2+}$, the larger the diameter size, the better the aspect ratio, and the distribution of large particles is larger.

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