

# Advance Aerobic Granular Sludge Development for the Treatment of Low Strength Wastewater

Hazlami Fikri Basri (✉ [hazlami@utm.my](mailto:hazlami@utm.my))

Universiti Teknologi Malaysia - Main Campus Skudai: Universiti Teknologi Malaysia

Aznah Nor Anuar

Universiti Teknologi Malaysia

Mohd Hakim Ab Halim

Universiti Teknologi Malaysia

Muhammad Ali Yuzir

Universiti Teknologi Malaysia

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## Research Article

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# Abstract

The aim of the present study was to assess the start-up performance of aerobic granular sludge for the treatment of low-strength ( $\text{COD} < 200 \text{ mg L}^{-1}$ ) domestic wastewater by the application of a diatomite carrier. The feasibility was evaluated in terms of the start-up period and stability of the aerobic granules as well as COD and phosphate removal efficiencies. A single pilot-scale Sequencing Batch Reactor (SBR) was used and operated separately for the control granulation and granulation with diatomite. Complete granulation (granulation rate  $\geq 90\%$ ) was achieved within 20 days for the case of diatomite with an average influent COD concentration of  $184 \text{ mg L}^{-1}$ . In comparison, control granulation required 85 days to accomplish the same feat with a higher average influent COD concentration ( $253 \text{ mg L}^{-1}$ ). The presence of diatomite solidifies the core of the granules and enhances physical stability. Diatomite granules recorded the strength and SVI of 18 IC and  $53 \text{ mL/g SS}$  which clearly superior to control granulation ( $19.3 \text{ IC}$ ,  $81 \text{ mL/g SS}$ ). Quick start-up and achievement of stable granules lead to an efficient COD (89%) and phosphate removal (74%) in 50 days of bioreactor operation. Interestingly, this study revealed that diatomite has some special mechanism in enhancing the removal of both COD and phosphate. The result of this research implies that the advanced development of granular sludge by using diatomite can provide a promising low-strength wastewater treatment.

## 1. Introduction

Aerobic granular sludge (AGS) is widely recognized as one of the promising technologies for modern wastewater treatment by the cause of imperative reduction in operational costs (20-25%), less electricity requirement (23-40%) and a (50-75%) land conservations [1]. A lot of studies on AGS have been conducted for the past decades and even scaled-up to full-scale reactors which are currently operational in the Netherlands, Portugal, and South Africa [2]. To date, the AGS technology have been most applied for the treatment of municipal wastewaters with high (COD) organics concentration [3]. The possibility of achieving successful (fast and stable) aerobic granules was higher in high strength wastewater due to the distinct microbial selection, F/M ratio and wash out pattern [4]. Most of the studies recorded successful granulation using more than  $400 \text{ mg L}^{-1}$  COD concentrations wastewater; such as mixture of industrial and municipal wastewater with average COD of  $2000 \text{ mg L}^{-1}$  [4], real municipal wastewater average COD of  $461 \text{ mg L}^{-1}$  [5] and high strength organic wastewater, average COD of  $4500 \text{ mg L}^{-1}$  that successfully achieved complete granulation in a very fast period 18 days [6].

With the global modernization advanced towards sustainable development, municipalities tend to receive industrial effluents mixed with domestic wastewater to unify their treatment processes, resulting in lower organic substrate concentrations. The reality nowadays indicates low strength wastewater treatment issues have become a major concern especially in the application of bio granulation technology. Recent studies have highlighted the main drawback in the development of granules by using low influent COD concentration was long start up period. The study conducted by Derlon et al. [7] tested low strength municipal wastewater with reported average COD influent concentration of  $304 \text{ mg L}^{-1}$ . The start-up

granulation took three months of operation, by the initiative of partial wash out of slow-settling sludge ( $<16\text{mh}^{-1}$ ). The granules size distribution was quite small ( $0.25 < \text{diameter} < 0.63 \text{ mm}$ ) with 30% of the sludge fraction considered flocs. Another attempt in using low strength wastewater was conducted by Laila et al. [8]. With the average COD concentration of  $147 \text{ mg mg L}^{-1}$ , successful start-up phase was achieved after 140 days. The formation of granules took a long period due to acclimatization phase of sludge during the early phase of the experiment. Later, the selection of high settling sludges strategy was applied and advance settling performance was achieved at the end of bioreactor operation (SVI:  $29 \text{ mL/g}$ ).

Owing to the long granulation issue, a lot of researchers have been working on to enhance the granules development. Up to now, most of the research acknowledges two main factors in affecting granulation and they are crystalline nuclei and selection pressure [3, 9]. Selection pressure approach could trigger the formation of the granules. This strategy is highly beneficial as it does not involve much cost for the bioreactor operation to enhance the granulation process [10]. Yet, this strategy has not provenly successful in enhancing start-up of granulation process. Meanwhile, crystalline nuclei are a concept of forming a nucleus in the initial phase of granulation process. Most of the previous research have tested this strategy by using crushed granules and addition of support material such as powdered activated carbon [11] and poly aluminium chloride [12]. The support material act as a carrier, and located at the core of the AGS, having two crucial roles. The first roles are building up the granular structure and the seconds are promoting the metabolism of bacteria attached to the granules [13]. Nonetheless, previous studies did not successfully achieve rapid start-up granulation by using the support material.

Latterly, an adsorbent known as diatomite has attracted increasing research interest in the field of water and wastewater treatment. Diatomite is a friable light-coloured sedimentary rock composed of the accumulation of siliceous shells of diatoms [14]. It has been widely used as an adsorbent, filter aid, as well as catalytic support because it has the characteristics of high surface area and porosity, optimum hydrophobicity and excellent adsorption capabilities. Chen et al. [15] mentioned that diatomite satisfied all the desired characteristics of good support material. Hu et al. [11] and Chen et al. [15] stated that diatomite has the ability to boost the quick formation of large bio flocs. It can also increase microbial diversity and promote aggregation process [16]. In modern wastewater treatment research, several studies have used diatomite as a support material for boosting treatment performance. For instance, diatomite was used for biofilm formation in dynamic membrane technology [11]. Meanwhile, Chen et al. [15] and Gu et al. [16] developed anaerobic granules with diatomite in up-flow anaerobic sludge bed (UASB) reactor. Yet, none studies had been investigated the diatomite potential in accelerating the start-up granulation process in SBR for low strength wastewater treatment. Therefore, this study focused on the influence of diatomite in enhancing the start-up process in SBR system for the treatment of low strength domestic wastewater.

## 2. Materials And Methods

### 2.1: Real domestic wastewater and bioreactor operation

The sample of real domestic wastewater was obtained from Bunus Sewage Treatment Plant (STP), Kuala Lumpur, Malaysia. The COD concentration was  $184 \pm 15.4 \text{ mg L}^{-1}$  which is considered very low strength domestic wastewater (less than  $200 \text{ mg L}^{-1}$ ) [3]. The accompanied parameter such as  $\text{NH}_3\text{-N}$ , TIN, and  $\text{PO}_4\text{-P}$  were all recorded to have higher concentration than the standard. The range of  $\text{NH}_3\text{-N}$  concentration for this study was  $21.8 \pm 5 \text{ mg L}^{-1}$ , while TIN and  $\text{PO}_4\text{-P}$  concentrations were  $63.5 \pm 8 \text{ mg L}^{-1}$  and  $13.30 \pm 3 \text{ mg L}^{-1}$  respectively. The experiments have been carried out in a solid cylindrical acrylic bioreactor, having a diameter of 17 cm, and a height of 125 cm, to operate with a working volume of 15 L. Clear view of the pilot-scale bioreactor used in this study can be seen in Figure 1. The development of aerobic granules in the bioreactor was divided into two conditions. The first condition was the development of naturally formed AGS (AGS control) and the latter was development of AGS with the addition of diatomite (AGS diatomite). The only difference between them was the diatomite. At the beginning of the experiment, 7.5 L of activated sludge from Bunus STP was added as inoculum to fill half of the working volume of the bioreactor during the start-up period. While,  $5000 \text{ mg L}^{-1}$  of diatomite dosage was added into the bioreactor during the early stage for the case of AGS diatomite development. The dosage was added only during the start-up period and no further addition of diatomite needed. The bioreactor operated under sequencing batch mode for 24 hours continuously with a cycle of 3 hours. The 3-hour cycle consists of 60 min of influent feeding, 110 min of aeration, 5 min of sludge settling, and 5 min of effluent discharge. A feeding, discharge, and an air pump with the setting time for each phase in the bioreactor was controlled with pre-programmed digital timers. Feeding and discharge of wastewater were managed by a set of two peristaltic pumps. As for the aeration pump, it supplies oxygen through a diffuser placed at the bottom of the bioreactor with a volumetric flow rate of  $0.18 \text{ m}^3 \text{ h}^{-1}$  ( $0.22 \text{ cm s}^{-1}$  superficial airflow velocity). The influent was fed into the inlet at the bottom of the bioreactor while the effluent was discharged through the outlet ports in the middle of the bioreactor. The effluent had a volumetric exchange ratio (VER) of 50% before the location of the outlet ports. The Solid retention time (SRT) and Hydraulic retention time (HRT) were 93 days and 6 hours respectively. The bioreactor was scheduled to run a specific period without excess sludge discharge; thus, the effluent was the only passage for biomass wasting to be transported. The temperature in the bioreactor was kept at  $27 \pm 1^\circ\text{C}$ .

## 2.2: Diatomite

The diatomite powder used in this study was collected and obtained from YunNan QingZhong Science Tech Co. Ltd (Beijing, China). After collection, the diatomite was stored in a plastic resealable storage bag and placed in the desiccator until the subsequent measures and experiments. The exact dosage for the diatomite is  $5000 \text{ mg L}^{-1}$ . During the experiment start-up, 1g of diatomite was mixed with 1 litre of distilled water for 1 hour using a magnetic stirrer.  $5000 \text{ mg L}^{-1}$  diatomite dosage was selected after a batch test of optimum diatomite dosage was conducted. The batch test was a modification of jar test and settling test to suit granulation properties. Different dosages were considered and the final result

indicates 5000 mg L<sup>-1</sup> as the optimum diatomite dosages due to fastest settling velocity and lowest turbidity.

### 2.3: Analytical methods

The sludge and wastewater (influent and effluent) were a crucial component in analysing the characteristic and removal performance of developed AGS. There were several parameters involved and all of them were measured according to the standard procedures. The analytical methods for this study can be divided into two sections, the characterization and removal performance of the AGS. For granules characterization, it consists of a physical parameter such as size distribution, settling velocity and sludge volume index (SVI) that was analysed by using the method explained by Ab Halim et al. [17]. Wet sieving separation method by using a sequence of sieves with apertures of 4.0 mm, 2.0 mm, 1.4 mm, 1.0 mm, 0.6 mm and 0.3 mm was carried out to obtain the distribution of granules size. Furthermore, the settling velocity was measured by calculating the average time required for a single granule to settle at a certain height while the strength was determined by measuring the integrity coefficient. Other physical characteristic parameters such as SVI and granulation rate were also monitored throughout the experiment using the method explained by Kreuk et al. [18] and Long et al. [19]. Next, for the morphological characteristic parameter, the granules' structure and microstructure were examined by using a stereomicroscope equipped with a digital image analyzer (Olympus SXZ7) and scanning electron microscope (SEM) (JSM-J800F, JEOL, Japan) respectively. Then, Chemical Oxygen Demand (COD), phosphate (PO<sub>4</sub>-P), Ammoniacal Nitrogen (NH<sub>3</sub>-N) and Total Inorganic Nitrogen (TIN) were determined using a spectrophotometer (DR 6000, Hach Co., USA) in accordance to Standard Methods for the Examination of Water and Wastewater [20] for the bioreactor removal performance. The measurements of MLSS and MLVSS biomass concentration also was performed according to APHA [20]. The biomass monitoring and removal performances procedure were shown in Table 2.

## 3. Results And Discussion

### 3.1. Formation and characteristics of developed aerobic granules

The development of AGS diatomite is different from natural granulation process (AGS control). Both granules were successfully formed in this study. The formation and characteristic of both granules were displayed in Table 1. It is important to highlight that the time taken to achieve granulation is the main difference between both types of granules. The transformation of AGS diatomite and AGS control was shown in Figure 2 and Figure 3. AGS control took a significantly longer period to become the granule. While, in an only short period, the loose and fluffy morphology of the inoculated seed sludge (Figure 2a) suddenly evolves into big and compact granules as shown in Figure 2d. It was noted that at the beginning of the experiment, both developed granules have the same startup condition with the majority of the sludge was considered flocculent sludge size of less than 0.3 mm. In just 10 days, the diatomite

effect was already noticed. A huge number of small granules was observed in the bioreactor system (Figure 2b). 70% granulation rate was recorded and only 30% of flocculent sludges were left in the system. A near majority (46.4%) of the sludge was in the range of 0.3 to 0.6 mm size. The granules colour varies from yellow, light brown and dark brown. This rapid transition of seed sludge to granular sludge indicates the influence of diatomite functioning as a carrier and nucleation agent for the granulation process. According to Sarma et al. [21], the first phase of AGS formation (cell to cell interaction), normally takes a long period before entering the second phase (micro aggregate formation). This could be seen by the control AGS having a passive granulation rate since the start of the experiment. The similar transformation could only be achieved after more than 50 days of the experiment. In day 10, the granulation rate of AGS control was 28% and majority of the sludges, 77% were in flocculent state (0-0.3 mm size).

Subsequently, the development of AGS was highly connected to the advancement of biomass and SVI in this stage. AGS diatomite recorded MLVSS/MLSS ratio of 0.67 and  $SVI_5/SVI_{30}$  of 0.67 while AGS control achieved 0.5 and 0.61 respectively. AGS diatomite clearly have a better ration for both biomass and settling properties compared to AGS control. The rapid enhancement of both settling ability and biomass concentration was presumably have something related to the presence of diatomite. Zhang et al. [22] in his study on the effect of diatomite towards the treatment of coal gasification wastewater stated that diatomite promotes the biomass and boost the performance of sludge settling. It was probably due to the high adsorption capability of diatomite to agglomerates with the bacteria and organic matter which result in faster settling rate. Diatomite plays a critical role in initiating the aggregation process during this early stage. Due to its chemical composition and microstructure, the surface of diatomite creates a Beta potential that could neutralise other particles. The beta potential enabled the particles to be adsorbed to the diatomite and agglomerated to become a floc and small granules [23]. With the achievement of initial aggregation, the subsequent granulations are easy to proceed [15].

Later, the AGS diatomite continues to develop and undergo clear morphological transformation becoming more compact and smoother surface. Ultimately, aerobic granulation for AGS diatomite was successfully achieved within 20 days as the granulation rate stabilized above 90%. According to Long et al [24], the aerobic granulation could be considered successful when the granulation rate firstly accounted for 90% of the total sludge in the bioreactor system. In this study, 92% of granulation rate was recorded at day 20 with AGS diatomite appeared bigger in size and solid structure The colour of the granules was shifted into a single dark brown as compared to previous cases. In this stage, the numbers of developed AGS diatomite inside the bioreactor were rapidly increased. On day 25, majority of sludges in the bioreactor consisting AGS diatomite were 0.6 to 1 mm in diameter. Correspondingly, the performance of the system towards the settling ability and the removal of organics and nutrients also became more efficient. Starting from day 25 onwards, the granulation rate and performance of the bioreactor were stable and maintained until the last day of the experiment (day 50). Mature granules with a bigger, compact and smooth surface were visualized on the 30th day as shown in Figure 2d. At day 50, vast majority (56.7%) of the AGS diatomite size ranging from 1 to 4 mm and achieved a desired  $SVI_5$  of 52.8 mL/g SS which

indicates high settling ability in the system. Referring to Ren et al. [25], the average SVI of high-quality granular biomass was less than 60 mL/g SS. Thus, AGS diatomite could be categorized in the group of high settling performance granules.

Meanwhile for AGS control on day 50, the majority (42%) of them was still flocculent sludge with less than 0.3 mm size. The  $SVI_5$  of AGS control was 102.3 mL/g SS and considered to be poor settling performance compared to AGS diatomite. At the end of the experiment (93 days), the  $SVI_5$  of 81 mg L<sup>-1</sup> SS was recorded and majority of the sludges have turn into small granules (1-1.4 mm). Remarkably, the feat that was achieved by AGS controlled at the end of bioreactor operation, was easily attained by AGS diatomite in less than 21 days. AGS diatomite accomplishes 92% of granulation rate at day 20 while AGS controlled approximately 85 days. Notably, the contrast towards the aerobic granulation period for both granules was distinct with 65 days' difference. In comparison with other support material such as zeolite [26] (90 days) and Granular activated carbon (GAC) [27] (6 weeks reduce to 3 weeks), the rapid granulation achieved with the addition of diatomite was clearly higher. Prominently, the short duration of AGS diatomite formation enhanced the sustainability of the treatment and at the same time, improving the efficiency and effectiveness of the system.

## 3.2. SEM analysis

Later, the inner and outer layer structures of 4 mm (diameter) AGS diatomite was visually examined via SEM after 50 days of cultivation. This method explores the whole granular structure, involving surface morphologies, bacterial cluster, as well as filamentous and extracellular polymeric substances (EPS) matrix that associated in the development of AGS diatomite. According to Othman [28], a different condition such as bioreactor technical set up, a different type of wastewater, or even different strategies to promotes granulation process could result in different microbial structures of granules. This study revealed the effect of diatomite on the structure of the granules in comparison to the controlled AGS. The SEM result illustrated the outer layer of AGS diatomite and identified various species of bacteria dominates the surface area. The most apparent bacteria among them were cocci-shape bacteria. The bacteria were observed occupying the granules as a single cell as well as a large group cell as shown in Figure 4a. Correspondingly, both granules displayed an irregular arrangement of cocci-colonies embedded with thick EPS matrix in a dense cluster. According to Zhang et al. [10], cocci-shaped bacteria functioned as a supporting consortium for the AGS development and normally exist in bigger size granules. Likewise, there were a lot of studies related to granulation found the same findings indicating the presence of cocci colonies in AGS [17;29].

Another element that detected throughout the AGS diatomite surface was Extracellular Polysaccharide Substances (EPS) as illustrated in Figure 4.3. The EPS appeared soft in texture with glue-like structures. According to Chen et al. [15], EPS is a complex mixture of polymers that normally present on the microbial cell surface, plays a crucial role in facilitating AGS formation by keeping the microbial aggregates bind together. In other words, this EPS produced by the microbes is a key component for the granulation process as its composition was similar to a biofilm structure. The major components of EPS

are Polysaccharides (PS) and proteins (PN), mainly involved in altering the physicochemical properties of sludge in biological wastewater [30]. Both PS and PN components were expressed in term of PN/PS ratio. As explained by Jiang et al. [29], the production of EPS was enhanced together with the granulation process as a reaction from the increasing trend of PN/PS ratio. Throughout the SEM observation, the EPS was seen on the surface and inner part of AGS diatomite, while the EPS in controlled AGS seems to accumulate more at the inner layer of granules compare to the surface. Other than microbes and EPS, a lot of micropores were observed during the SEM analysis. These micropores were known as cavities and located in any part of AGS diatomite surface adjacent to bacteria clusters. According to Ab Halim et al [17], the cavities were formed due to the continuous flow of liquid produce by EPS and microbes activities close to the internal part of granules. This channel-like structure cavities, act as a transportation passage delivering the needs such as nutrients, oxygen and substrates to the inner part including the core of the granules. The presence of multiple cavities ensure enough supply of needs for every granules component and lead to a rapid granular formation with high stability characteristic. This could be one of the factors that influence the rapid granulation of AGS diatomite. The formation of compact AGS diatomite, at the same time, possess porous structure consisting of loads of cavities might have some relationship with diatomite addition. The presence of diatomite presumably boosts the numbers of bacteria which enhance EPS secretion. Therefore, both components caused the formation of cavities that ensure healthy granules development.

Apart from that, a closer observation towards the inner part of AGS diatomite discovered more than one diatomite was appeared at different location of the granules as compared to none in AGS controlled, which undergo a natural process to become granules. Figure 5a illustrates the image of a single diatomite, it was noticed that the surface of diatomite was enclosed and tightly bonded with microbial cells and EPS. This circumstance verified the role of diatomite as a carrier and nucleation agent during the formation of AGS. Similar observation by Chen et al. [15] reported the diatomite was found in the centre of the granular sludge and act as a substrate for the microbes. Also, two different types of diatomite were found in the granules, they are centric and pennate based primarily on cell shape and frustule morphology. Centric typically have a discoid or cylindrical shape, with radial symmetry, as well as longitudinal and transverse axes as shown in Figure 4b. While pennate have a range of shapes from elongated to filiform (Figure 4c). In the comparison of both diatomite figures, the microbial cells were more attracted to pennate diatomite compared to Centric diatomite. The microbes enclosed nearly 75% of the pennate diatomite while in centric diatomite, only the side part was filled and attached with microbes. The result was comparable with Van Leeuwe et al. [31], claiming that the microbes clearly dominated the pennate diatomite but less abundant in centric diatomite. According to Măicăneanu et al. [32], the pennate diatomite has superior technical properties, that elevates the adsorption ability. In the SEM analysis, the pennate diatomite was discovered to have open-pores dispersed in clay matrix. The pores and open voids provide natural filtration and adsorption properties to the pennate diatomite compare to centric diatomite. Besides, with more than one diatomite were discovered in the 4 mm diameter granules, it was believed that each diatomite and microbial cells undergoing aggregation process to form one small aggregate. Afterwards, it was bonded by EPS together with other aggregates filled with diatomite as

the nuclei, to formed larger granules. Therefore, the AGS formation was presumably catalyst by the existence of diatomite.

### **3.3. Removal performance of AGS control and AGS diatomite**

In this section, the removal performance of COD, PO<sub>4</sub>-P, NH<sub>3</sub>-N and TIN of both types of granules were compared throughout the experiment. It was noted that the performance of AGS diatomite was on par with controlled after the system achieved stable state condition. However, an important element to be highlighted was the significant difference in the duration of the experiment with AGS diatomite in 50 days while controlled, 93 days. It shows the capability of AGS diatomite to effectively removed the pollutants and ensure the stability of the treatment system within a short period.

#### **3.3.1. Chemical oxygen demand removal**

The performance of AGS diatomite towards the elimination of organic matter (COD) from the treatment system during the whole period of bioreactor operation was displayed in Figure 6. As shown in the figure, the overall profile of COD removal performance indicates satisfying performance since the beginning of the experiment and rapidly increased achieving more than 90% removals before stabilized to a steady-state after a short period. Different circumstances occurred in the controlled experiment as it took a long period to accomplish stable removal performance. Nevertheless, it was noticed that the COD removal performance successfully stabilized at advance levels after both AGS achieved granulation.

During the early stage of the experiment, the bioreactor cultivating AGS diatomite already demonstrated better performance with the average COD removal percentage beyond 70% compared to control with only 46%. The effluent COD also lower than controlled for the first 10 days. Two factors might influence this condition. Firstly, it was due to the differences in the influent COD concentration with the controlled average influent COD was higher than the latter. This condition was due to the additional residential areas (Construction for other Sewage treatment plant) that contributes towards the increase of raw sewage concentration in Bunus STP. The average influent COD concentration for controlled was 335 mg L<sup>-1</sup> while the AGS diatomite bioreactor recorded only 175 mg L<sup>-1</sup> for the first 10 days of the experiment. However, the low concentration of COD normally have lower removal percentages because of the difficulties of achieving low concentration COD. Therefore, the biggest factor might be the aftermath of diatomite addition. It was mentioned by Xu et al [33] that diatomite high adsorption capability of organic matter. Diatomite surface comprised of electronegative charged that managed to absorb the cationic organic chains. The organic chains then shrunk and attached to the diatomite. This could highly reduce the concentration of suspended organic matter (in term of COD) in the treated effluent.

Afterwards, the COD removal performance was drastically increased in a short period. The performance achieved the highest of 91% COD removal percentages after only 22 days of the experiment. Meanwhile, different circumstances occurred in the controlled experiment as it only recorded 67.2% COD removals.

This achievement has a strong relationship with the granulation process as high numbers of mature granules would surely enhance the system performance. This was supported by the fact that the granulation rate for AGS diatomite accomplished 92% while controlled, only a handful of 43%. According to Rosman [34], one of the reasons behind the high COD removal performance was numerous contacts between the granules and surrounding particles. It enhances the granules to mineralize organic matter and intermetabolites in the bioreactor. In this case, the presence of diatomite which possessed high adsorption ability could intensify the contact between the particles and microbes that attach to its surface and effectively increased the COD removal performance.

In the 25th day until the end of the experiment (day 50), the COD removal performance was stabilized with a range of 83–90%. The performance could be considered excellent achievement as a vast amount of organic matter were removed from the supernatant. The stable performance was also supported by 0.8 MLVSS/MLSS ratio obtained in the final day of bioreactor operation which demonstrated the optimum level of organic and inorganic solids in the system. In comparison, the controlled AGS attained only 70% removal after 50 days of the experiment. It finally accomplished the same feat as AGS diatomite after a long period of 93 days. The accomplishment of AGS diatomite in this study was comparable to Chen et al [15], that developed granules with the presence of diatomite for treating petroleum wastewater. The author mentioned that diatomite greatly improved the system due to its' unique characteristic, high surface area and high adsorption capacity. With those advantages, the particulate substrates were effectively removed by adsorption processes at the granule surface, followed by hydrolysis. As for the effluent quality, the average COD concentration after the treatment met the sewage discharge standard A, (COD <120 mg L<sup>-1</sup>) as regulated in Environmental Quality (Sewage) Regulations Malaysia (2009).

### **3.3.2. Phosphate removal**

In general, the elimination of phosphorus demands alternating anaerobic-aerobic condition (Bassin et al, 2019). It suits the application of granulation technologies in the SBR system that consists of non-mixed anaerobic feeding phase, aeration phase, settling phase and effluent withdrawal. Both AGS diatomite and controlled were developed in the same system and the presence was the biggest factor separating the two. In this study, the PO<sub>4</sub>-P removal performance profile for AGS diatomite was illustrated in Figure 7. The overall pattern of PO<sub>4</sub>-P profile indicates increasing removal performance after going through rapid granulation process. In the earlier 10 days of operation, both types of bioreactor recorded a very low PO<sub>4</sub>-P removal performance averaging only 30–40%. The low-performance issue was also experienced by Ab Halim et al [17] with PO<sub>4</sub>-P removal of 30 to 50%. It was suggested that the sludge was still going through an adaptation phase in this period. The microbes in the sludge do not grow instantly after the start-up process and required a certain amount of time to adopt a new living environment.

In the period between 9 to 18 days, there is a significant rise in phosphate removal performance from 32–56% showing positive changes after AGS diatomite formation implying its' capability for phosphorus biodegradation. According to Nancharaiah and Reddy [36], for low strength real domestic wastewater, a small concentration of substrates is a limiting factor for phosphorus removal process which required

COD. This obstacle could be hindered with the enrichment of microbes in granules that able to simultaneously undergo denitrification and phosphate removal. Interestingly, the rapid increased of phosphate removal performance for AGS diatomite might have some relation to this theory. The presence of diatomite possibly attracted polyphosphate-accumulating organisms (PAO) to attach to the granules and grows. The PAO stored the influent organic matter as intracellular polymers during the influent feeding period (anaerobic condition), and remove it from the wastewater in the aeration phase.

The removal performance kept increasing until it reached 74% at the end of the experiment. Meanwhile, controlled AGS recorded 70% removals after 93 days of the experiment. AGS diatomite has a better  $\text{PO}_4\text{-P}$  removal performance compared to control with a significantly shorter period. Furthermore, a study conducted by Zhang et al [37] towards the ability of diatomite in nutrient removals from agricultural wastewater also proved the effectiveness of diatomite for  $\text{PO}_4\text{-P}$  removals. The removal performance of  $\text{PO}_4\text{-P}$  with the addition of diatomite was reportedly more than 88%. The mechanism for the removals was via chemical adsorption which involves valence forces or electron exchanges. This suggests two different abilities for diatomite in removing phosphate, involving biological and chemical adsorption. Yet, the performances of AGS diatomite in removing  $\text{PO}_4\text{-P}$  were still average compared to other parameters at the end of the experiment. According to Nancharaiah and Reddy [36], this incident seemingly due to the fair amount of PAO which result in passive bioactivity of the organisms in the bioreactor. Nevertheless, AGS diatomite illustrated the increasing pattern of removal as compared to control which indicates its' potential to achieve exceptional performance in the long run.

### **3.3.3. Complete removal of nitrogen comprises nitrification and denitrification process.**

Generally, AGS is well known for its capability in undergoing both processes simultaneously which led to an effective nitrogen removal for the treatment. In this study, the nitrogen removal performance was analysed and compared between AGS diatomite and AGS controlled. The nitrogen removal was measured in term of TIN and  $\text{NH}_3\text{-N}$ . The profiles removals for both parameters were shown in Figure 8 respectively. As illustrated in the figures, the first weeks of the experiment indicate average TIN removal performance for AGS diatomite, managing less than 70% removal percentage. It was probably due to the low influent COD concentration that reduces the denitrification ability and affect the low removal of  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  [38]. However, the removal percentage of  $\text{NH}_3\text{-N}$  displays a positive sign given that the performance kept on increasing and achieved more than 70%, suspected due to diatomite addition. The performance was comparable to AGS controlled achieving an average of 69% removal percentages for both TIN and  $\text{NH}_3\text{-N}$  parameter in the beginning, 7 to 10 days of the experiment.

The subsequent period demonstrated large numbers of granules were formed in AGS diatomite bioreactor at day 20. Surprisingly, in this short period, it already accomplished the state of granulation after successfully attained a granulation rate of 92%. The advancement of AGS in the system undoubtedly influences the enrichment of nitrifying bacteria that responsible for nitrogen degradations. As a result, TIN and  $\text{NH}_3\text{-N}$  removal performance increased exponentially just after 20 days of experiment achieving 72%

and 87% removals respectively. This condition proved the effect of diatomite towards rapid granulation and at the same time enhanced the system to perform efficiently. Correspondingly, Basri et al [39] mentioned the process of granules formation could enhance the growth of nitrifying bacteria and improved the nitrification process which might be the case of this study. Furthermore, according to Derlon et al [7], the extent of simultaneous nitrification-denitrification was directly associated with the fraction of granules that exposed to the anoxic condition. In this case, the diameter of the granules was already in the range of 0.6 to 1 mm at day 14 proving the effectiveness of the removal performance of TIN and  $\text{NH}_3\text{-N}$  from that period onwards.

Starting from day 33, the  $\text{NH}_3\text{-N}$  was kept above 87% while TIN, 76% until the end of the experiment. The AGS diatomite reached the highest removal of 93% and 85% for  $\text{NH}_3\text{-N}$  and TIN respectively. Notably, the maturation of AGS enhance the removal performance with the presence of aerobic and anaerobic/anoxic layers, which make the granules able to carry out the removal of carbon, nitrogen, and phosphorous simultaneously [24]. Specifically, the performance was highly influenced by the population of nitrifiers and denitrifiers (slow growers) [40]. Xia et al [41] stated ammonia-oxidizing bacteria (AOB) responsible for ammonia removals primarily located at the outer layer of granules while nitrite-oxidizing bacteria (NOB) normally reside in the inner layer. Large size granules presumably favour AOB growth and cause inhibition to NOB growth. The ratio of AOB to NOB in the granules also rise along with the increase in AGS size. This explained the excellent performance of AGS diatomite in removing  $\text{NH}_3\text{-N}$  compared to TIN in this study.

Eventually, both AGS successfully achieved excellent removal of  $\text{NH}_3\text{-N}$  with more than 90% removal percentages at the end of the experiment. To achieve such a feat in a short period, AGS diatomite was seen as the better performer compared to control. Previously, Dong et al [42] conducted a study to investigate the performance of diatomite in removing  $\text{NH}_3\text{-N}$  in coking wastewater. The result indicates excellent  $\text{NH}_3\text{-N}$  removals with more than 90% removal percentage. This suggests that diatomite might be one of the main factors enhancing the ammonia removal performances for AGS diatomite. Nonetheless, controlled AGS recorded a better TIN removal performance compared to AGS diatomite. Liu et al [40] explained that to ensure the excellent simultaneous performance of  $\text{NH}_3\text{-N}$ ,  $\text{NO}_3^- \text{-N}$  and  $\text{NO}_2^- \text{-N}$  removal, sufficient microbial load was needed and demanded a longer duration. In this case, AGS controlled going through a significantly longer period to accomplish the desired state as compared to AGS diatomite. This condition was one of the main reasons for control to have a slightly better performance than AGS diatomite in term of nitrogen removal. Even so, the excellent biological activity of the microbes in the AGS diatomite could ensure a better nitrogen removal performance in the future.

## 4. Conclusions

In the development of aerobic granules fed with low strength domestic wastewater, diatomite had proven to be a good enhancer for granulation. The diatomite addition granules have successfully achieved granulation (granulation rate > 90%) within 20 days of operation, significantly faster than control (85

days). AGS diatomite possessed compact physical characteristic with high settling properties (SVI: 52 mL/g SS). The microscopic analysis discovered more than one diatomite present in the inner part of granules. This phenomenon suggested each diatomite and microbial cells undergoing aggregation process to form one small aggregate. Afterwards, it was bonded by EPS together with other aggregates filled with diatomite as the nuclei, to formed larger granules. As the formation of granules was enhanced by diatomite, the removal performance of the treatment was also highly efficient and stabilized at a faster rate compared to AGS control. AGS diatomite recorded an average removal performance of 90% for organic matter (COD) removals, 74% phosphate nutrients removals, 92% and 85% for  $\text{NH}_3\text{N}$  and TIN removals at 50 days of experiment. While, AGS control accomplish 87% COD removals, 71% phosphate removals, 95% and 91% for  $\text{NH}_3\text{N}$  and TIN removals respectively at 93 days of experiment. The huge difference in period for both AGS along with AGS diatomite's excellent performance were a crucial point that demonstrated the effectiveness of diatomite. For the record, this study is the first attempt that applied diatomite for rapid granulation fed with very low strength wastewater. The successful application of the diatomite in granulation system for low strength domestic wastewater treatment will promote the growth of green economy that not only support the income generation to the country but also promoting the sustainable development.

## Declarations

### Availability of data and materials

All data generated or analyzed during this study are available to view on all articles.

### Competing interests

The authors declare they have no competing interests.

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### Authors' contributions

Corresponding author, Hazlami Fikri Basri provided the data analysis and manuscript. All authors read and approved the final manuscript.

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## References

1. Bengtsson S, de Blois M, Wilén BM, and Gustavsson D. A comparison of aerobic granular sludge with conventional and compact biological treatment technologies, *Environ. Technol.* 2019;40:21:2769-2778.
2. Pronk M, Abbas B, Al-Zuhairy SHK, Kraan R, Kleerebezem R and Van Loosdrecht MCM. Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. *Appl. Microbiol. Biotechnol.* 2015;99:12:5257-5268
3. Sguanci S. Lubello C. Caffaz S. and Lotti T. Long-term stability of aerobic granular sludge for the treatment of very low-strength real domestic wastewater. *J. Clean. Prod.* 2019;222:882-890.
4. Pishgar R, Dominic JA, Sheng Z, and Tay JH. Influence of operation mode and wastewater strength on aerobic granulation at pilot scale: startup period, granular sludge characteristics, and effluent quality. *Water Res.* 2019;160: 81-96.
5. De Sousa Rollemberg SL, Barros ARM, de Lima JPM, Santos AF, Firmino PIM, and dos Santos AB. Influence of sequencing batch reactor configuration on aerobic granules growth: Engineering and microbiological aspects. *Clean. Prod.* 2019;238:117906.
6. Hamza RA, Iorhemen OT, Zaghoul MS, and Tay JH. Rapid formation and characterization of aerobic granules in pilot-scale sequential batch reactor for high-strength organic wastewater treatment, *J. Water Process. Eng.* 2018;22:27-33.
7. Derlon N, Wagner J, da Costa RHR., and Morgenroth E. Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume. *Water Res.* 2016;105:341-350.
8. Purba, LDA, Yuzir A, Halim MH, Zamyadi A, and Abdullah N. Performance of Aerobic Granular Sludge for Domestic Wastewater Treatment. *Int. J. Eng. Innov. Technol.* 2020;8:12.
9. Zou J, Pan J, Wu S, Qian M, He Z, Wang B, and Li J. Rapid control of activated sludge bulking and simultaneous acceleration of aerobic granulation by adding intact aerobic granular sludge. *Sci. Total Environ.* 2019;674:105-113.
10. Zhang, Y, Dong X, Nuramkhaan M, Lei Z, Shimizu K, Zhang Z, Adachi Y, Lee DJ. and Tay JH. Rapid granulation of aerobic granular sludge: A mini review on operation strategies and comparative analysis. *Bioresource Technology Reports*, 2019a;7:100206
11. Hu Y, Wang XC, Sun Q, Ngo HH, Yu Z, Tang J, and Zhang Q. Characterization of a hybrid powdered activated carbon-dynamic membrane bioreactor (PAC-DMBR) process with high flux by gravity flow: Operational performance and sludge properties. *Technol.* 2017;223:65-73.
12. Liu Z, Liu Y, Zhang A, Zhang C, and Wang X. Study on the process of aerobic granule sludge rapid formation by using the poly aluminum chloride (PAC). *Eng. Sci.* 2014;250:319-325.
13. Zhang D, Li W, Hou C, Shen J, Jiang X, Sun X, Li, J, Han W, Wang L and Liu X. Aerobic granulation accelerated by biochar for the treatment of refractory wastewater. *Chem. Eng.* 2017;314:88-97.
14. Sogut EG, and Caliskan N. Isotherm and Kinetic Studies of Pb (II) Adsorption On Raw and Modified Diatomite by Using Non-Linear Regression Method. *Fresenius Environ. Bull.* 2017;26:4:2720-2728.

15. Chen C, Liang J, Yoza BA, Li QX, Zhan Y, and Wang Q. Evaluation of an up-flow anaerobic sludge bed (UASB) reactor containing diatomite and maifanite for the improved treatment of petroleum wastewater. *Bioresour. Technol.* 2017;243:620-627.
16. Gu QY, Liu XY, and Wang ZN. Enhanced Performance of Sulfate Reducing Bacteria (SRB) Granules Process by Using Diatomite as Functional Carrier Material. *Key Eng. Mater.* 2017;730:206-211.
17. Ab Halim MH, Anuar AN, Azmi SI, Jamal NSA, Wahab NA, Ujang Z, Shraim A. and Bob MM. Aerobic sludge granulation at high temperatures for domestic wastewater treatment. *Technol.* 2015;185:445-449.
18. De Kreuk MK. Aerobic granular sludge. State of the art, *Water Sci. Technol.* 2007;55:75 -81
19. Long B, Yang CZ, Pu WH, Yang JK, Jiang GS, Dan JF, Li CY and Liu FB. Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor. *Bioresour. Technol.* 2014;166:57-63.
20. Standard Methods for the Examination of Water and Wastewater. American Public Health Association: Washington, DC; 2007.
21. Sarma SJ, Tay JH and Chu A. Finding knowledge gaps in aerobic granulation technology. *Trends Biotechnol.* 2017;35:1:66-78.
22. Zhang W, Pinhua RAO, Zhang H, and Jingli XU. The role of diatomite particles in the activated sludge system for treating coal gasification wastewater. *Chin. J. Chem. Eng.* 2009;17:1:167-170.
23. Kamaruddin NF. Diatomite as Natural Coagulant in the Removal of Water Turbidity. Master of Philosophy, UniversitiTeknologi Malaysia, Kuala Lumpur; 2017.
24. Long B, Yang CZ, Pu WH, Yang JK, Jiang GS, Li CY, Liu FB, Dan JF, Zhang J and Zhang L. Rapid cultivation of aerobic granule for the treatment of solvent recovery raffinate in a bench scale sequencing batch reactor. *Sep. Purif. Technol.* 2016;160:1-10.
25. Ren Y, Ferraz F, Lashkarizadeh M, and Yuan Q. Comparing young landfill leachate treatment efficiency and process stability using aerobic granular sludge and suspended growth activated sludge. *J. Water Process. Eng.* 2017;17:161-167.
26. Wei D, Xue X, Chen S, Zhang Y, Yan L, Wei Q, and Du B. Enhanced aerobic granulation and nitrogen removal by the addition of zeolite powder in a sequencing batch reactor. *Appl. Microbiol. Biotechnol.* 2013;97:20:9235-9243.
27. Zhou JH, Zhao H, Hu M, Yu HT, Xu XY, Vidonish J, Alvarez PJ and Zhu L. Granular activated carbon as nucleating agent for aerobic sludge granulation: Effect of GAC size on velocity field differences (GAC versus flocs) and aggregation behavior. *Bioresour. Technol.* 2015;198:358-363.
28. Othman I. Aerobic Granular Sludge from Different Cycle Time for Livestock Wastewater Treatment. Doctor of Philosophy, UniversitiTeknologi Malaysia, Skudai; 2017.
29. Jiang Y, Wei L, Yang K, and Wang H. Investigation of rapid granulation in SBRs treating aniline-rich wastewater with different aniline loading rates. *Total Environ.* 2019;646:841-849.
30. Wei D, Wang B, Ngo HH, Guo W, Han F, Wang X, Du B. and Wei Q. Role of extracellular polymeric substances in biosorption of dye wastewater using aerobic granular sludge. *Bioresour. Technol.*

- 2015;185:14-20.
31. Van Leeuwe MA, Tedesco L, Arrigo KR, Assmy P, Campbell K, Meiners KM, Rintala JM, Selz, V, Thomas DN and Stefels J. Microalgal community structure and primary production in Arctic and Antarctic sea ice: A synthesis. *Elementa*. 2018;6.
  32. Măicăneanu AS, Chicinaş RP, and Bedeleian H. Treated diatomite for Toluidine Blue removal from wastewater. Is it worth it?. *Stud. Univ. Babeş-Bolyai Chem*. 2019;64:4.
  33. Xu, K., Liu, Y., Wang, Y., Wang, T., Wang, H., Liang, X., Lu, C., Tan, Y., Liu, X. and Wang, P. (2018). A Novel Wastewater Treating Material: Cationic Poly Acrylamide/Diatomite Composite Flocculants. *Journal of Polymers and the Environment*, 26(7), 3051-3059.
  34. Rosman N. Biogranular Sludge for Rubber Processing Wastewater in a Sequencing Batch Reactor. Doctor of Philosophy, UniversitiTeknologi Malaysia, Skudai;2017.
  35. Bassin, JP, Tavares DC, Borges RC, and Dezotti M. Development of aerobic granular sludge under tropical climate conditions: The key role of inoculum adaptation under reduced sludge washout for stable granulation. *J. Environ. Manage*. 2019;230:168-182.
  36. Nancharaiah YV and Reddy GKK. Aerobic granular sludge technology: mechanisms of granulation and biotechnological applications. *Bioresour. Technol*. 2018;247:1128-1143.
  37. Zhang B, Wang X, Li S, Liu Y, An Y and Zheng X. Preferable Adsorption of Nitrogen and Phosphorus from Agricultural Wastewater Using Thermally Modified Zeolite–Diatomite Composite Adsorbent. *Water*, 2019b;11:10:2053.
  38. Sarma SJ, and Tay JH. Aerobic granulation for future wastewater treatment technology challenges ahead. *Environ. Sci. Water Res. Technol*. 2018;4:1:9-15.
  39. Basri HF, Anuar AN. and Ab Halim MH. Pilot scale study on characterization and performance of aerobic granular sludge to treat domestic wastewater. *Mal. J. Fund. Appl. Sci*. 2020;16:1:38-43.
  40. Liu J, Li J, Xie K, and Sellamuthu B. Role of adding dried sludge micropowder in aerobic granular sludge reactor with extended filamentous bacteria. *Bioresour. Technol. Rep*. 2019;5:51-58.
  41. Xia J, Ye L, Ren H, and Zhang XX. Microbial community structure and function in aerobic granular sludge. *Appl. Microbiol. Biotechnol*. 2018;102:9:3967-3979.
  42. Dong J, and Lv BN. Diatomite strengthen COD and ammonia removal from a micro-aerobic EGSB reactor treating coking wastewater. *Energy Environ. Sci*. 2018;191:1:012075.

## Tables

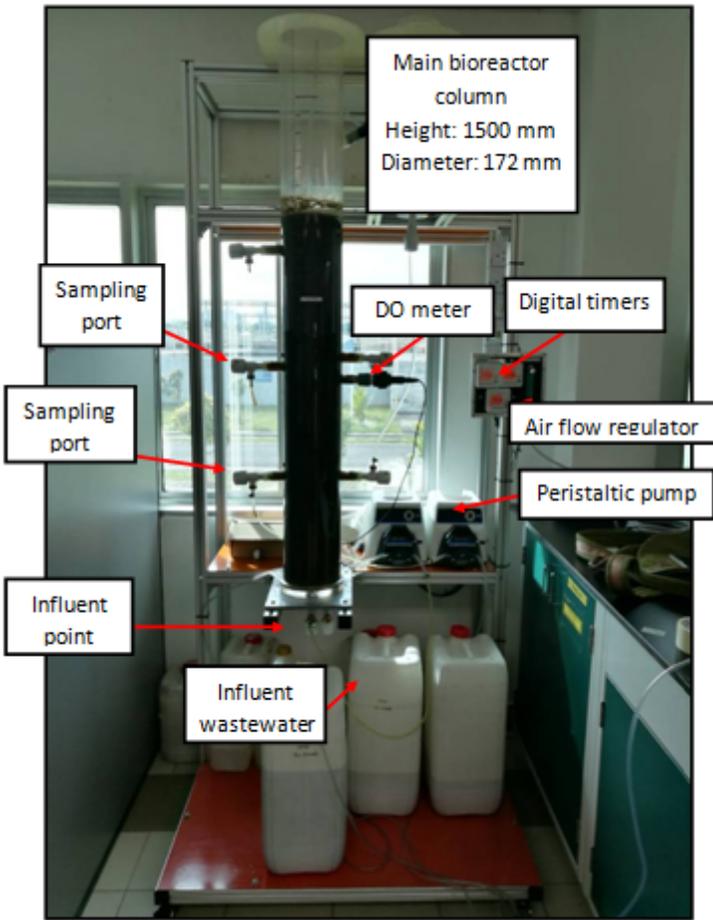
**Table 1** Biomass and physical characteristic monitoring throughout granules development

Parameter	Time	AGS diatomite			AGS Control		
		Initial (day 10)	Intermediate (day 25)	Final (day 50)	Initial (day 10)	Intermediate (day 50)	Final (day 93)
MLSS (mg/L)		7000	8000	9400	7400	10520	12120
MLVSS/MLSS		0.67	0.70	0.81	0.50	0.59	0.75
SVI <sub>5</sub> (mL/g SS)		120	98.2	52.8	147.0	102.3	81.4
SVI <sub>5</sub> / SVI <sub>30</sub>		0.67	0.75	0.76	0.61	0.79	0.79
Granulation (%)		70	92	94	28	58	91
Size distribution (majority) (mm)		0.3-0.6	0.6-1.0	1.0-1.4	0-0.03	0-0.3	1.0-1.4

**Table 2** Biomass monitoring and removal performance

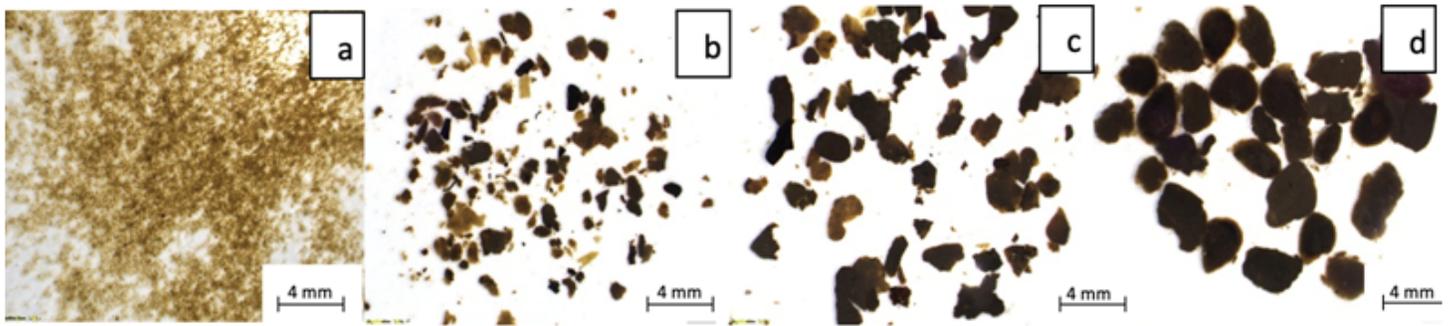
No	Measurements	Methods	Unit	Time
1.	Mixed Liquor Suspended Solid (MLSS)	1 hr drying at 105 °C	$\text{g L}^{-1}$	daily to weekly
2.	Mixed Liquor Volatile Suspended Solid (MLVSS)	15 min heating at 550 °C	$\text{g L}^{-1}$	daily to weekly
3.	Sludge Volume Index (SVI)	SVI was determined by reading the height of the settled bed after 5 mins settling and calculated from the settled bed volume and the MLSS in the bioreactor	$\text{mL g}^{-1}$	daily to weekly
4.	Chemical oxygen demand (COD)	Measured using HACH spectrophotometer (Method No. 8000)	$\text{mg L}^{-1}$	daily to weekly
5.	Phosphate ( $\text{PO}_4\text{-P}$ )	Measured using HACH spectrophotometer (Method No. 8048)	$\text{mg PO}_4\text{-P L}^{-1}$	daily to weekly
6.	Ammonia nitrogen ( $\text{NH}_3\text{-N}$ )	Measured using HACH spectrophotometer (Method No. 8038)	$\text{mg NH}_3\text{-N L}^{-1}$	daily to weekly
7.	Nitrite ( $\text{NO}_2\text{-N}$ )	Measured using HACH spectrophotometer (Method No. 8507)	$\text{mg NO}_2\text{-N L}^{-1}$	daily to weekly
8.	Nitrate ( $\text{NO}_3\text{-N}$ )	Measured using HACH spectrophotometer (Method No. 8039)	$\text{mg NO}_3\text{-N L}^{-1}$	daily to weekly

## Figures



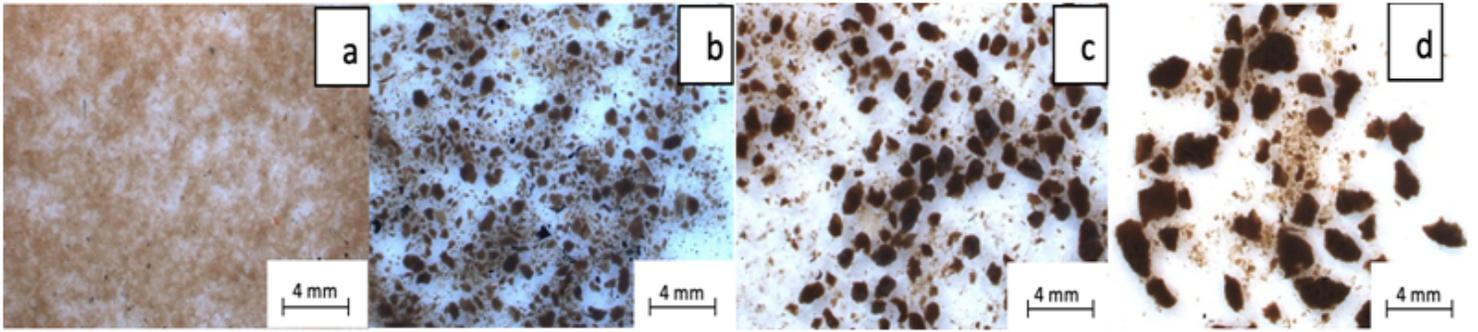
**Figure 1**

Pilot Sequencing Batch Reactor (SBR)



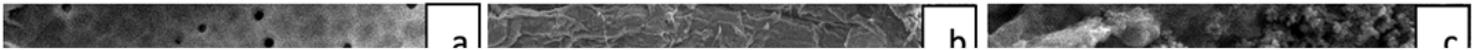
**Figure 2**

Evolution of seed sludge to AGS diatomite a) Day 0: Seed sludge, b) Day 10, c) Day 20, d) Day 30: Mature granules



**Figure 3**

Evolution of seed to AGS control a) Day 0: Seed sludge, b) Day 30, c) Day 60, d) Day 90: Mature granules



**Figure 4**

Images of a) Cavities; b) Extracellular polymeric substances (EPS); c) Coccus bacteria on the surface of granules.



**Figure 5**

Images of a) Single diatomite; b) centric-diatomite; c) pennate-diatomite in the inner part of granules.

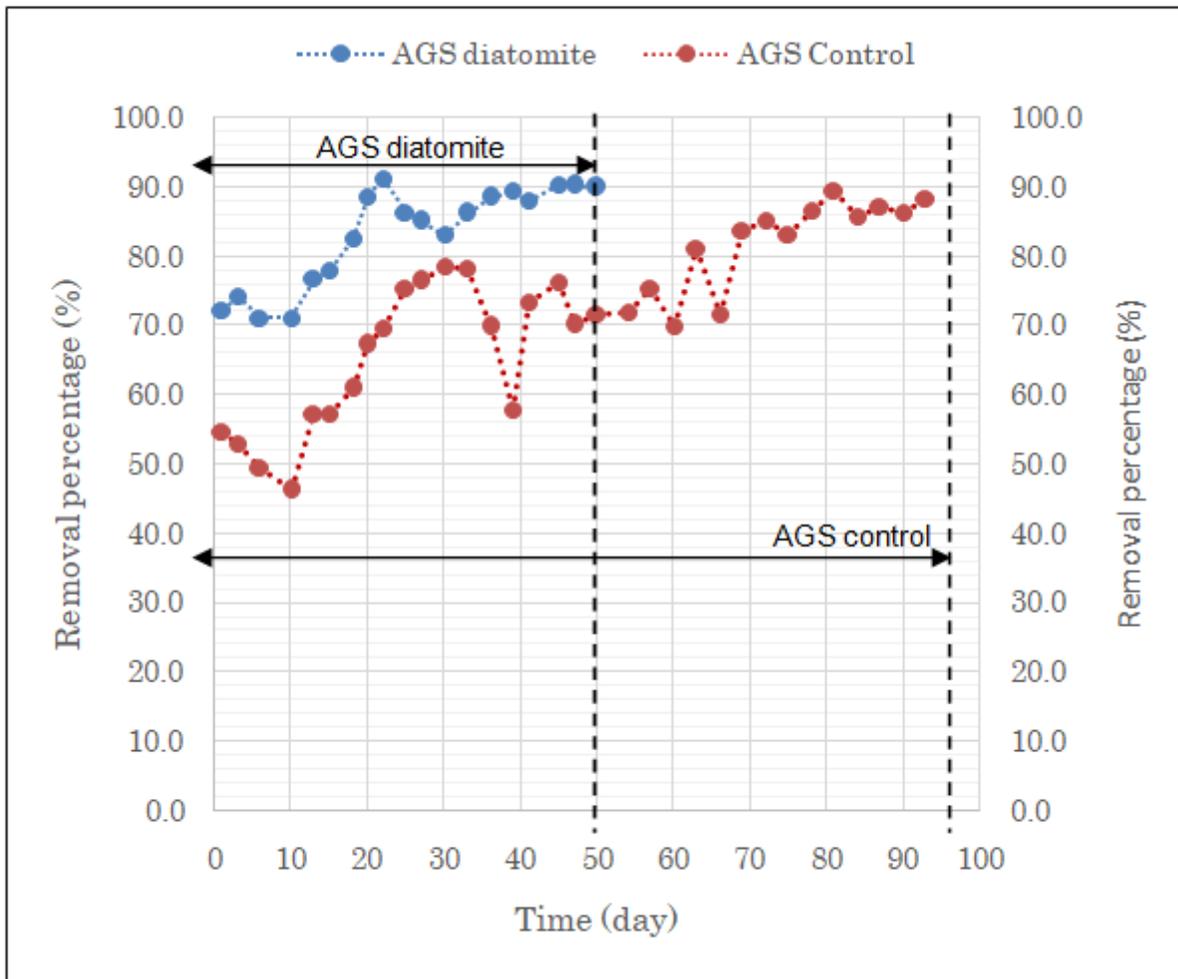
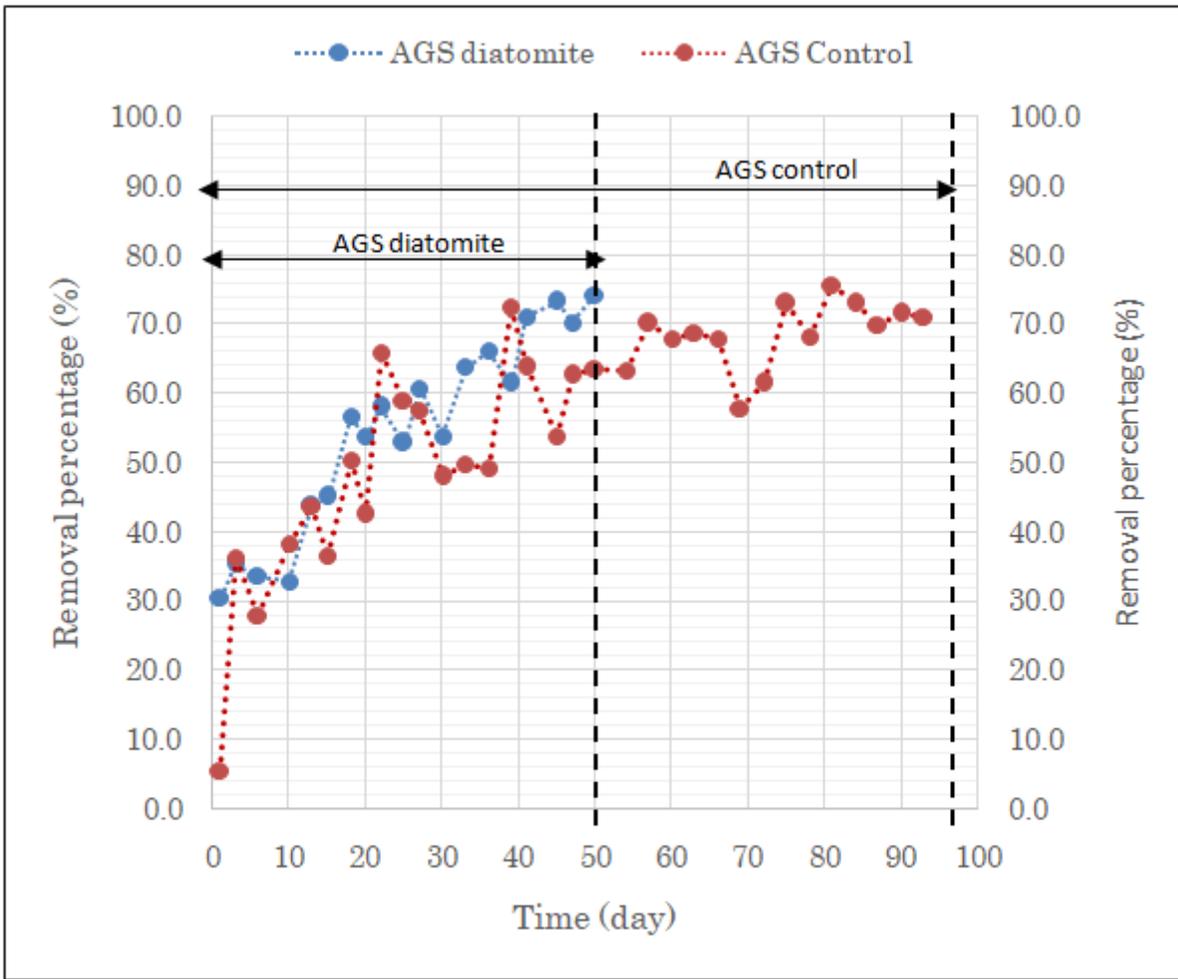


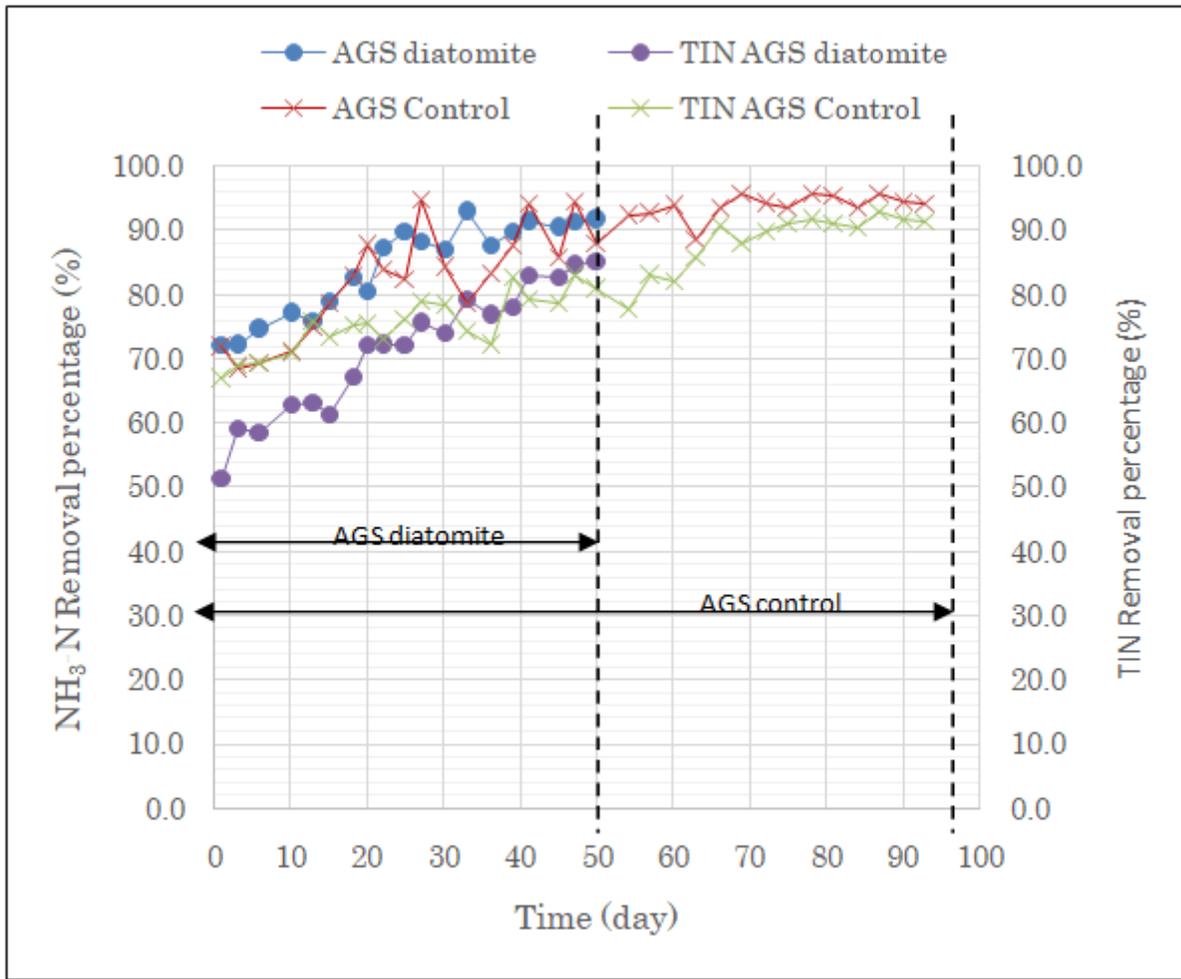
Figure 6

Removal Performance of COD



**Figure 7**

Removal Performance of Phosphate



**Figure 8**

Removal performance of Ammonia Nitrogen and Total Inorganic Nitrogen