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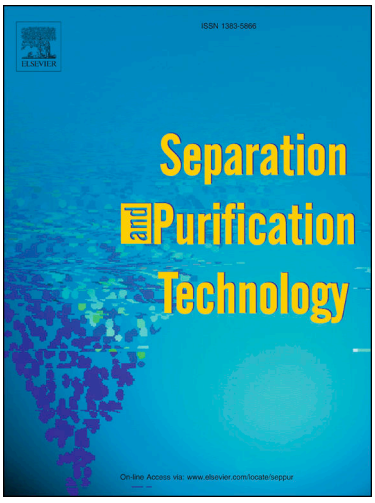

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PII: S1383-5866(20)32693-9
DOI: https://doi.org/10.1016/j.seppur.2020.118220
Reference: SEPPUR 118220

To appear in: *Separation and Purification Technology*

Received Date: 29 August 2020
Revised Date: 8 December 2020
Accepted Date: 13 December 2020

Please cite this article as: N. Shahidah Aftar Ali, K. Muda, M. Faiz Mohd Amin, M. Zuhaili Mohamed Najib, E. Henry Ezechi, M.S.J. Darwish, Initialization, enhancement and mechanisms of aerobic granulation in wastewater treatment, *Separation and Purification Technology* (2020), doi: https://doi.org/10.1016/j.seppur.2020.118220

INITIALIZATION, ENHANCEMENT AND MECHANISMS OF AEROBIC GRANULATION IN WASTEWATER TREATMENT

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Highlights of Review Article

- A comprehensive review on factors initiating formation of aerobic granules.
- Effective approaches adopted to enhance initial development of aerobic granules.
- Different mechanisms of aerobic granules development at initial stage were discussed.
- Natural polymer as a new approach in accelerating aerobic granules development.
- Aerobic granules fed with enhancer showed excellent properties and pollutant removal.

ABSTRACT

Aerobic granulation is a promising technology that has increasingly attracted global attention due to its potential as a cost effective system, and its application in a wide range of wastewaters. Aerobic granules are highly structured suspended microbial aggregates capable of degrading biodegradable compounds with excellent settling properties, good pollutant removal and high resistance to toxic compounds. However, evidences show that granules formation in high strength wastewaters takes relatively much longer time at initial stages. This article reviews the state-of-the-art of the researches done on the factors influencing granulation at initial stage, with particular focus on the aggregation of microbial cells. In addition, this review discusses the effective approaches adopted for the enhancement of initial development of aerobic granules. Moreover, the current article highlights the mechanism of aerobic granulation at its initial stage, as well as other different approaches. Finally, future research directions to improve aerobic granules formation at the initial stage are discussed.

Keywords: Aerobic granules, Aggregation, Enhancement, Initial Development, Mechanism

1.0 Introduction

Interest in biogranulation technology has constantly grown due to its unique characteristics, particularly in the treatment of a wide range of wastewaters. Research work on biogranulation technology has been extensively studied in wastewater treatment. Anaerobic granular sludge was successfully developed in upflow anaerobic sludge blanket (UASB) reactor since 1970 [1], while formation of aerobic granular sludge in sequencing batch reactor (SBR) was observed in 1997 [2]. Biogranulation process involves the aggregation of aerobic and anaerobic microorganisms through the interaction of their biological, physical and chemical properties [3]. These biogranules form a network of bacteria community that has the ability to degrade complex wastewaters.

The conventional wastewater treatment technology typically consists of ponding systems, evaporation and open or close digesting tank [4]. Although these methods are suitable for wastewater treatment in terms of cost and operation, they are considered less effective due to their major drawbacks including application of long hydraulic retention time (HRT), requirement of large working area and presence of large amount of undigested suspended solids capable of causing sludge bulking [5, 6].

Aerobic granulation is a promising wastewater treatment method that has several advantages over conventional activated sludge (AS) systems. Aerobic granulation systems can be operated at high organic loadings, short retention time, low energy consumption and low operational cost [7, 8]. Additionally, aerobic granules are strong, compact microbial structures which have excellent settling and high biomass retention

capabilities that enable them to withstand high recalcitrant organic loadings and toxicity from high strength wastewaters [8, 9]. Furthermore, land requirements, sludge generation, energy consumption and cost are significantly reduced by 50-75%, 20-25% and 23-40%, respectively under aerobic granulation system as compared to conventional activated sludge process [10]. Over the years, aerobic granulation systems have effectively treated low and high strength wastewaters.

However, the major drawback in aerobic granulation development is the long start-up period, which has remained unsolved and this lead to unpredictable granule morphology that influence aerobic granules properties (granular strength and stability), growth of irregular filamentous bacteria, scum formation and stable foaming, washout of slow settling biomass and inefficient nutrient removal capabilities [11-13]. Recent studies showed that granules took relatively longer times to be initially developed while treating high strength wastewaters. High strength wastewaters such as palm oil mill effluent (POME), livestock, textile and rubber wastewaters contain excess amounts of organic constituents (suspended solids, COD, BOD, oil and grease) and lignocellulose materials (lignin, cellulose, phenolic and humic acid). Indiscriminate disposal of improperly treated wastewaters has severe deleterious effects on the ecosystem. Fulazzaky et.al [12] noted that granules' formation did not occur after 190 days of palm oil mill effluent (POME) treatment. Pijuan et.al [14] also noted that aerobic granules were initially appeared after 133 days of operation. During the cultivation of aerobic granules at high suspended solids concentrations, Cetin et.al [13] indicated that smaller granules were formed on day 44 (0.6 mm). The concentration of Mixed Liquor Suspended Solids (MLSS) fluctuated due to the presence of high suspended solids, causing system instability and low pollutants removal. At stable period, smaller matured granules were cultivated and caused the

outgrowth of irregular filamentous granules [13]. Thus, focus on the initial process of aerobic granules' development is essential to reduce the long start-up period.

The overall granulation mechanism involves four stages, including cell to cell attachment, microbial aggregation, pre-maturation and post-maturation. The formation of aerobic granules starts from (i) 0-30 days; (ii) 30-60 days (iii) 60 – 200 days or more [10]. Each phase performed different mechanism as they are affected by different factors. In granulation, out of four stages, the first stage (cell to cell attachment) plays a major role in determining a successful development of aerobic granules. The factors that are involved in the first stage, also known as the initial stage, are identified as primary factors. According to Sarma et.al [10], the common factors that are responsible for the formation of aerobic granules are classified as primary factors. The initial stages of granulation are influenced by different primary factors such as surface hydrophobicity, EPS, charge neutralization and hydrodynamic shear force. During the initial granulation process, cell aggregations are likely to occur due to the factors that act as a possible mechanism to facilitate initial cell to cell attachment. Primary factors play a crucial role in the initiation of cell aggregation by strengthening the interaction between microbial cells, which, in further, leads to the attachment and formation of microbial aggregation at the second stage. It is essential to give more attention to the initial process of granulation, as this could help to promote rapid formation of aerobic granules by reducing long granulation time. In addition, this can assist in strengthening the initial development of aerobic granules in terms of structure and physical properties, which will be discussed in Section 3. Meanwhile, the factors that are less likely to be involved in the formation of aerobic granules are classified as secondary factors, because the granulation may occur even

without their presence. Secondary factors include organic loading rate (OLR) and solid retention time.

During the past decade, a number of review papers on granulation technology have been published, discussing factors influencing granulation [7, 15, 16], mechanisms [8], structural stability [17], application of biogranules in wastewater treatment and mathematical modelling of biogranules [18]. In addition, the main gap knowledge in granulation technology was addressed for better understanding of the granulation process [10]. In several reviews, operational conditions, such as settling velocity, shear force, settling time, biomass concentration and feeding strategy, have been suspected as key factors to promote the granulation. Nancharaiah and Kiran Kumar Reddy [8] discussed the formation mechanism of aerobic granules, where EPS and high selective pressure such as hydrodynamic shear force and feast/famine condition were selected as triggering factors that lead to biogranule formation. Besides, Kent et.al [17] reviewed the aerobic granulation formation in continuous flow reactors (CFRs), by considering settling velocity, hydraulic and shear force as main factors to accelerate the granulation process. Bengtsson et.al [16] and Winkler et.al [19] also addressed the operational conditions as parameters associated with the formation of aerobic granules. Rollemberg et.al [7] stated that selection pressure is the driving force for a successful cultivation of aerobic granulation.

To date, most recent studies indicated that formation mechanism of granulation is mainly dependent on environmental and operational parameters. However, there is a lack of comprehensive and critical review on the roles of microbial conditions toward the formation of aerobic granules. Apart from operation conditions, microbial conditions, such as EPS, surface hydrophobicity and bacterial strain, also play a major role in the

initiation of microbial aggregation, which are considered for a successful development of aerobic granules [20, 21]. Granulation is a complex phenomenon process, and the method of accelerating it involves numerous parameters, including seed sludge, exchange ratio and organic loading rate. It is clearly seen that the dominant factors influencing the initiation of aerobic granules are still unknown. Moreover, reviews highlighting the alternative approaches on the enhancement of aerobic granules are still limited. Up to the present, only Zhang et.al [22] reported the recent strategies that have been used to accelerate aerobic granules formation and their contributions to rapid granulation. Therefore, the purpose of this article is, firstly, to briefly review the primary factors associated with the formation of aerobic granules, and their influence on the initiation of cell aggregation in the granulation process, where microbial conditions are selected as triggering factors. Secondly, to assess the influence of alternative approaches in enhancing initial development of aerobic granules and their mechanisms. This review compared the main physicochemical characteristics of aerobic granules, as well as the different development approaches, and analysed their contribution to the rapid granulation. Future research directions in improving aerobic granules formation are suggested, which could be useful for a better understanding of the enhancement of aerobic granules development at initial granulation process.

2 Factors Affecting Initiation of Aerobic Granules Development

Formation of aerobic granules is a complex process influenced by a number of parameters. In many studies, it was illustrated that selection pressure is the major factor involved in successful aerobic granules cultivation. Some of the selection pressures, which primarily influence aerobic granulation development, include shear force [8], volumetric exchange ratio [23], settling time and aeration intensity [24]. Wastewater

characteristics are also important in the development of aerobic granules as they are difficult to be controlled in the reactor. On the other hand, organic loading rate (OLR) and substrates are more likely to influence the characteristics of developed granules, rather than the process development of aerobic granulation [3]. Very weak selection pressures such as low shear force and long settling time could hinder granulation development process. Relatively strong selective pressures are essential to initiate aerobic granules development.

2.1 Selection Pressure and Wastewater Characteristics

According to Nancharaiah and Kiran Kumar Reddy [8], hydrodynamic shear force is one of the major triggering forces in SBR for successful development of aerobic granules, which is beneficial for the structural formation of aerobic granules. Previous studies emphasized that high shear force is required for cultivation of aerobic granules as it contributes to the compactness and stability of aerobic granules [7, 8]. In addition, the presence of high shear force increases the secretion of EPS and cell surface hydrophobicity. The increased production of EPS at a high shear force could promote granules formation and produce stronger structure of aerobic granules. When the reactor operated with high shear force, compact and strong granules will be developed, thus lower the SVI value. In SBR system, shear force is represented by superficial upflow air velocity (SUAV). Several studies have shown that shear force under 1.2 cm/s is unable to produce granules with good properties. According to Tay et.al [25], loose and irregular biofilm were formed under low shear force of 0.3 cm/s and no granulation occurred as SVI value was increased to 170 ml/g at steady state. However, the study conducted by Tay et.al [25] differed from the findings of Lochmatter and Holliger [26], which reported the development of aerobic granules under shear force of 0.8 cm/s. Nonetheless, the structure

of aerobic granules developed were loose and irregular. Lot of researches claimed that strong and dense granules could be formed under superficial air velocity of higher than 1.2 cm/s. Based on the studies of Ab Halim et.al [27] and Ibrahim et.al [28], higher superficial air velocity of 2.1 cm/s and 2.3 cm/s, respectively, were able to develop aerobic granules with better settling ability. However, Bindhu and Madhu [29] stated that excessive increasing of shear force may not be effectives in enhancing removal performance of aerobic granules. The result showed that the increased of upflow air velocity from 3 cm/s to 4 cm/s did not give significantly impact towards the COD removal efficiency. Therefore, the selection of optimal shear force is essential in order to produce aerobic granules with excellent properties and maximum removal efficiency.

Another important factor for granules cultivation is the settling time. A number of studies indicated that short settling times are necessary for aerobic granules formation [24, 30, 31]. Linlin et.al [32] emphasized that short settling time is important for the formation of aerobic granules, because it determines the amount of sludge accumulation in the reactor. Qin et.al [24] indicated that, after 7 days, aerobic granules with a diameter of 0.35 mm were successfully cultivated in the reactor operated at 5-min settling time. The authors also claimed that it was difficult to develop aerobic granules at a settling time higher than 15 min. Aerobic granules with larger size appeared initially at settling time of 5 to 10 min. Bindhu and Madhu [29] stated that a settling time of 3 min can sufficiently produce compact granules with excellent settleability. However, too short settling time will cause accumulation of insufficient granules due to the wash-out of large amount of sludge at the initial stage of aerobic granules development. Similarly, prolonged settling time could cause the formation of flocculated biomass and retention of filamentous

bacteria in the bioreactor. This can disturb the settling ability of granules and cause failure to the granules development.

Many studies have shown the role of wastewater composition as key factors influencing the development of aerobic granules. The presence of divalent ion in wastewaters, such as Ca^{2+} and Mg^{2+} can promote aggregation by acting as nuclei, initiate bacterial attachment by acting as a bridge and further accelerate the initiation of aerobic granules development [33]. As reported by Gao et.al [34], Ca^{2+} produces larger aerobic granules with higher organic loading rate and is capable of inducing higher secretion of polysaccharides-EPS content. Due to their positive effects in improving the aerobic granules development, attempts have been made by several studies to supplement these divalent ions in the reactor, in order to enhance the aerobic granulation process as discussed in section 3.2. Further, according to Li et.al [35], the existing of chemical elements in wastewaters such as Fe, P and Si may contributes to microbial aggregation. Presence of Al and Fe are necessary to produce aerobic granules with excellent properties [36]. Si could increase the growth of microbial metabolism, build the foundation for the structure of aerobic granules and increase the strength of granules.

Despite the well-established factors that affect aerobic granules formation, there are complex processes that are involved during the initialization of bacteria aggregation, which are influenced by some primary factors, including bacterial aggregation strain, cell surface hydrophobicity, EPS secretion, surface charge and proton translocation. The effects of these factors on the mechanism of granulation processes are largely unknown. Bacteria is not likely to aggregate naturally due to the repulsive electrostatic forces and hydration interactions among it. It prefers to disperse, rather than aggregate, without force. Thus, these factors become the triggering force that is essential for the initiation of

cell aggregation by coalescing the bacteria and, further, make them aggregate. The formation and stability of aerobic granules are crucial for a successful granulation system.

2.2 Bacterial Aggregation Strain

Microbial aggregation is the process of coalescing bacteria cells that belong to the same bacterial strain (auto-aggregation) or to two or more different bacterial strain (co-aggregation). It is well established that bacterial strain (auto aggregation and co-aggregation) plays a major role in the development of aerobic granules at the initial stage. Auto-aggregation refers to the aggregation formed through the interaction between bacterial cells from same species, while co-aggregation refers to the interaction of bacteria from different species. Both bacterial strains have different characteristics and conditions that influence their ability to be classified as good and poor aggregators in the aggregation process. According to Chen [37], auto-aggregation ability is scaled by aggregation index. High aggregation index indicates high aggregation ability and denotes a strong tendency of the cells to agglomerate into an aggregate. Basically, high aggregation index is a result of high settling ability caused by increased cell size at early stage of granules development.

Auto-aggregation can be classified based on its aggregation strength. According to Rahman et.al [38], auto-aggregation ability can be classified into high, medium and low auto-aggregation. The aggregation ability decreases when auto-aggregation is high and increases at medium and low strain. *B.longum* strain is a bacterium with good aggregation ability. A study by Ibrahim et.al [39] classified the bacteria strain ability by aggregation behaviour such as sensitive, moderate and resistant. Besides, media compositions, pH and temperature can affect auto-aggregation [38, 39]. The auto-aggregation ability decreases when there are changes in pH and media composition.

Rahman et.al [38] observed that, high auto-aggregation strain at high temperature shows a significant decrease in aggregation ability.

Auto-aggregation can facilitate the aggregation of bacteria by enhancing the formation of microbial aggregates and contribute to the structural stability of cells. The characteristics of auto-aggregation strains are unique, indicating that the bacterial strains are species-dependent and have different aggregation capabilities at different growth stages. Besides, the behaviour of auto-aggregation is regulated in different ways. However, the characteristics of co-aggregation show that their relationship with bacteria is complex and varies at different growth times. According to Jiang et.al [40], bio augmentation of two co-aggregation strains has significantly improved the aerobic granulation development at initial stage. This could be attributed to the association of bacterial co-aggregation with an integral component of the granulation process.

2.3 Surface Hydrophobicity

Cell surface hydrophobicity plays an important role as a triggering force to initiate cell-to-cell aggregation during the initial stage of aerobic granules development. It is one of the most important forces in microbial aggregation that influences the bacteria to coalesce and aggregate. Previous studies demonstrated that cell surface hydrophobicity could induce, strengthen cell-cell interaction and initiate the granulation [20]. High cell surface hydrophobicity accelerates the formation of a denser structure and enhance microbial aggregation, particularly aerobic granules [3]. The increase of cell hydrophobicity could be influenced by the high shear force or hydraulic selection pressure imposed on microorganisms. Nevertheless, cell hydrophobicity is not sensitive to OLR. Based on the thermodynamic theory, the increase in cell surface hydrophobicity would decrease the excess Gibbs energy on the surface and, therefore, promote cell-to-cell interaction of

bacterial self-aggregation [41].

Several studies have investigated the factors that influence cell hydrophobicity, including starvation phase, addition of cations, type and concentration of substrate, pH, temperature, composition of the media, hydrodynamic shear force, settling time and others [8, 42, 43]. Under starvation period, the bacteria change their surface properties and become more hydrophobic, allowing them to coalesce and aggregate [20]. Cations have been examined for their influence on aggregation and cell surface hydrophobicity. Alias et.al [44] studied the effect of cations on the aggregation and cell surface hydrophobicity and found that the addition of Ca^{2+} increased aggregation up to 62%, and enhanced the cell surface hydrophobicity of aerobic granules. The aggregation and cell surface hydrophobicity of microbial cells increased with increasing ionic strength. Moreover, Ca^{2+} additions can cause a shorter starting period and a faster aerobic granulation process [3, 45]. This indicates that high cell surface hydrophobicity is important for the integration between microbial cells, and to form compact aggregates. Several studies have found that variation of microbial community structure in bacteria aggregation and contribution of extracellular polymeric substances affect cell surface hydrophobicity in aerobic granulation [20, 46]. The increase of cell surface hydrophobicity can improve the settling ability of sludge biomass and enhance the granulation process.

2.4 Surface Charge

Surface charge is one of the parameters that can contribute to microbial attachment or aggregations. In general, bacterial cell surface is negatively charged, and the microbial cell surface of the same charge undergoes electrostatic repulsion and hinder cell aggregation [20, 43]. Therefore, microbial aggregation can be enhanced by reducing the

repulsion between bacterial surfaces. Previous studies demonstrated that the addition of metal ions such as Ca^{2+} can neutralize negatively charged cells and facilitate initial cell-to-cell interaction [10, 20, 45]. Ren et.al [47] noted that the addition of Fe^{2+} and Fe_3O_4 can accelerate microbial aggregation by reducing the negative charges on bacterial surface while acting as nuclei for bacterial attachments. Moreover, the metal ions can facilitate the attachment between microorganisms, and promote microbial aggregation by decreasing and neutralizing the negatively charged microbial cells.

2.5 Extracellular Polymeric Substances

Extracellular polymeric substances (EPS) is an important component in aerobic granular sludge, which plays a major role in aggregating microbial cells and forming granules [10, 21, 48]. EPS are biopolymers secreted by microorganisms, which mainly compose of polysaccharides, protein, humic acid and lipids. Polysaccharides and protein are the predominant constituents of EPS [10, 30, 48]. EPS can influence microbial aggregation by acting as an adhesion to attach single bacterial cells, enhance aggregation and help aggregated cells to develop granules [49]. According to Sheng et.al [21], the interaction between EPS and microbial cells can induce the attachment of bacterial cell by closely binding the cells through ion bridging interactions, hydrophobic interactions and polymer entanglement, which serves to enhance and promote the formation of microbial granules, as shown in Fig.1. Liu and Tay [3] hypothesized that high shear force can enhance EPS production to produce compact and stronger structure of aerobic granules.

In general, besides hydraulic selection and OLR, the abilities of microbial communities such as *Zoogloea spp. and Rhodocyclales*, *Xanthomonadaceae*, and *Comamonadaceae* to produce EPS are well known. According to Fra-Vázquez et.al [50], it was observed that EPS in aerobic granules were highly secreted by *Brachymonas,*

Zoogloea and *Thauera* . Moreover, the study indicated that these bacteria were attributed to the granule structure and pollutant removal of aerobic granules. Studies by Szabó et.al [51] and Zhang et.al [52] claimed that, *Rhodocyclaceae, Xanthomonadaceae, Sphingomonadaceae, Meganema* and *Devosia* are highly associated with EPS secretion. Also, these EPS-producing bacteria are reported to be denitrifiers, which help in removing nitrogen in wastewater [53]. Therefore, it is clear that apart from being a good EPS producer, the microbial community also tends to play significant role in maintaining granule stability and is responsible for removal performance of aerobic granules, especially in nitrification-denitrification process.

EPS have been found to surround the microbial aggregates; they localize both outside the bacterial cell surface and in the inner part of the aggregates. Sheng et.al [21] stated that the structures of EPS are divided into two categories: bound EPS and soluble EPS. The bound EPS structure includes capsular polymers, condensed gel and microbial sheaths, whereas soluble EPS structure includes soluble macromolecules, colloids and slimes. Bound EPS are more likely to attach to the bacterial cells, while soluble EPS are weakly bounded with cells. However, Li and Yang [54] reported that soluble EPS could positively affect the microbial activity of aggregates, although soluble EPS are unattached to the bacterial cells. The structural layer of bound EPS consists of a tight bound and a loose bound. Tight-bound EPS (TB-EPS) are closely bounded with the bacterial cells, while loose-bound EPS (LB-EPS) only cover the outer layer of the cells. Yan et.al [55] claimed that, although the structure of LB-EPS contains less polysaccharides and protein compared to TB-EPS, LB-EPS have shown a greater performance impact on sludge flocculation, sedimentation and dewatering.

Cell adhesion can be enhanced by polymeric interaction with high EPS content,

but can be inhibited at low EPS content. Wang et.al [56] stated that low secretion of EPS may result in cultivation of a weak structure of aerobic granules. EPS content increases during the cultivation period of aerobic biogranule development, but remains constant at maturity and steady state conditions. Adav et.al [30] noted that high EPS production can increase aerobic granules stability and accelerate the aggregation process. According to Wang et.al [57], the increase of EPS is a key to promoting rapid granulation. Although EPS is important for the initiation of microbial aggregation, there are contradictory findings regarding the roles of protein and polysaccharides in EPS**.** This is due to both protein and polysaccharides are comprised of different functional groups and have different structures which then resulted to the different effects towards their roles in EPS. Torres et.al [58] observed a sharp increase in protein-EPS content as larger granules appeared. The protein content in EPS was found higher than polysaccharides during granulation process. Similar observations was evidenced by Campo et.al [59], where the proteins were dominant in EPS as compared to polysaccharides in aerobic granulation. McSwain et.al [60] showed that the protein content is rich in the core layer of granules, while polysaccharides are presented only in the outer layer of granules. During the separation of hydrophilic and hydrophobic EPS fractions, approximately 7% were hydrophobic that consisted of proteins, whereas the hydrophilic fraction contained more polysaccharides. Hydrophobic fractions of EPS, which are mainly proteins, could favour the granulation by reducing electrostatic repulsion between cells, neutralizing bacterial cell charges and increasing hydrophobicity of sludge, thus leading to development of compact and denser granules [21, 42, 48, 59]. This is because protein content has a high content of negatively charged amino acids and higher ability to facilitate interaction of electrostatic force towards multivalent cations compared to sugars [61]. Guo et.al [46]

reported that protein content contributed to high cell surface hydrophobicity compared to the polysaccharides. This is due to the presence of inactive hydrophilicity in polysaccharides.

In contrast, high polysaccharides content in EPS can facilitate cell-to-cell adhesion, bridge the bacterial cells into aggregate and strengthen the microbial structure by maintaining the structural integrity of granules in a community of immobilized cells [30, 62]. In addition, Sutherland [63] stated that polysaccharides play an essential role in the initiation of cell aggregates, as well as in determining the physical properties of the formed aggregates. Polysaccharides in EPS act as adhesion substances for binding purposes, and coalesce the bacteria to form bacterial flocs [15]. The polysaccharide polymers that attach on the surface of microbial aggregates can act as a bridge to link small aggregates, or aggregates at long distances, together to form larger sized granules. However, the removal or low amount of polysaccharides in EPS may affect cell adhesion and cause instability in the aggregation. Previous studies reported that bacterial alginate, one of the extracellular polysaccharides components, plays an important role in biogranule formation. Bacterial alginate improves development of aerobic granules by increasing the density and hydrophobicity of microbial cells [64, 65].

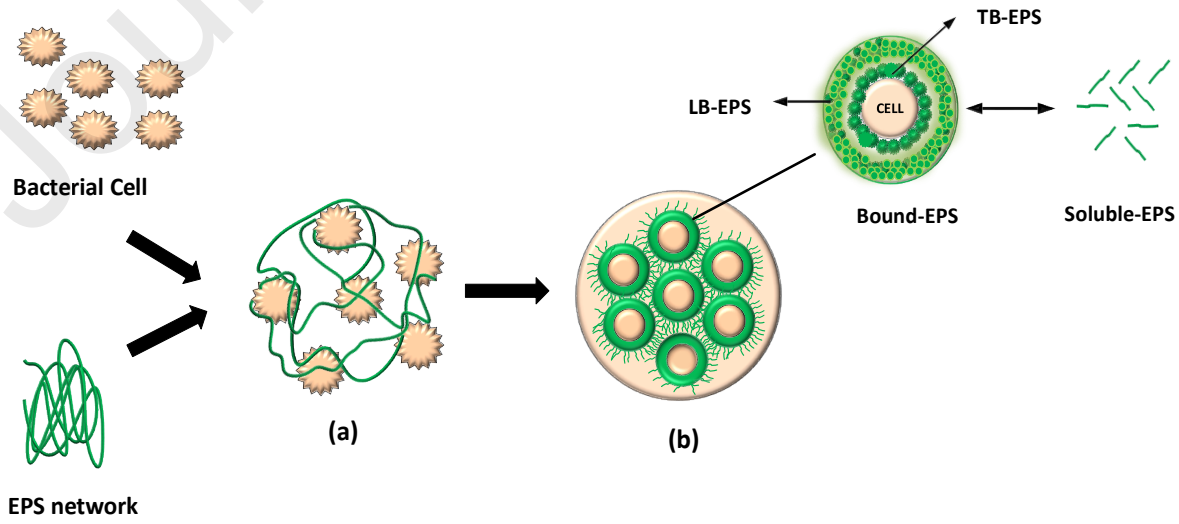

Fig. 1: Graphical representation of the influence of EPS to microbial aggregation (a) EPS act by bridging single bacterial cells. (b) Formation of microbial aggregation with further illustration of the structural layer of EPS.

2.6 Proton Translocation

Proton translocation involves the transfer of proton from the inner mitochondria membrane across the cell membrane to create a proton gradient [10]. Proton translocation theory is another mechanism that can influence the microbial aggregation and initiate granules formation. Based on this theory, activation of proton pumps involved in proton translocation across the cell membrane is due to substrates fermentation. The established proton gradient can cause cell surface protonation, neutralize the negatively charged particles and make the bacterial surface slightly hydrophobic through a dehydration process. According to Liu et.al [66], in proton translocating activity, hydrophobic interaction between bacterial surfaces is necessary for the initiation of bacterial adhesion. Proton translocations across a bacterial surface could induce dehydration of bacterial surfaces. During this phase, proton pumps on the membrane of fermentative bacteria are activated. Proton translocation activity energize the bacterial surface which cause breaking of hydrogen bonds between water molecules and negatively charged surface, neutralize negative charges and induce dehydration of bacterial surface.

Neutral and hydrophobic acidogens, acetogens and methanogens can interact and attach together to form microbial aggregates during the formation of embryonic granules. This scenario can be attributed to the weaker hydration repulsion [67]. Effective metabolites transference causes further dehydration of the bacterial surfaces and strengthen the cell aggregates at initial stage. Those aggregates which can obtain energy and nutrients from the environment are selected for the remaining biomass. The original

bacterial community continues to grow in the biogranule maturation stage, while dispersed bacteria will attach to embryonic granules [67]. Proton translocating activity keeps bacterial surface in mature aerobic granules at hydrophobic state during post maturation phase. At this stage, these activities are responsible for maintaining the structure of mature granules. This theory is applicable for microbial adhesion, as well as sludge granulation process.

2.7 Quorum Sensing

Quorum Sensing (QS) is a process by which bacteria cells communicate with each other and survive in continuously changing surrounding. The process of QS includes the monitoring of population density by bacteria and secretion of autoinducer molecules. Autoinducers are defined as signalling molecules, which control the bacterial gene expression depends on the cell density. According to Sarma et.al [10], acyl homoserine lactone (AHL) and autoinducer-2 (AI-2) are the major autoinducers that mainly involved in QS. Many studies have shown the importance of QS activity in granulation process. QS is known to be essential to the initiation of aerobic granules As reported by Tan et.al [68], high concentration of acyl homoserine lactone (AHL) were positively correlates with granules formation, as the AHL ranging from C4-C8 were observed to be increased up to 100-fold along with the aerobic granules development at initial stage. Further, it was found that the secretion of EPS was higher when the AHL concentration increases, with higher hydrophobicity, thus leading to the acceleration of microbial aggregation. In a study conducted by Ren et.al [69], it was observed that autoinducers release by the bacteria could initiate the bacterial cells attachment and positively affects the growth of aerobic granules. Moreover, it was indicated that addition of granular sludge (GS) intracellular substances could accelerate the granulation process. This is due to the GS

cellular extracts containing QS molecules (autoinducers), where it is able to induce the bacterial gene expression, resulting in the rapid cell attachment and formation of aerobic granules. In addition, the author stated that the higher concentration of autoinducers at initial stage could lead to the higher microbial attachment and fast aerobic granules formation. Nonetheless, the production of quorum quenching (QQ) enzymes has resulted in inhibition of QS activity, which in turn leads to delayed granulation processes, reduced EPS production and negatively affected the properties of formed aerobic granules [8, 10, 70]. Fig.2 shows the mechanism of aerobic granulation development at initial stage, which influenced by different factors.

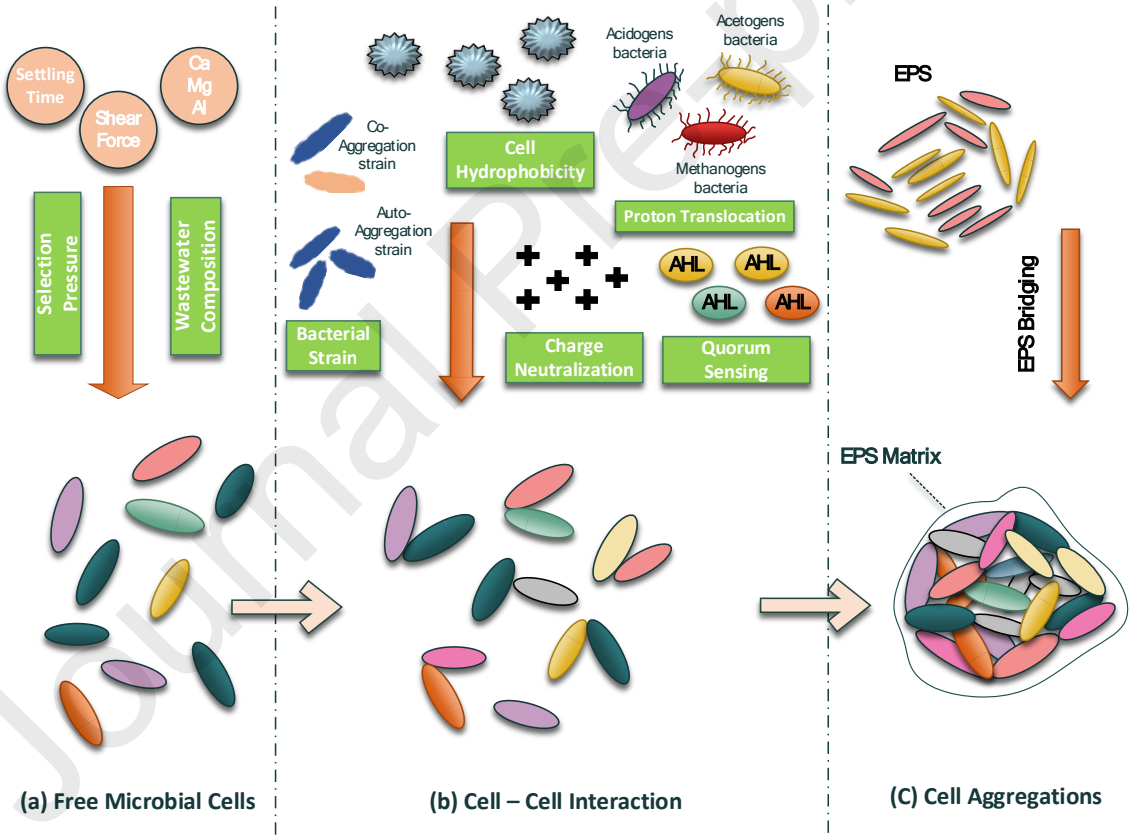

Fig.2: Graphical illustration of factors affecting the initiation of aerobic granules development

3 Enhancement of the Initial Stage of Aerobic Granulation Development

Some studies have attempted to enhance biogranule development through the manipulation of a number of reactor design parameters including aeration phase, shear force, volumetric exchange ratio and settling time. Recent studies have primarily focused on the improvement of the initial stage of aerobic granulation process. Various approaches have been investigated by researchers worldwide to achieve fast granules development. Application of additives including cations and synthetic polymers have shown positive effects on aerobic granules formation. Recently, research on natural polymeric coagulant as alternative approach in improving granulation system is increasing due to the advantages of natural polymeric coagulant over synthetic polymer and chemical additives [71]. Some approaches used selected sludge biomass, either in the form of aerobic granules, anaerobic suspended sludge or granulated biomass aiming at enhancing the aerobic granulation process. Additionally, different types of microbes were added to improve aerobic granules development process and increase the removal percentage of targeted pollutants in wastewater. The influence of magnetic field on the aerobic granules development has been relatively studied and the results show that the magnetic field positively affects the initial condition of aerobic granules, particularly on the percentage of aggregation and cell surface hydrophobicity.

3.1 Biomass

The performances of different biomass have been evaluated to enhance aerobic granules development at initial stage. Sludge including sewage sludge taken from municipal wastewater treatment plant, anaerobic granular sludge, aerobic sludge and dewatered sludge were used to improve the start-up period of aerobic granules development [23, 57, 72]. Aerobic granules can be successfully cultivated with different kinds of sludge. The

bacterial community in sludge is important for granulation process, in which the presence of hydrophobic bacteria can strengthen the formation of aerobic granules. Addition of anaerobic granular sludge in aerobic granulation can cause better sludge settling and proper granule formation. Muda et.al [73] demonstrated that anaerobic granular sludge can initiate the formation of aerobic granules. After 30 days, aerobic granules seeded with anaerobic granules formed a structure containing patches of fragmented anaerobic granules with an average size of 0.02 ± 0.01mm. The application of anaerobic granules could greatly reduce the start-up period of granulation process. Verawaty et.al [74] demonstrated that mixture of crushed granules with flocculated biomass as seed sludge significantly reduced the start-up period of aerobic granulation system and maintained the nutrient removal performance. However, aerobic granules formed under these conditions can destabilize and be washed out before a new granulation occurred.

Coma et.al [75] reported that aerobic granules were successfully cultivated in 40 days with 10% of crushed granules as seeding and the granules formed at early stage had an average diameter of 0.2 mm. Long et.al [76] used 25% of mature aerobic granules as a seeding material and successfully developed aerobic granules in 4 days. The addition of mature granules has greatly shortened the granulation time. Nevertheless, Bashiri et.al [72] found that the granules initially appeared on day 52 when pre-formed aerobic granules was used to reduce granulation time. However, granules had poor structure due to the tension present in the reactor. Krishnen et.al [77] seeded a pilot scale granular system with sludge from sewage and pineapple wastewater. The average size of the developed granules was 0.2 mm on day 60. The granulation had the longest time to cultivate aerobic granules, due to the longer start-up time required in pilot scale reactor, compared to lab scale reactor. Wang et.al [57] claimed that seeding dewatered sludge

could accelerate aerobic granulation. Small granules initially appeared after 2 days with average size of 0.5 mm and the granulation completed within 5 days.

Applying different types of microbes is another strategy used to improve the initial stage of aerobic granulation development. The addition of microbes can enhance cell aggregation by facilitating the interaction between cells, and forming compact and stable granules. However, in practice, this can be costly, and the accumulation for the specified pure culture is complex. Ivanov et.al [78] noted that the application of selected bacterial strains with high cell aggregation can accelerate and enhance the formation of microbial aggregates. Aerobic granules were cultivated with the addition of *Pseudomonas veronii* strain F having a self-aggregation and co-aggregation index of 51 % and 58 %, respectively. Aerobic granules were successfully formed after 3 days, indicating that adding microbial strains with higher aggregation abilities can reduce the granulation time from several weeks to 3 days. In addition, Ivanov et.al [79] noted that the augmentation of *Pseudomonas veronii* strain B species with an aggregation index higher than 50% could reduce the granulation time to 3 days.

Adav and Lee [80] reported that aerobic granules were successfully cultivated from single-bacterial strain, *Acinetobacter calcoaceticus.* The aerobic granules were initially formed on day 7 of operation with a diameter of 0.7 mm. The granules demonstrated a good settling ability and were able to degrade phenol effectively. The effect of *Rhizobium sp.* and *Shinella granuli* co-aggregative strain on the production of aerobic granules in pyridine wastewater was examined by Liang et.al [81]. After 42 days, the mixture of both strains showed strong co-aggregation index of 62%, produced granules with a mean size of 0.2–0.5 mm and produced higher EPS content compared to other bacterial strains. Combination of *Rhizobium sp*. and *Shinella granuli* as seeds could

enhance granulation due to the high aggregation index of both strains. Nevertheless, there are other bacterial strains that cannot produce granules with excellent properties or take longer time to form granules. The addition of mixed bacterial culture could only produce aerobic granules with an average size of 0.02 mm after 7 days of operation [28]. Granules fed with *Rhizobium* sp have the longest cultivation time of 120 days [82]. Thus, this shows that fast granules development was largely dependent on the involved bacterial strains. Table 1 shows details information on the aerobic granules formed with addition of granules, sludge and microbes for rapid granulation.

As discussed in section 2.5, microbial community especially, *Zoogloea spp. and Rhodocyclales*, are associated with EPS production and able to store poly-b-hydroxyalcanoates (PHA) in the presence of high organic loading, and thus promote formation of granules. PHA confers higher density and settling velocity to bacterial cell, in which their storage is highly dominated by *Zoogloea spp.* However, biomass consisting of more filamentous bacteria can form slow-settling fluffy aggregates. This can cause deteriorations to the settling properties of granules, poor nutrient removal performances at early stage and dephosphatation.

Fig.3 shows the mechanism of microbial aggregation with addition of two bacterial strains. Microbial aggregation requires secretion of both EPS and the second messenger cyclic diguanylate (c-di-GMP). EPS act as important mediators in the adhesion of bacteria onto carrier surface or other bacteria during aggregate formation. The combination of both *Rhizobium sp.* and *Shinella granuli* co-aggregative strains increases the protein and polysaccharides content in EPS and promotes microbial aggregation during pyridine degradation. The first stage of microbial aggregation involves the secretion of c-di-GMP through the activity of diguanylate cyclases (DGCs). The

concentration of c-di-GMP is highly dependent on this activity. Then, FleQ (master regulators of the flagella gene) acts as a transcriptional repressor and binds with c-di-GMP to form polysaccharides (PS). After c-di-GMP binding, FleQ occupies a different promoter region and activates PS operon transcription. Besides, the c-di-GMP regulates PS production in the post-translational level. The velocity, flexibility and precise control of c-di-GMP regulation represented great advantages in PS synthesis and secretion systems. The increased secretion of PS and c-di-GMP promoted microbial aggregation, which is beneficial for the aerobic granulation process.

Another perspective involving the mechanism of biomass is anaerobic granules used as seeding materials. Anaerobic granules may undergo a process of disintegration during initial process of aerobic granules development. Fig.4 shows the process of aerobic granulation development seeded by anaerobic granules. At an early stage, the regular structure of anaerobic granules begins to shrink and disintegrate (Fig.4 (a, b)). This may be attribute to the outgrowth of filamentous bacteria in the reactor which resulted to the formation of loose and unstable granules. This condition led to the granules broke up into pieces and washed out from the reactor. Another possible reason associated with the disintegration of granules is due to the shear force which caused by high aeration applied in the reactor. At this phase, the granules have changed in colour from black into smaller grey granules. Next, new granulation occurs where granules are mainly dominated by aerobic microorganism in the presence of anaerobic patches (Fig.4 (c)). These anaerobic patches acted as the seeding for the microbes to clump together and gradually grow bigger and finally form compact aerobic granules (Fig.4 (d)). The interior of the granules is darker and consists of small fragments of anaerobic granules with various sizes and shapes. On the other hand, the exterior of granules is light brown in colour and consists

of aerobic bacterial that was newly attached to the patches of anaerobic granules seeding during granulation development process. The changes of colour to lighter brown shows that the aerobic granules are no longer dominated by anaerobic microorganisms.

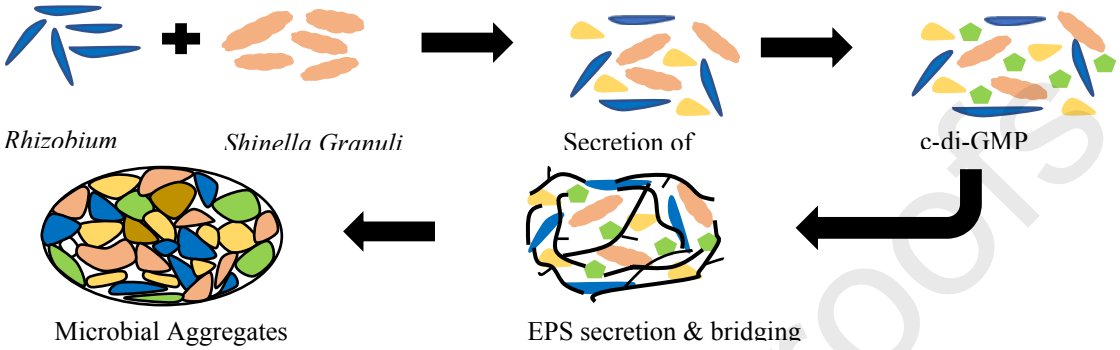

Fig.3: Microbial aggregation with combination of both co-aggregative strains [81]

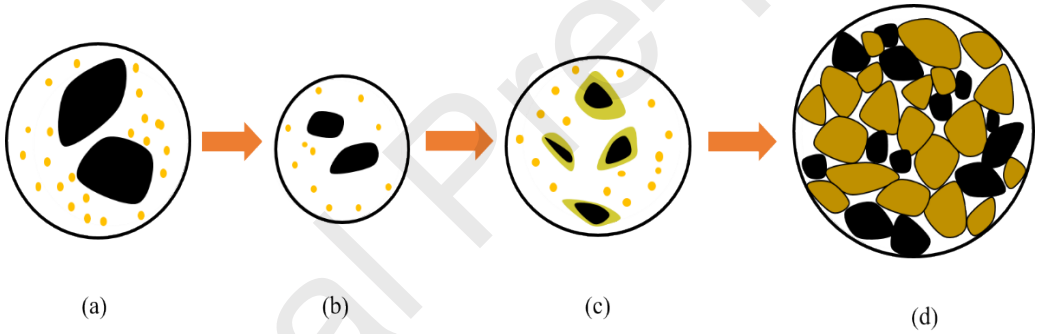

Fig.4: The illustration of aerobic granulation development seeded with anaerobic granules: (a, b) anaerobic granules shrink and disintegrate, (c) aerobic microorganisms become dominant and (d) formation of compact aerobic granules

3.2 Cations

Microbial agglomeration is initiated by bacterial adhesion through physical cell-to-cell interaction to form granules under suitable conditions. The presence of cations is an important factor that strengthens the granulation through the strong effect of the ions on the self-immobilization of microbial biomass during the start-up of aerobic granulation

process. Cations may facilitate the granulation process by bridging the negatively charged groups on cell surfaces with EPS. Ion bridging between cations and EPS is essential in bacterial aggregation. Multivalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} tends to bridge with EPS, enhance the agglomeration process and eventually form stable complexes. Previous studies, as shown in Table 2, indicated that addition of divalent metal ions, particularly Ca^{2+} and Mg^{2+}, can accelerate aerobic granulation process and decrease the start-up period [45, 83]. Calcium ions enhance granulation by neutralizing the negative charges on the bacterial surface, interconnect bacterial surfaces with EPS by acting as a bridge, accelerate microbial aggregation and increase physical strength of granules [84]. Ren et.al [33] noted that addition of Ca^{2+} improved granulation and shortened the time from 32 to 16 days. Aerobic granules augmented with Ca^{2+} develop faster and granules show a strong and dense structure at steady state [45].

Li et.al [85] reported that addition of Mg^{2+} can accelerate aerobic granulation and increase microbial diversity of aerobic granules. However, according to Liu et.al [45], Mg^{2+} has a weak effect on the structure of aerobic granules and unsuitable for bridging functions. Granular sludge has been successfully developed with the addition of Fe^{2+} and Fe_3O_4. The ions promote aerobic granules formation by decreasing the negatively charged microbial cells, contribute to extracellular polysaccharides production and act as nuclei for bacterial attachment. The addition of Fe^{3+} could accelerate microbial aggregation by promoting EPS secretion [86]. Despite the wide usage of these chemical additives, their application is correlated to several drawbacks including high cost, health related issues and production of voluminous sludge. The presence of high concentration of Fe^{2+} could damage the granulation environment, reduce bacterial activity, induce negative effect on the biomass and cause granules degeneration.

The mechanism of cations involved in the initial development of granules is complex due to the varying effects of different charges of cations on granulation development. Positive charges on multivalent cations can neutralize all negative charges on the bacterial surface and accelerate granules development. Addition of cations mostly effects the production of EPS and creates binding site for various EPS compounds when different types of cations are added in the granulation system. Calcium ions (Ca^{2+}) has a strong ability to attach with polysaccharides through OH (hydroxyl) groups, due to their reduced hindrance compared with that of proteins [83]. The COO (carboxyl) groups of humic acids do not produce any effect when they interact with Ca^{2+}, due to their small value compared to polysaccharide and protein. In contrast, Mg^{2+} has a strong interaction with amide group of protein, which shows depressed behaviour towards bonding with polysaccharides. Differing reaction between both divalent cations in bonding with polysaccharides and protein may be influenced by the size of metal ions. Basically, larger size Ca^{2+} could easily interact with OH groups of polysaccharides due to its availability compared to amide group of protein. The amide group of protein is surrounded by a carbon atom attached with high molecular weight alkyl or aryl group. Primarily, it is difficult for larger size Ca^{2+} to interact with protein due to this steric hindrance. Therefore, Ca^{2+} initially bonds with polysaccharides and secondarily with protein. Meanwhile, smaller size Mg^{2+} fits well with amide group of proteins, where there are no obstacles in bonding with nitrogen of amide groups in protein. Light metals are retained in protein, whereas heavy metals retain polysaccharides due to the structure of EPS. Fig.5 shows the illustration of EPS mechanism with addition of metal ions. In contrast, combination of both Mg^{2+} and Ca^{2+} as additives could cause a decrease of polysaccharides production. Mg^{2+} causes ion exchange with Ca^{2+} inside the granules, which affects the interaction

between Ca^{2+} and polysaccharides. Nevertheless, augmentation of Mg^{2+}, Ca^{2+} and Fe^{3+} together could increase the granulation [86].

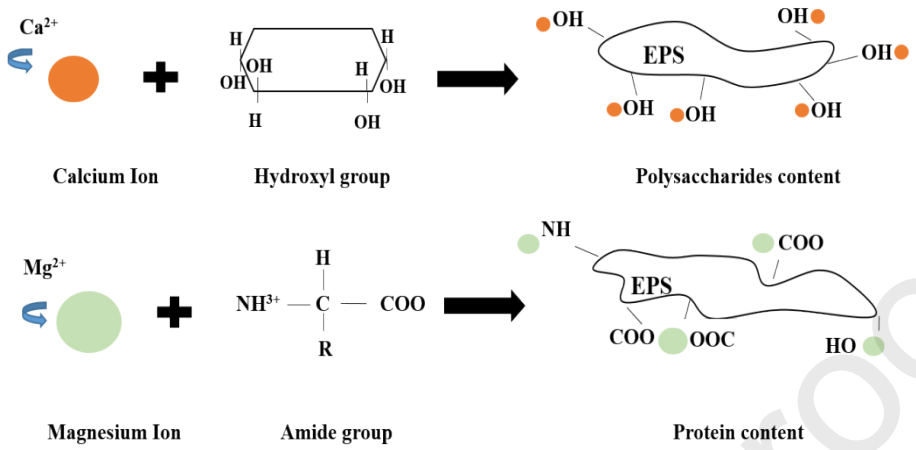

Fig.5: Mechanism of EPS with presence of Ca^{2+} and Mg^{2+}

3.3 Magnetic Fields

Magnetic field plays a major role in particle aggregation by manipulating the positive and negative charges of bacterial cells. In general, the particle charges are well separated and easily aligned in the direction of the magnetic field. This allows the magnetic field to strengthen the electrostatic force and polymeric interaction, which, therefore, enhances the cell adhesion. Moreover, the application of magnetic field could enhance the biomass settleability in wastewater treatment and accelerate the development of aerobic granules at initial stage by increasing the cell surface hydrophobicity. Table 3 shows the effects of adding magnetic field on the formation of aerobic granules, particularly in terms of size and structure of granules at initial stage. According to Omar et.al [87], magnetic field of moderate field intensity could positively influence particles aggregation. The percentages of cell surface hydrophobicity and aggregation were reported to increase to 54% and 90.4%, respectively, after being exposed to magnetic intensity of 48 and 10 hours, respectively. Wang et.al [88] noted that static magnetic field could reduce the long

granulation time from 41 to 25 days, by enhancing the settling properties of granules and stimulating the secretion of EPS. Omar et.al [89] demonstrated that static magnetic field intensity of 20 mT suitably decreased granulation time by 20 days and produced granules with good properties.

The four factors that contribute to the application of magnetic field include magnetization of magnetic field, magnetic gradient, Lorentz force and magnetic memory. At magnetization of magnetic field, molecules consist of positive and negative charges. Molecular substances can be categorized as polar or nonpolar. Polar molecules are difficult to attach to each other in the absence of magnetic field, even though collisions between molecules occur. Non-polar molecules randomly attach to each other without the presence of magnetic field. This could prevent the occurrence of coagulation. However, when polar molecules are influenced by magnetic field, they easily align in accordance with their charges and direction of magnetic field. When the molecules are in arrangement, coagulation and aggregation occur.

With regards to Lorentz force, it can influence the mechanism of magnetic field. Lorentz force could affect charged particles, which increases linearly with particle charge, velocity and component of magnetic field strength. Lorentz force is produced when charged particles flow in a direction perpendicular to the magnetic field in the same plane. Aggregation of particles usually occurs when the particles become unstable, and their surface charge is displaced from original position, causing them to collide with each other in order to form aggregates. Additionally, magnetic memory helps the particles to sustain their magnetization properties after exposure to different intensities of magnetic field [90].

3.4 Inert Carrier

Activated carbon has been successfully applied in various types of wastewaters. Rapid granulation of aerobic granules could be achieved by using activated carbon as support materials, as shown in Table 4. This is due to their characteristic that have fast settling velocity and large surface area which could initiate the formation of granules although under unfavourable conditions, such as low organic loading rate [91]. Li et.al [91] investigated the effect of granulated activated carbon (GAC) on the formation of aerobic granules under low OLR. The study was conducted in two phases and there was no granulation occurred in phase I. By adding 0.2 mm GAC, small granules with diameter of 0.15 mm was visible after 10 days in phase II and full granulation was completed after 20 days despite the low strength influent. However, it was observed that the granulation is quite difficult to be achieved in the reactor with no GAC addition. A similar, but more extensive, study was conducted by Li et.al [92] for partial nitrification treatment of ammonia-rich wastewater. The result showed that dosing 0.2 mm GAC has successfully shortened the granulation period from 42 days to less than 21 days. The granule was initially appeared on day 14 with size larger than 0.2 mm. GAC facilitated the cell aggregation by providing the core for the attachment of bacterial cells thus accelerating the formation of aerobic granules. At maturation phase, GAC addition led to the formation of larger granules with an average diameter of 0.36 mm. On the other hand, slow formation of granules was observed when powdered activated carbon was added in the reactor.

Omar et.al [93] studied the effects of magnetic activated sludge (MAC) on the granulation development at initial stage. Cell surface hydrophobicity was used as an indicator for microbial aggregation. It was reported that dosing 3.08 mg/L of magnetic

activated carbon in SBR has increased the cell surface hydrophobicity by 56%. Higher cell surface hydrophobicity will cause an increase in cell aggregation, which further accelerates the granules formation. It is summarized that addition of MAC was an effective strategy due to its ability to increase the aggregation rate and cell surface hydrophobicity of microorganisms by acting as nuclei. Based on the finding by Tao et.al [94], GAC addition produced granules with excellent physical properties. Aerobic granules initially appeared on day 21^{th} with size increasing from 0.1 mm to 0.5 mm and were fully granulated after 71 days of operations. Also, the mature granules reached a maximum size of 0.6 mm, which higher than granules cultivated in the reactor that run without GAC. This shows that GAC has the ability to enhance aerobic granulation by providing strong core to the granules and reduce their compaction during granulation process.

The mechanism of granular activated carbon (GAC) is still unclear though GAC has demonstrated promising enhancement that has a potential for initial aggregation of microbial particles. The mechanism of granulation development involves three phases: lag, granulation and granule maturation phases [91, 95]. During the lag phase, GAC acts as a supporting medium and provides nucleus for bacterial attachment. At this stage, GAC can increase collisions between aggregated strain and cause a decrease in SVI. During the following phase; granulation phase, GAC can initiate the aggregation of bacterial cell through physical interactions. As a strong nucleating agent, GAC decreases the double layer repulsive potential on bacterial surface and facilitates the aggregation to withstand high shear force. Once the bacteria agglomerated, the attached particles start to grow until larger aggregation is formed. However, aerobic granules development through the addition of GAC is unlikely to be affected by biological and chemical processes. In fact,

GAC has no effect on the bacterial community in granules and is unlikely to initiate the aggregation through chemical interactions. At maturation phase, GAC is no longer needed after the granules attained steady state and became stable.

The GAC size, which is used as a supporting media, is important for a successful development of aerobic granules at initial stage [95]. GAC of suitable sizes can serve as the nucleating agent to accelerate cell aggregation. Previous studies demonstrated that GAC size of 0.2 mm can effectively facilitate initial microbial aggregation. In contrast, no microbial attachment was observed when GAC size of 0.6 mm was added [95]. An appropriate GAC size is required to initiate aggregation and form granules. Larger GAC size is unfavourable due to the disruption of attached flocs, disintegration of granules and inhibition of flocs-GAC co-aggregation. However, too small size of GAC, particularly in the form of powder, could be easily washed out from the reactor along with poorly settled bacterial cells at the initial process of microbial aggregation. Fig.6 shows the effect of GAC size during initial process of microbial aggregation.

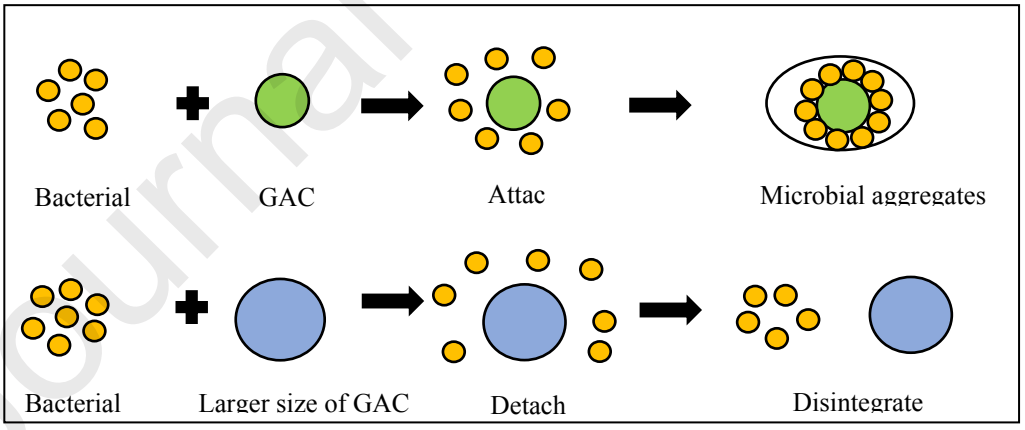

Fig.6: Aggregation of flocs-GAC at initial process of granules development

3.5 Polymer

Various types of polymers have shown potential application in water and wastewater treatment. Polymers help to initiate the flocculation of colloidal particles so that it can be

separated from wastewater in a relatively easy manner. Addition of polymers (synthetic and natural) can enhance the agglomeration process by interacting and bridging the bacterial cells at the initial stage of granules formation [96, 97]. Polymers are primarily used in the reactors to immobilize sludge, enhance the strength of granules and improve the mechanism process at the early stage of granulation development. With the application of polymers, the drawbacks of chemical additives in aerobic granules development can be essentially overcome.

3.5.1 Synthetic Polymer

Synthetic polymers have been widely used in wastewater treatment to enhance agglomeration process, as summarized in Table 5. Several polymers such as polyacrylamide (PAM), polyaluminium chloride (PAC) and polyhyrdoxybutyrate (PHB) have been used to accelerate aerobic granules development in wastewater treatment [96, 98, 99]. Polymers enhance agglomeration process, increase the flocs size and promote floc removal during the wastewater treatment process.

Polyaluminium chloride (PAC) can potentially accelerate sludge granulation process. PAC promotes rapid granules formation in less than 7 days. The granules have good sludge characteristics in terms of settling ability, biomass retention, high EPS and nutrient removal [97, 99]. Liu et.al [100] found that granulation time significantly decreased from 17 to 7 days when PAC was added. The granules were visible with an average size of 0.1 mm - 0.55 mm, which increased to 3.5 mm on day 50 with about 10% visibility. However, the granules that appeared on day 7 had 3% of its sludge larger than 2.5 mm [97]. Meanwhile, Nasrullah et.al [99] reported that addition of PAC enhanced the surface area of flocs and increased the settling ability. Applying PAM and PHB can enhance microbial agglomeration at the initial stage of granulation. Addition of these

polymers could increase the surface charge, cell surface hydrophobicity, EPS content, produce compact structure and show good removal performance of pollutants [96, 98]. This indicates that synthetic polymer is a suitable enhancer for granules formation. However, synthetic polymers exhibit several drawbacks due to their neurotoxic and carcinogenic nature. Kerr et.al [101] reported that both anionic polyacrylamide and cationic polymers are highly toxic and unsafe for aquatic invertebrates and fish. Synthetic cationic polymers accumulate in fish gills, hence interfere with gill function and ion regulation, which causes fish death and reduces the supply of healthy fish for consumption. As a result, regulatory authorities have restricted the use of chemical polymers in various industrial applications.

There are four different mechanisms in synthetic polymers that can accelerate microbial aggregation including polymer adsorption, polymer bridging, charge neutralization and sweep flocculation [102]. Polymer adsorption occurs when the interaction between polymer segment and particle surface involves other types of interactions, such as electrostatic interaction, hydrogen bonding and ion binding. In electrostatic interaction, cationic polymers with positive charges easily interact with negatively charged particle surface, due to the attraction between opposite charges. Hydrogen bonding occurs when a polymer such as polyacrylamide and polyethylene oxide contains hydroxyl group and interact with particle surface by forming h-bonding. Ion bonding usually occurs in anionic polymers and interact with negatively charged particle surface without repulsion with similar charges. Adsorption occurs with the addition of cations, which acts as bridges to support the interaction between a polymer and the bacterial surface.

Polymer bridging involves the attachment of long chain polymers on colloidal particles, linking the particles together, which consists of loops and tails. Bridging mechanism can produce strong and large flocs under high shear conditions. Aggregates formed by polymers are more resistant to breakage. At low polymer dosage, charge neutralization takes place to stabilize the particles. Generally, charge neutralization is the mechanism that uses cationic polyelectrolyte to neutralize the negatively charged particles. Cationic polyelectrolytes are considered effective flocculants due to their capability to stabilize colloidal particles by decreasing the repulsion between them, as well as achieving zero zeta potential. Nevertheless, at sufficiently high dosage, a mechanism called sweep flocculation (Fig.7) likely occurs. This mechanism occurs through the precipitation of metal Hydroxide (M (OH_3)), due to the addition of metal salt to water at sufficiently high concentration. This causes colloidal particles to be enmeshed in these precipitates. Hence, this mechanism eliminates suspended particles that are harmful for mesh composition because of the superior rate of aggregation.

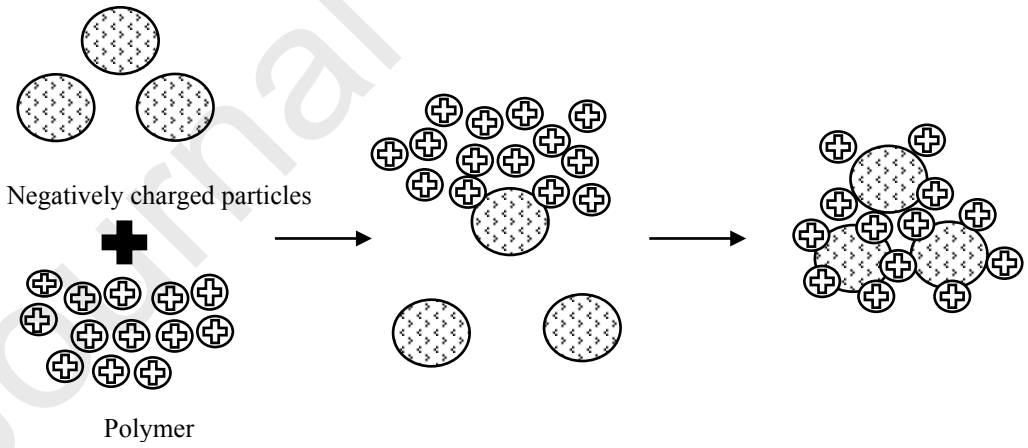

Fig 7: Mechanism of sweep flocculation

3.5.2 Natural Polymer

Due to global concerns over the harmful effects of synthetic coagulants, the application of natural materials has become a promising solution. Recently, more attention was paid

to the application of natural polymers, because of their advantages that outweigh synthetic polymers. Natural polymers are highly biodegradable, non-corrosive, generate less amount of sludge, environmentally friendly and cheap. On the other hand, they are rarely abundant. Several studies have demonstrated that green polymeric coagulants extracted from plants, animals and microorganisms such as chitosan, *Moringa Oleifera*, *Cicer arietinum*, *Jatropha Curcas* seeds and dragon fruit foliage can be used in wastewater treatment [58, 103-106]. The addition of coagulant extracted from nature can reduce the cost and enhance quality effluent in terms of turbidity removal. Table 6 evaluates recent studies of natural coagulants applied in treating different wastewaters and their effectiveness. These natural coagulants are efficiently used in treating wastewater containing high turbidity, COD and other organic pollutants. Most of the studies used higher pH values in the range of 6-9 that can influence COD reduction and decolourization efficiency.

Chitosan is an important natural polymer that is widely used in the treatment of water and wastewater. It has unique properties such as high cationic charge density, long polymeric chains and bridging of aggregates and precipitations. However, based on previous studies, it has been observed that chitosan is only added in anaerobic granulation system. No studies have been reported on the addition of chitosan in aerobic granulation system. Application of chitosan can effectively enhance anaerobic granules development by increasing the production of EPS, accelerate bacterial agglomeration at the initial stage of granulation and produce dense and compact granules [58, 96]. Based on Table 6, it is clear that application of natural coagulant was effective for the removal of wastewater pollutants. Chitosan was found to reduce start-up period, improve UASB reactor performance, enhance biogranule formation (average diameter 2 mm), produce high EPS

content, exhibit high settling velocity (35 m/h) and achieve high COD removal (90%) [58]. The usage of chitosan in anaerobic microbial granulation increased EPS production by 50% [96]. Application of chitosan mushroom for POME treatment achieved 90% total suspended solid and residual oil removal [107]. In addition, utilization of chitosan as coagulant has successfully removed water turbidity by 99.9% [108].

Application of *Moringa Oleifera* seeds for pollutants removal from synthetic and POME wastewaters shows more than 95% degradation of COD and turbidity, and high suspended solids removal [109, 110]. These studies indicate that *Moringa Oleifera* seeds is suitable alternative polymer for wastewater treatment. Application of *Cicer arietinum* for POME treatment showed high turbidity and suspended solid removal of 86% and 87%, respectively [105]. However, COD removal was relatively low due to the presence of soluble organic groups released during the treatment. Papaya seeds demonstrated high turbidity removal (100%) with no pH neutralization effect [111].

With regards to biopolymers, such as starch, there is an increasing application in wastewater treatment due to their renewability, non-toxicity, biodegradability, low cost and ability to treat wastewater containing negatively charged particles [71]. Cassava peel starch can achieve 90% removal of total suspended solids in synthetic wastewater (Table 6). In addition, rice starch alone and rice starch combined with alum can achieve total suspended solid removal of 84.1% and 88.4%, respectively [112]. Basically, rice starch has bigger polymeric chain, high efficiency in shortening settling time, produces larger and resistance flocs. Moreover, studies suggest that sago trunk starch can effectively remove turbidity, and decolourize landfill leachate by 98.9% and 94.7%, respectively [113].

Table 6 clearly shows that *Plantago Major L* and *Tamarindus Indica* Seeds can effectively decolourize different wastewaters up to 92.4% and 97%, respectively [114, 115]. *Opuntia ficus indica* mucilage, *Jatropha Curcas* seeds and *Sterculia Foetida* seeds achieve high turbidity removal of 98%, 99% and 97%, respectively [103, 116, 117]. Generally, most of the natural coagulants summarized in Table 6 have good turbidity and suspended solid removal capacity. This implies that the application of natural coagulant can increase the agglomeration between cell particles and improve the settling properties of the sludge.

Natural polymers are commonly composed of several macromolecules such as carbohydrates, protein and lipids. The major building blocks are the polymers of polysaccharides and amino acids. Natural polymers contain numerous charged functional groups in polysaccharides chain such as –OH, –COOH, and –NH, which are categorized as long polymer chains. The major mechanisms of natural polymer are polymer bridging and charge neutralization [118]. Polymer bridging (Fig.8) usually occurs when long chain polymers are adsorbed on more than one particle surface, leaving the dangling polymer segment to bridge and attach particles together. Bridging is known as the ability of polymer to aggregate and gather particles. Linear polymers that have high molecular weight are effective in bridging mechanism. Long chain polymers can form a strong and large aggregate by bridging at high shear condition. Fundamentally, the mechanism of polymer bridging involves: (i) mixing of polymer chain among the particles, (ii) adsorption of polymer chain to destabilize the particles, (iii) conformation of adsorbed polymer chain, (iv) aggregation (floc) and (v) re-stabilization. Cationic polyelectrolyte acts as a bridge that attaches to negatively charged particle surface and forms flocs. Adsorbed polymer chain adopts a conformation which consists of trains, loops and tails.

Trains are polymer segments that attach to the particles, while the unattached polymer form loops and tails. Firstly, polymer is adsorbed onto a particle's surface, while loops and tails as the main structure help to allow attachment of one surface to another.

In polymer bridging, sufficient space is required to enable the divisions of polymer chain to be attached with other particles at optimum polymer dosage. At high dosage, colloid particles undergo re-stabilization, because of the excess adsorption of the polymer and the steric repulsion of polymer that covers the particles. At low dosage, however, insufficient polymer chains form bridges and link to the particles. Principally, the optimum dosage of adsorbed polymer is directly proportional to the total particle surface area and particle concentration [102]. Aggregation can be improved by dosing the polymer solution slowly to a stirred suspension during mixing. This is because the growth of flocs is more likely to increase with the addition of polymer. When polymers are added slowly, they may help to prevent overdosing, thus inhibit the rapid growth of flocs at early stage of the process. However, during sudden changes, irreversible floc breakage can happen due to the breakage of bond in long polymer chain. Although long chain polymers have higher resistance to flocs breakage, they tend not to re-form if breakage does occur. To resist breakage, bridging contact is required.

Charge neutralization (Fig.9) is a possible mechanism for natural polymers. When a positively charged polymer is adsorbed on negatively charged particles and neutralizes the surface charge of colloid particles, charge neutralization occurs. The added polymers penetrate into the particles that are surrounded by diffuse double layers, causing the particles to move closer to each other. Polymers with high charged density and low molecular weight are the most effective in charge neutralization. To achieve zero zeta potential, an optimum coagulant dosage is required. The reduction of negatively charged

surface decreases electrostatic repulsion between particles, making the formation of large and dense floc possible. However, when polyelectrolytes have maximum charged density adsorbed on low-density negatively charged particles, electrostatic patch mechanism likely emerge.

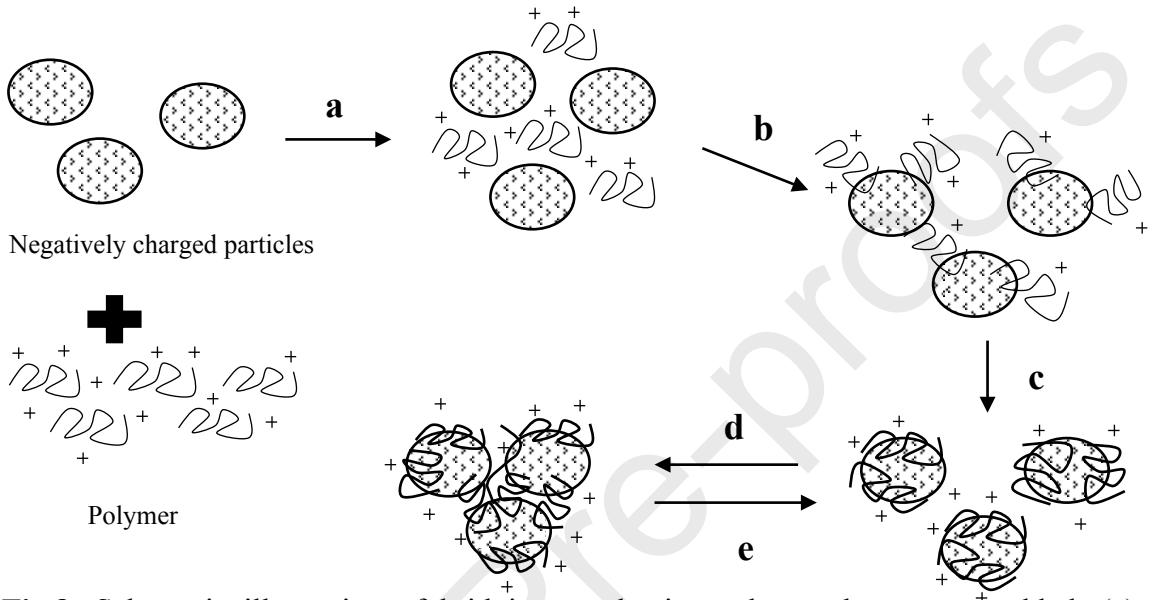

Fig.8: Schematic illustration of bridging mechanism when polymers are added: (a) mixing of polymer chains with particles (b) adsorption of polymer molecules on the particles (c) rearrangement of adsorbed chain (d) aggregation (e) break-up of flocs

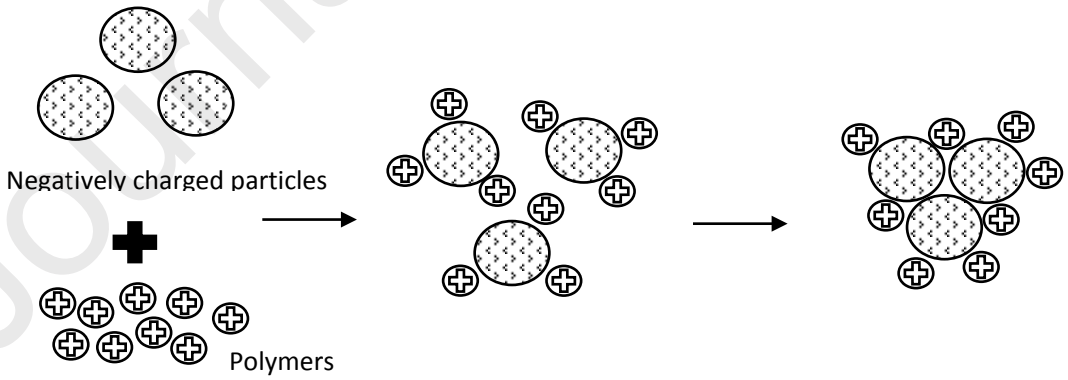

Fig.9: Schematic illustration of charge neutralization mechanism

4 Characteristic of Aerobic Granules Developed Using Different Types of Enhancer

This section further discusses the characteristic of aerobic granules formed with the addition of different types of enhancers (Table 7). Nevertheless, this section mainly focuses on the formed granules at maturation phase especially the morphology and size of granules. This is due to the lack of information on the characteristic of aerobic granules at initial stage as most studies only reported the properties of granules when they reached steady state. Several studies discussed in section 3 have shown that the addition of enhancers could positively increase the aggregation rate, accelerate granules formation and thus, increase the quality of the formed granules. In order to obtain good aerobic granules, a number of parameters have been examined including the properties of granules. Physical properties such as settling velocity, sludge volume index (SVI), granulation time, size and granular strength, specified by different research studies, are evaluated in this section. Most studies reported that good formation of granules is mainly dependent on the size and granulation time. Shorter granulation time is important mainly to avoid the loss of biomass during the start-up period. Settling properties are essential parameters in wastewater treatment, particularly in aerobic granulation. Low SVI and high settling velocity are the indicators of excellent settling properties of granules [119].Aerobic granules with excellent settling properties have strong and compact structure. Additionally, the investigation of the granular strength is crucial to determine the capability of granules to withstand shear force [120]. An extensively high shear force might cause breakage and reduce the stability of the formed granules. Studying the biomass concentration such as MLSS and MLVSS is also necessary to determine a good formation of granules. Generally, the increase of MLSS and MLVSS concentrations shows a good accumulation of biomass in the reactor, as the biomass is not easily washed

out from the reactor, hence it has more ability to settle. High biomass concentration may improve the settleability of granules with wastewater, thus resulting in formation of granules with good settling properties. Furthermore, the high MLVSS/MLSS ratio indicates high concentration of microorganisms within the reactor system. This section also focuses on the comparison between aerobic granules facilitated by enhancers and aerobic granules without addition of enhancers, based on the properties discussed above and removal performances.

4.1 Properties of Aerobic Granules

Settling velocity is related to structure and size of granules, varying above 18 m/h and significantly higher than sludge floc. Chemical additives such as sodium chloride demonstrated the highest settling velocity (92 m/h), while carbon sources can achieve a slightly lower velocity of 90 m/h (Table 7). A study by Ren et.al [47] observed that addition of Fe_3O_4 increased the settling velocity to 49.68 m/h, however, addition of Fe^{3+} has resulted to decrement of settling velocity to below 18.72 m/h. This indicates that Fe_3O_4 could accelerated the granulation process and produced aerobic granules with better settling properties. As reported by Sajjad and Kim [83], aerobic granules fed with Ca^{2+} achieved higher settling velocity than aerobic granules fed with Mg^{2+} , by 48.60 m/h and 41.80 m/h, respectively. This is due to Ca^{2+} was capable to cultivated denser and fast settling aerobic granules, which resulted to the increasing of settling velocity. Meanwhile, aerobic granules obtained settling velocity of 35.7 m/h, with addition of acetate [121]. Omar et.al [89] reported that when magnetic field was applied in the reactor, aerobic granules produced the highest settling velocity of 92.54 m/h. High settling velocity can withstand the biomass with good settling properties from being washed out, resulting to more biomass retained in the reactor. Further, Muda et.al [122] proved the ability of

anaerobic granules as substrate to formed aerobic granules with good physical properties, where the settling velocity obtained average of 42 m/h. It was monitored that settling velocity reached a maximum of 61.4 m/h and resulted in the increase of biomass concentration at the end of granulation process. Another study by Muda et.al [73] also revealed that addition of anaerobic granules could developed aerobic granules with higher settling velocity of 80 m/h. On the other hand, granular activated carbon produced the lowest settling velocity of 6 m/h [91], while addition of 10% crushed granules has resulted to 10 m/h of settling velocity [75]. However, these values are considered as fast settling velocity when compared to conventional activated sludge. Meanwhile, it was observed that without any additives, the settling velocity of 33 m/h could be achieved by mature aerobic granules [119]. Othman et.al [123] indicated the properties of aerobic granules formed without addition of any substrates in the reactor, where aerobic granules could obtained higher settling velocity of 88 m/h. However, it was noticed that the increased of settling velocity in the study was due to the presence of minerals such as Fe^{2+} and Al^{3+}. High concentrations of minerals may contribute to the microbial aggregations, thus leading to the formation of aerobic granules with compact structure and higher settling ability.

The sludge volume index (SVI) denotes the degree of compactness of granules with relative to the microbial structure of the granules. Low SVI indicates the aerobic granules have good settling ability which contributed by compact and denser granules. According to Tao et.al [94], low SVI of 20 mL/g can be achieved when aerobic granules is fed with GAC. Nevertheless, it was reported that GAC addition had no correlation with SVI_{30} values, as both control and GAC-fed reactor obtained similar SVI_{30}. In contrast, Li et.al [91] demonstrated that GAC has improved the sludge settleability in the reactor,

where the sludge obtained SVI_{30} of 30 mL/g. Further, it was observed that the SVI_{30} and SVI_5 obtained similar values, indicating complete granulation was achieved after 25 days of operations with addition of GAC. Ren et.al [47] reported that when Fe_3O_4 was added, SVI_{30} and SVI_5 decreased to 28.5 mL/g and 30 mL/g at steady state. Also, it was monitored that Fe_3O_4 –fed aerobic granules has successfully achieved full granulation in 11 days as the ratio of SVI_{30} / SVI_5 exceeded 90.10%. Based on the findings by Liu et.al [124], SVI values is attributed to the structure of aerobic granules. It was observed that the granules with loose and irregular structure might lead to the higher SVI.

Sajjad and Kim [83] noted that at the end of experiment, aerobic granules with Ca^{2+} addition showed rapid decreased of SVI_{30} compared to that of aerobic granules with Mg^{2+} addition, by 28 mL/g and 37 mL/g, respectively. Ca^{2+} allows high secretion of polysaccharides content in the reactor, which helped to accelerated the formation of granules with good properties, thus reduced SVI_{30} values more rapidly. Lower SVI was obtained by de Sousa Rollemberg et.al [121] when acetate was used as substrate in SBR. The SVI was decreased from 198 mL/g to 33.7 mL/g after the system was stabilized. Also, it has been reported that lower SVI of 30 mL/g was attained with addition of glucose [125]. On the contrary, Zhou et.al [126] noted that the augmentation of glucose under alkaline pH could lower the SVI value, where sludge reached below 50 mL/g on 60 days of operation. However, addition of acetate and glucose under acid pH were resulted in higher SVI, by 110 mL/g and 90 mL/g, respectively. Thus, it was inferred that SVI of aerobic granules was highly influenced by reactor pH, rather than carbon sources. Addition of preformed aerobic granules exhibited lower SVI of 28 mL/g [72] . It was reported that, within 35 days of granulation process, disintegration of granules occurred and resulted to the increase of SVI up to 60 mL/g. Nevertheless, SVI was decreased

gradually when the system stabilized.

On the other hand, several research studies revealed that without any support material, aerobic granules could formed with good settling properties, mainly in terms of SVI [13, 119, 123]. Rosman et.al [119] reported that aerobic granules formed without enhancer had a lower SVI of 22.3 mL/g. At initial stage, the increasing of SVI value was observed due to biomass washout. During maturation phase, SVI decreased substantially along with the formation of denser granules. Similar trend was also observed by [123], where the SVI significantly decreased from 131 mL/g to 42 mL/g, without additives in the reactor. Also, Cetin et.al [13] noted that aerobic granules reached a maximum SVI value of 38 mL/g during the last period of granulation. Therefore, it can be concluded that the lower SVI achieved by most granules are not mainly influenced by addition of enhancers as it did not have any significant effect on the SVI values, while it is clear that the lower SVI could be obtained without addition of enhancer, as reported by most studies.

The higher biomass concentration (MLSS) is an indication of good settling properties of aerobic granules. However, most studies have identified that the reduction of settling time at the beginning of granulation process is the major factor that can lead to high biomass washout, resulting in a decrease of biomass concentration. A study by Coma et.al [75] revealed the decreases of biomass concentration (MLSS) during the initial stage of granulation. This is due to the settling time was reduced significantly from 23 min to 2 min, which then resulted to washout of biomass from the reactor. Nonetheless, on day 30, it was monitored that addition of 10% crushed granules increased the MLSS concentration from 3.4 g/L to 5.8 g/L along with the increased of VER. In contrast, without addition of enhancer, the reactor obtained MLSS concentration of below than 1

g /L on day 40, and causes the decrease of VER to minimize biomass loss. In addition, Guo et.al [127] also noted the reduction of settling time had a significant impact on the biomass concentration. The loss of biomass occurred as the settling time was progressively decreased from 20 min to 5 min. Consequently, the initial MLSS concentration was deteriorated from 3.6 g/L to 1.9 g/L. At stabilization phase (day 100), it was observed that the MLSS concentration has eventually reached maximum of 4.5 g/L, in the reactor operated under the exposure of electric field. Higher MLSS concentration was obtained by Muda et.al [122], where the MLSS increased from the initial concentration of 4.8 g/L to 7 g/L. Initially, due to short settling times, the biomass concentration declined to 3.14 g/L. However, with the increase in settling velocity, high biomass could have retained in the reactor which resulted to higher MLSS value. According to Muda et.al [73], high settling velocity has the capability to avoid the biomass from being flushed out, mainly during the reduction of settling times.

Meanwhile, Wang et.al [57] obtained maximum biomass concentration of higher than 5 g/L in SBR operated with addition of dewatered sludge. After 2 days of operation, it was monitored that the MLSS was significantly decreased from 4 g/L to 2.9 g/L due to the disintegration of sludge into flocs. Then, the MLSS rapidly increased along with the increasing of granulation rate and stabilized within 22 days. Similar trends was evidenced by Bashiri et.al [72], where the MLSS faced massive washout from day 57 to the day 81. Insufficient nutrients due to the presence of more than 60% granules larger than 1 mm in the reactor have resulted in a reduction of biomass activity, which then affects the growth rate and further decreased the MLSS concentration. Subsequently, the MLSS concentration increased when only 50% of the granules over 1 mm were present in the reactor, as it enabled higher diffusion of nutrients in the smaller granules. At the end of

experiment, the aerobic granules reached the average MLSS of 2.08 g/L.

Rosman et.al [119] found that aerobic granules could achieved higher MLSS concentration even without addition of enhancer to support the formation of granules. During the start-up period, the decreases in biomass concentration (MLSS) was observed in the reactor, from 5.3 g/L to 2.2 g/L, due to the short settling time applied in the reactor. Concentration of biomass kept increasing as the granules appeared until MLSS achieved steady state at 8.2 g/L. Cetin et.al [13] noted that MLSS concentration reached minimum of 0.4 g/L when the settling times was shortened to 3 min. Despite the short settling time, it was monitored that the MLSS concentration was rapidly increased when the first aerobic granule was appeared. Also, it was inferred that the increase in MLSS concentration was attributed to the increase in size of granules. Higher MLSS concentration of 3 g/L was achieved by the reactor fed with settled wastewater. Moreover, the ratio of MLVSS/MLSS was higher than 90%, indicating a good accumulation of biomass in the reactor.

A number of studies has indicated that the size and structure of granules are important parameters in the characterization of aerobic granules. Compared to conventional activated sludge, aerobic granules have a minimum diameter of 0.2 mm and typically range from 0.2 mm up to 10 mm. The effective granulation time are also essential to avoid retention of poor biomass in the reactor which might causes the slow growth of bacterial cells and lead to the formation of granules with poor properties. Based on Table 7, it is summarized that addition of enhancers displayed excellent properties of aerobic granules. Addition of 0.5 mm dewatered sludge fastened the growth rate of aerobic granules. It was discovered that on day 2 of operation, the proportion of granular size ranging between 0.5 –1 mm increased up to 59% and becomes dominant in the

reactor. Wang et.al [57] explained that the increasing of size was mainly attributed to the larger particle size of dewatered sludge that could avoid disintegration. On day 20, 2-4 mm granules dominated the reactor with proportion reached up to 40%. Moreover, the microscopic examination shows that the mature granules were clear, regular, compact with yellowish-brown in colour. Aerobic granules with size of 0.28 mm were observed on day 11 with addition of Fe_3O_4 [47]. 96% of mature granules had size larger than 2 mm on day 30^{th} of operations. It was demonstrated that Fe_3O_4 inhibits the overgrowth of filamentous bacteria on the structure of granules. The result shows that the granules had a dense and compact microbial structure, where the bacteria had structure of rod-like species. Also, it was observed that addition of Fe_3O_4 lead to the presence of mineral crystal in the important fraction of granules structure, which contributed to the fast formation of granules.

Feng et.al [128] noted that addition of glucose produced larger granules of 2.5 mm while only 1 mm granules were observed with a mixture of glucose and acetate. However, the structure of aerobic granules-fed glucose was mainly dominated by filamentous bacteria, which resulted to loosed and irregular structure. Bacilli and cocci became dominant in aerobic granules-fed glucose and acetate, thus further exhibited a very strong and compact structure of granules. It was inferred that the changes of pH could influenced the formation of granules. The changes of solution pH to acid has favoured the growth of filamentous bacteria while the alkaline pH inhibits the growth of fungi, which then increased the amount of short bacilli and cocci in aerobic granules. Apparently, the granulation time could not be identified as the study does not focus on the formation of granules at initial stage. The author only reported the properties of granules when they achieved maturation phase, which was on day 21 of operations.

However, the observation contradicts the findings of Chen et.al [125], as the author indicated the formation of aerobic granules had excellent settleability when glucose was added in the reactor. The structure of mature granules was observed to be more compact and smoother with 50% of particle size greater than 0.46 mm. Despite the excellent properties, the granule cultivation period was longer (day 35) and smaller in size compared to synthetic wastewater treatment where the cultivation process took less than 7 days with larger granules. This could be attributed to toxicity in wastewater, leading to slower microbial growth and, hence, inhibition of rapid granule formation. Similar findings was obtained by Krishnen et.al [77], where aerobic granules required 60 days to be developed. Despite the inefficient start-up period, the mature bioganules were larger in size, compact and mainly dominated by coccid bacteria with long rod shape. Therefore, adding suitable enhancers during aerobic granule cultivation can reduce the start-up period of granules development and reduce the long granulation time. Also, it clearly seen that, by adding the enhancers, aerobic granules formed had compact and denser structure with mean size larger than 1 mm. On the contrary, the mature granules formed without enhancers had a smaller structure with maximum diameter of 0.6 mm. Moreover, the development period is longer and can reach 133 days [14]. A study conducted by Cetin et.al [13] noted the cultivation time took 44 days to developed aerobic granules in SBR. From these studies, it can be concluded that when aerobic granules are cultivated without enhancers, the granulation time is longer and the size of mature granules appeared smaller than the granules-fed enhancers.

Granular strength is expressed as integrity coefficient (IC). Lower value of integrity coefficient indicates higher granular strength. The strength of aerobic granules could be determined using method suggested by Ghangrekar et.al [129]. The granules are

considered high strength when the integrity coefficient reached higher than 80%. According to Chen et.al [130], high shear force contributed to the formation of high strength granules as it may facilitate the aggregation of microbial cells. Tay et.al [25] reported the possible formation of high strength aerobic granules under high shear force, which represented as up-flow superficial air velocity. The reactor was operated at superficial air velocity of higher than 1.2 cm/s, and this resulted to compact and stronger granules. This finding was supported by Zhu et.al [42], who reported that high shear force could positively affects the production of EPS, which may help in strengthening the structure by forming a cross-linked network and further stabilize the structure of aerobic granules. Also, high shear force improves the hydrophobicity of granular sludge and eventually, the hydrophobicity induce the interaction and attachment of bacterial cells.

However, only few reports have emphasized on the strength of granules. According to Muda et.al [73], when anaerobic granules patches were used as enhancer, the aerobic granules formed was strong, stable and have low integrated coefficient value of 9.4, indicating a high strength granules. It was observed that the reactor was operated at 1.6 cm/s of superficial air velocity. Similar studies by Muda et.al [122] obtained higher integrity coefficient value of 11 under superficial air velocity of 2.4 cm/s. This may be partially attributable to the longer granulation period took in the reactor which might affects the stability of the formed granules. Also, this could be influenced by intermittent aerobic-anaerobic conditions applied in the study as bacteria in anaerobic phase grow slowly. Ibrahim et.al [28] cultivated aerobic granules with lower integrity coefficient of 3.7 in 42 days, at higher superficial air velocity of 2.33 cm/s. The integrity coefficient of granular sludge formed by dewatered sludge and glucose were 99%, respectively, indicating that the granules had strong structure and can be stabilized in the system [57,

125]. As reported by Wang et.al [57], the study was carried out under high superficial air velocity of 2.0 cm/s and the results showed that the integrity coefficient value of aerobic granules had similar increasing trend with EPS content. It was inferred that EPS was the main factor affecting the strength and stability of aerobic granules. Therefore, application of shear force higher than 1.6 cm/s is required to accelerate the production of EPS, which further enhance the formation of high strength granules. Strength of granules are also likely to be dependent on HRT, where longer HRT may resulted to the reduction of size and causes instability to the structure of granules [131]

4.2 Removal Performance of Aerobic Granules

Aerobic granulation has been applied in the treatment of various wastewaters for the purpose of removing toxic substances, such as phenols and metals, nutrients and organic matters such as nitrogen, phosphorus and ammonia, and degradation of dyestuff [7, 8]. Many studies have shown that aerobic granules supplemented with enhancers achieved excellent performances in removing pollutants, as shown in Table 8. The average removal of COD and nutrient was obtained by Guo et.al [127] using low intensity electric field as enhancer in aerobic granulation process. The removal efficiencies were 97.1% COD, 80.5% TP, 68.1% TN and 99 % ammonium. At first 40 days, it was reported that COD was slightly fluctuated due to biomass washout. When the system stabilized, the COD removal increased. Also, phosphorus removal efficiencies were found to be highly dependent on the characteristic of aerobic granules, where the removal increased as the granules reached maturation phase. However, at the end of experiment, it was noted that capability of denitrification was insufficient as the effluent concentration of nitrate does not reached the environmental-friendly concentration of discharge. Omar et.al [89] achieved over 90% removal of COD, TP, TN, orthophosphate, nitrite and nitrate

simultaneously in SBR fed with magnetic field. It was monitored that application of magnetic field influenced the biological activities in the system, resulting in higher removal efficiencies of COD and nitrogen, by 97% and over 80%, respectively. Phosphorus removal reached maximum of 99% and the increased was observed along with the development of aerobic granules. In a recent study by Wang et.al [57], aerobic granules accomplished excellent removal of COD and ammonium, by over 90% and 98%, respectively, with the aid of dewatered sludge. It was shown that the removal rate of ammonium was stabilized at 98%, during the initial phase of operations. This is due to sludge dewatering influenced the granular sludge to accumulate slow growing bacteria especially nitrifying bacteria, thus increased the ammonium removal rate.

Effects of different carbon sources on nitrogen removal efficiency of aerobic granules were investigated by Feng et.al [128]. Addition of a mixture of glucose-acetate in the reactor resulted to 97.5% of COD removal while 96.1% was obtained with only glucose. It was inferred that COD removal efficiency in the reactor was less affected by carbon sources. Meanwhile, the removal efficiency of TN was 83% for granules-fed glucose-acetate and 74.6% for granules-fed glucose. It was observed that denitrification rates of aerobic granules fed with glucose-acetate was higher than glucose, by 18.75%. This is due to acetate produced intracellular storage polymer, which is poly-β-hydroxyalkanoates (PHB) to act as electron donor and further replaced the reduction of carbon source during denitrification process. Electron donor is essential to achieved high simultaneous nitrification and denitrification (SND) rate and accelerated nitrogen removal efficiency of aerobic granules [75, 128].

de Sousa Rollemberg et.al [121] monitored the effect of different carbon sources: acetate, ethanol and glucose on aerobic granulation in SBR. The COD removal efficiency

showed no impact with addition of carbon sources, as COD achieved over 90% removal in all systems. Acetate-granules has better removal efficiency of TN and TP removal, by 72% and 42%, respectively, compared to ethanol and glucose. On 72nd day of operations, partial disintegration of granules occurred and resulted to lower removal of ammonium. This is due to the loss of nitrifying bacteria presence in the broken granules. Formation of granule larger than 3mm is among the factor affecting granules disintegration when acetate was the substrate. Long et.al [132] also found that addition of acetate resulted to granules disintegration over long operational periods. Nevertheless, when the granules reintegrated, the ammonium, TN and TP removal increased. In the study, glucose-granules presented the lowest removal performance of TN and TP of 44% and 21%, respectively. Glucose caused the growth of filamentous bacteria, leading to continuous biomass washout and resulted to the lowest SRT value of 5-7 day. It was monitored that low phosphorus removal was due to the absence of storage polymer in the glucose, such as polyhydroxyalkanoates (PHA), to act as electron donor when extra carbon was depleted.

Compared with the study by Feng et.al [128] and de Sousa Rollemberg et.al [121], higher removal efficiency was obtained by Chen et.al [125] with addition of glucose. TN and ammonium achieved 100% removal when glucose was added in the reactor after 120 days of operations. It was monitored that the removal efficiency of TN and ammonium were fluctuated by less than 40% when petroleum wastewater increased to 100%. Dissolved oxygen (DO) value was increased from minimum 5 mg/L to maximum 8 mg/L in the reactor to improve nitrification, however, there is no significant difference found in TN and ammonium removal. The bioavailability of glucose as carbon source recovered the high nitrification and denitrification capacity. The influent of petroleum wastewater

was increased to 200 mg/L during the experiment and achieved COD removal of 95% after 200 min. However, there is no correlation observed between the removal rate of COD and the increased proportion of petroleum wastewater.

A study by Ren et.al [47] showed that addition of cations including Fe_3O_4, Fe^{2+} and Fe^{3+} have no significant effects on the efficiency of COD, TP and ammonium removal in aerobic granulation system. Granules fed with Fe_3O_4, Fe^{2+} and Fe^{3+} achieved removal of 94.76% COD, 97.68% ammonium and 59.29% TP. Similar observation was also evidenced by Cai et.al [133]. In the study, it was noticed that the removal efficiency of constant Fe^{2+} dosing and pulse Fe^{2+} dosing was similar. Both strategy achieved more than 96% dissolved organic carbon (DOC) removal and 99% ammonium removal. At the same time, constant Fe^{2+} dosing showed higher removal efficiency of TP compared to pulse Fe^{2+} dosing, by 91.9% and 80.8%, respectively. The study indicated that PAOs were found to be enriched under constant Fe^{2+} dosing strategy. Meanwhile, lower removal of TP was driven by high Fe^{2+} concentration. High Fe^{2+} concentration might inhibit the activity of PAOs group of bacteria, which lead to lower anaerobic P release and further resulted to lower TP removal rate. After all, it was inferred that Fe^{2+} dosing strategy was effective for TP removal in wastewater.

Liu et.al [100] reported that addition of PAC showed no significant difference with COD and ammonium removal rate of aerobic granules. Both PAC-fed granules and control granules obtained almost same removal efficiency of COD, by 93% and 92%, respectively. Also, it was observed that both granules achieved ammonium removal in range between 64% and 75%. Tao et.al [94] indicated that addition of granular activated carbon did not affect the removal efficiencies of COD, TN and ammonium in SBR. Both reactor, control and with addition of GAC achieved high COD removal (80%), total

nitrogen removal (80%) and complete removal of ammonium (100%). On the other hand, augmentation of GAC in the reactor enhanced the TP removal rate. GAC-fed aerobic granules obtained excellent efficiency of TP removal, by higher than 80%, on day 53. On day 18, it was observed that TP removal rate significantly decreased due to the absence of regular removal of biomass which then affects the phosphorus removal ability. GAC was found capable of enhancing the recovery ability of aerobic granules, resulting in a rapid increased of the TP removal. The findings are supported by Zhou et.al [95], who observed almost similar removal efficiencies of COD in control reactor and reactor added with GAC. High COD removal of 90.8%, 94.4% and 91.8% were achieved by control reactor, 0.2 mm GAC-fed reactor and 0.6 mm GAC- fed reactor, respectively. At low OLR of 1.5 kg COD m^{-3} d^{-1}, 0.2 mm GAC reactor showed higher removal rate of ammonium (98%) and TN (75.2%). Mature granules formed with addition of GAC enhanced the occurrence of SND, along with the presence of high DO, resulting in higher nitrogen removal efficiency. Apparently, based on previous research, it is summarized that most removal efficiency of COD was not affected by addition of enhancers. Nonetheless, the augmentation of enhancers has positively influenced the removal performance of aerobic granules in terms of biological nutrients such as ammonium, TN and TP.

Othman et.al [123] found that the aerobic granules achieved maximum COD, TN and TP removal of only 74%, 73% and 70%, respectively, without addition of support material. It was monitored that lower removal efficiencies obtained in the study were affected by high OLR. Similar findings was obtained by Di Bellaa and Torregrossa [134], where the result showed that aerobic granules reached lower COD removal of 50%. Initially, the efficiency decreased up to 20% due to the changing of influent from synthetic

wastewater to landfill leachate. This resulted to de-flocculation of granules and biomass washout, which affects the organic removal. When leachate was fed, granulation occurred and increased the removal efficiency. After the change of influent, ammonium dominated the granules, lead to the inhibition of ammonium oxidizing bacteria (AOB) and subsequently decreased the denitrifying performance lower than 20%. Due to higher nitrate concentration, aerobic granules only obtained 50% of nitrogen removal.

On the other hand, a study by Rosman et.al [119] demonstrated that aerobic granules successfully achieved higher removal efficiencies in COD (96.5%), TN (89.4%) and ammonium (94.7%), without presence of enhancer. COD removal rate increased from 70% to 89.9% after 30 days and eventually increased to a maximum of 96.5%, demonstrating the occurrence of high biological activity in the system. When aerobic granules developed, ammonium removal rate increased and the concentration was maintained below 10 mg/L. Simultaneous COD and nutrient removal was also obtained by Liu et.al [23] treating slaughterhouse wastewater in SBR, without support of additives. Removal efficiencies reached 95.1% (COD), ammonium (99.3%) and phosphate (83.5%). COD achieved lower removal efficiency of 80% on the first 60 days of operation, probably due to biomass washout and sludge acclimatization of slaughterhouse wastewater. When aerobic granules become matured, stable removal performance of COD were achieved. The ammonium concentration remained below 2.0 mg/L at the end of experiments. It was reported that matured aerobic granules promoted the nitrifying bacteria which resulted to high ammonium removal. Furthermore, PAOs was found enriched in aerobic granules. The phosphate concentration was remained at 4.5 mg/L, indicating higher removal efficiency of phosphate. Therefore, it is well summarized that without addition of enhancers, aerobic granules could also achieve COD and biological

nutrients removal above 90%. However, as discussed in section 4.1, the granulation process took longer time (exceeding 49 days) to develop the granules. This indicates the deficiencies of aerobic granules formed without enhancers in rapid granulation, although high removal performances can be achieved.

Based on the characteristic and removal performance of aerobic granules developed as summarised in Table 7 and Table 8, it can then be justified that addition of enhancers is the most effective approach to cultivate aerobic granules with excellent physical properties. The granules formed initially appeared as early as day 2 with a diameter of 0.5 mm. The granulation could be completed within 5 days with average size of 2-4 mm. The mature granules were compact, regular, fast-settling and having high granular strength. Most aerobic granules fed with enhancers achieved over 90% removal of COD, biological nutrients and phosphorus, while only certain studies have reported that aerobic granules without enhancers could achieved higher removal performance over 90%.

5 Future Research

At present, although the research on aerobic granulation has made significant progress, studies conducted still does not convey the knowledge of various aspect. The scenario requires further investigation to fully understand the whole aspect. Several recommendations for future research directions are listed below, as follows:

As summarized in section 4.1, the addition of enhancers has successfully minimised the granulation time and positively affects the properties of aerobic granules developed, but only in terms of size and structure of granules. Aerobic granules formed without enhancers could also achieved excellent properties, including higher settling

velocity, lower SVI value and higher MLSS concentration. Apart from enhancers, there may be other factors involved that could influence the properties of the granules developed, including operational parameters or other environmental factors which require further investigations.

The article has discussed and reviewed thoroughly on the strategies to enhance the development of aerobic granular sludge. However, studies on anaerobic granules formation with addition of enhancers are limited. Evaluations on the efficiencies of enhancers on the removal performance and characteristic of anaerobic granules specifically in terms of physical and biological properties should be taken into consideration for further research.

Further in-depth investigations are demanding at initial process of aerobic granules development, as it is still far from being understood. Knowledge about the interaction between bacterial strain, surface hydrophobicity, EPS, surface charge and proton translocations that contributes to the formation of granules is required. This would give further insight towards achieving rapid development of granules. The mechanisms of aerobic granules development have to be thoroughly explored, as this can further explain the clear mechanism involved during the initiation of granulation process.

A detailed analysis on the physical properties of aerobic granules is required. Research studies should indicate the amount of granulated biomass retained after granulation is completed. Some studies reportedly enhanced aerobic granules development after few days, however, they did not indicate when the system was fully granulated with certain amount of granulated biomass. In certain conditions, some experiments can produce compact and dense granules of large sizes at early stages of operation, however, they are still unable to attain full granulation. This should be

investigated in further, as the condition can be caused by several factors including design parameter of the reactor system, settling ability, as well as surrounding factors such as shock loading and toxic substances in wastewater. Based on the findings, only a few studies have focused on the strength of aerobic granules. Investigation on physical strength (IC), is essential because it is one of the parameters that determines the strength and stability of developed granules. Investigations on the properties of aerobic granules at initial stage are warranted to enhance the granulation development.

As shown in Table 8, with addition of enhancers, aerobic granules have been successfully applied for carbon, nitrogen and phosphorus removal in wastewaters. Nonetheless, the strategies of aerobic granules fed with enhancers to deal with toxic compounds are not well explored by many researchers. Thus, further investigation is required to determine the capability of enhancers to improve the removal performance of aerobic granules on toxic and recalcitrant pollutants in high strength wastewaters.

Little attention has been paid to the application of natural polymers on aerobic granulation technology. Natural polymers appear promising because they are excellent pollutant removal, and have high potential in improving start-up period of aerobic granulation due to their characteristics as a good coagulating agent which helps in promoting aggregations. Research on factors affecting long start-up period of granules is also crucial to obtain the best approach for shortening the granulation time along with addition of enhancers. However, as summarized in table 1- 8, different operational and environmental parameters involved in a single reactor, including wastewater composition, cycle period, operating pH, OLR and inoculum, may cause difficulties to evaluates the factors that mainly contributed to the long start-up period. Therefore, extensive lab-scale studies or alternative methods need to be conducted in further research

to determine the main factors which resulted to longer granulation time, in order to improve the start-up period of granulation and the characteristic of aerobic granules.

6 Conclusion

Aerobic granules have demonstrated superior settling characteristics, nutrient removal and biodegradation of recalcitrant pollutants. The knowledge accumulated evidently shows that aerobic granules is reliably cultivated in SBR with the addition of enhancer. The aerobic granules fed with the enhancer showed excellent properties and pollutant removal, compared to granules formed without any enhancer. Strategies to enhance aerobic granulation development as well as reducing start-up time required were analysed to improve the formation of granules in future research. This review provides an insight on the importance of enhancers in accelerating aerobic granules development at initial stage.

Acknowledgements

The authors would like to acknowledge Universiti Teknologi Malaysia and Ministry of Education Malaysia for their assistance in the production of this article.

Funding Sources

The authors declare that this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figure Legends

Fig.1: Graphical representation of the influence of EPS to microbial aggregation (a) EPS act by bridging single bacterial cells. (b) Formation of microbial aggregation with further illustration of the structural layer of EPS.

Fig.2: Graphical illustration of factors affecting the initiation of aerobic granules development

Fig.3: Microbial aggregation with combination of both co-aggregative strains [81]

Fig.4: The illustration of aerobic granulation development seeded with anaerobic granules: (a, b) anaerobic granules shrink and disintegrate, (c) aerobic microorganisms become dominant and (d) formation of compact aerobic granules

Fig.5: Mechanism of EPS with presence of Ca^{2+} and Mg^{2+}

Fig.6: Aggregation of flocs-GAC at initial process of aerobic granules development

Fig.7: Mechanism of sweep flocculation

Fig.8: Schematic illustration of bridging mechanism when polymers are added: (a) mixing of polymer chains with particles (b) adsorption of polymer molecules on the particles (c) rearrangement of adsorbed chain (d) aggregation (e) break-up of flocs

Fig.9: Schematic illustration of charge neutralization mechanism

Table Legends

Table 1: Findings on aerobic granules formation with addition of granules, sludge and microbes for rapid granulation

Table 2: Findings on aerobic granules formation with addition of cations (Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Fe_3O_4) for rapid granulation

Table 3: Findings on granules formation with addition of static magnetic field and electric field for rapid granulation

Table 4: Findings on aerobic granules formation with addition of granular and magnetic activated carbon for rapid granulation

Table 5: Findings on aerobic granules formation with addition of synthetic polymers for rapid granulation

Table 6: Natural coagulant used for different wastewater treatment

Table 7: Comparison on the physical properties of aerobic granules developed using different types of enhancers

Table 8: Main works on the removal performances of aerobic granules with and without addition of enhancers

Table 1: Findings on aerobic granules formation with addition of granules, sludge and microbes for rapid granulation

Ref	Biomass	Operational Conditions	Findings
[73]	Anaerobic granules	1) SBR (lab scale): 4 L; working volume 2) VER: 50%; Temp: 30 ˚C; pH: 6.0- 7.8; Airflow rate: 1.6 cm/s; Cycle period: 6 h; HRT: 12 h 3) Inoculum: mixture of sludge from municipal WWTP and textile mill WWTP 4) 100 mL of anaerobic granules (size: < 1 mm) were used as additives; taken from anaerobic sludge blanket reactor treating paper mill industrial effluent 5) The reactor was operated for 66 days	1) Biomass conc. decreased on the initial few days of operation due to half of sludge washed out from reactor. 2) Anaerobic granules were disintegrated into smaller fragments of granules during the initial stage and washed out from reactor due to poor settling ability 3) On 7th day, Anaerobic granules were observed to have changes in colour and size (from 1mm granules and black in colour into smaller grey granules). On 30th day, granules were clearly observed in the reactor. 4) The average diameter of aerobic granules developed on day 66 were 2.3 mm and max size reached 4 mm
[14]	Crushed granules (AGS)	1) SBR: 2 L, working volume; VER: increased from 12.5% to 25%- 50%; Temp: 20 - 23 ˚C; pH: 6.8-8.6; Airflow rate: 1 L/min; Cycle period: 8 h; Inoculum: floccular sludge from full scale WWTP 2) Mixture of crushed aerobic granules (5%, 10%, 15%, 25%, 30% and 50%) and floccular sludge were seeded in 6 reactors. 3) Initial settling time: 20 min; reduced to 10 min on the first 10 days and reduced to 5 min in the next 10 days; further reduced to 2 min when 50% of granules higher than 0.2 mm; constantly reduced to 1.5 min when reactor achieve full granulation	1) Biomass conc. did not decrease during granulation; no loss of biomass occurred in the reactor 2) Granulation had the shortest time (18 days) with addition of 50% crushed granules; The longest time was obtained (133 days) with only 5% crushed granules 3) The reactor seeded with crushed granules (0.5 mm- 1.0 mm) attain full granulation in 35 days – 40 days; seeding 0.1mm crushed granules took 80 days to achieve full granulation
[75]	10% crushed granules (AGS)	1) SBR (lab scale): 2 L; working volume; VER: increased from 25% to 62.5%; Temp: 20-22 ˚C; pH: 7.2- 8.2; Cycle period: 6h; Inoculum: floccular sludge (90%) 2) Seed 10% crushed granules (mean size: 0.3 mm), taken from a lab-scale SBR treating abattoir wastewater 3) Settling time was decreased from 23 min to 2 min to enhance granulation time and HRT decreased from 24 h to 9.6 h	1) On 40th day of operation, the visible granules appeared with average size of 0.2 mm, when settling time decreased to 10 min 2) Full granulation was achieved on day 80, where the granules had size > 0.2mm 3) 10% crushed granules was able to formed granules with compact structure and achieved stable biomass

Table 1: Findings on aerobic granules formation with addition of granules, sludge and microbes for rapid granulation (continue)

Ref	Biomass	Operational Conditions	Findings
[76]	25% mature granules (AGS)	1) SBR (pilot scale): 150. 46 L, working volume; VER: 60%; Temp: 15-20 ˚C; Cycle period: 6 h; Inoculum: activated sludge. Initial settling time: 25 min; Initial MLSS conc.: 3000 mg/L 2) 25% of mature AGS (size: 1.61 mm) was added into the reactor when settling time reduced to 10 min on 11th day	1) 1 day after inoculum seeded in reactor, a few small bacteria called zoogloeas appeared. A small granules appeared on day 4 and AGS dominated the reactor on the 17th day, the structure was irregular and pale yellow 2) On 11th day, AGS granules gradually increased due to the addition of 25% of mature AGS (the particle size of > 0.3mm increased).
[77]	Mixed sludge	1) SBR (pilot scale): 70 L, working volume; Airflow rate: 3 L/min; pH: 10; Cycle period: 24 h; Inoculum: sewage sludge (50% v/v); 2) Additives: mixed sludge (50% v/v sewage and textile) 3) Sewage sludge was reduced from 50% to 20% within six weeks; After 90 days, sewage replaced with pineapple wastewater (7% v/v) 4) Settling time was initially 30 min; reduced to 10 min within six weeks; further reduced to 5 min on week 8	1) Small granules appeared on 15th day of operation 2) After 56th day, granules were observed to have size ranging from 0.2 mm to 9.5 mm 3) 67% of biomass formed biogranules and AGS (size: 0.2 mm to 0.4 mm) dominated the reactor 4) Maximum size of biogranules reached 10 mm; the average size obtained: 2.7 mm ± 3.0 mm
[72]	Preformed aerobic granules	1) SBR (lab scale): 4 L, working volume; VER: 50%; Airflow rate: 4L/min; Temp: 27˚C; pH: 8.69-9.09; Cycle period: 6h. 2) Inoculum: Preformed aerobic granules (mean size: 0.7mm), which formed by activated sludge and slaughterhouse ww	1) On 52th day, 95% granules had size > 1 mm 2) On 57th day to 81th day, the structure of granules weakened and only 60 % of granules had size > 1mm due to present tensions in the reactor
[57]	Dewatered activated sludge	1) SBR (lab scale): 2 L, working volume; VER (anaerobic condition): 50%; Temp: 15-20 ˚C; pH: 6.9-7.3; Cycle: 4 h; Inoculum: activated sludge taken from aerobic tank 2) Activated sludge was dewatered for 4 min and cut into granules (mean size: 0.5 mm) 3) Granules were seeded into the reactor on day 0 with short settling time (3 min) 4) Initial MLSS: 4000 mg/L	1) During the initial 2 days, granules were disintegrated into smaller particles. Flocculent sludge washed out from reactor 2) On 5th day of operation, AGS dominated the reactor and had structure with smooth surface, clear outline, regular and the colour changed to yellowish brown. 3) Full granulation was achieved on day 5; The granules size increased from 0.52 mm on day 2 to 0.88 mm on day 5 4) 58.48% of granules formed achieved size between 0.5-1 mm, on day 2; the percentage of granules size (2-4 mm) increased from 3.52% to 39.98% 5) Mature granules had average size of 2.8 mm on 15th day

Table 1: Findings on aerobic granules formation with addition of granules, sludge and microbes for rapid granulation (continue)

Ref	Biomass	Operational Conditions	Findings
[80]	*Acinetobacter calcoaceticus* strain	1) SBR: 2 L, working volume 2) VER: 50%; pH: 7.02 3) Reactor was seeded with 2 L of free cell of *A. calcoaceticus* strain 4) Autoaggregation index of *A. calcoaceticus* strain reached maximum of 81%	1) After 9 h of operation, cells began to aggregates 2) After 27 h, visible aggregates were formed and 0.7 mm granules appeared within 7 days 3) In 49 days, granules size increased to 2.3 mm with addition of *Acinetobacter calcoaceticus* strain
[79]	*Pseudomonas veronii* strain B bacteria	1) GSBR: 2.5 L, working volume 2) VER:50%; Airflow: 2.5 L/min; Temp: 25-32 ˚C; Cycle period: 3 h; HRT: 6 h 3) Inoculum (GSBR1): activated sludge; Reactor (GSBR2) was seeded with 100 ml cell suspension of *P. veronii* strain B. Aggregation index *P. veronii* strain B is higher than 50%	1) AGS from GSBR2 were initially cultivated in 3 days with mean size of 0.5 mm 2) AGS from GSBR1 were initially cultivated after 9 days of operations; wit mean size increase from 0.08 m to 0.75 mm. 3) P. veronii becomes dominant in AGS for 14 days AGS formed faster with addition of P. veronii strain B due to higher aggregation index
[82]	*Rhizobium* sp	1) SBR: 2.2 L, working volume 2) VER: 50%; Flowrate: 0.2 m^3/h; Temp: 30 ˚C; Airflow rate: 0.02 m/s; Cycle period: decreased from 24 h to 6 h 3) Settling time reduced from 12 min to 2 min 4) 2g of *Rhizobium* sp strain were initially added into reactor	1) Fragile and small granules were observed on 14th day of operation. 2) AGS with diameter 0.2- 0.5 mm were formed after 98 days with changes in colour from white to yellowish brown; AGS becomes denser and regular in the following 28 days 3) After 120 days, mature AGS with diameter 0.5- 1.0 mm appeared in the reactor 4) *Rhizobium* sp strain is a good autoaggregator and have high potential in fastening start-up period of granulation
[81]	Mixture of *Rhizobium* sp and *Shinella granuli* bacterial strain	1) SBR: 2.2 L, working volume 2) VER:50%; Temp: 30 ˚C; Cycle period: decreased from 24 h to 12 h 3) Settling time: decreased from 20 min to 1 min 4) The reactor was initially seeded with 1g *Rhizobium* sp strain and 1g *Shinella granuli* strain	1) AGS with diameter 0.2 – 0.5 mm were observed on day 42 of operation 2) Mixture of two bacterial strain (*Rhizobium* sp and *Shinella granuli*) showed higher coaggregation ability, leading to shortening of AGS cultivation period

*Notes: AGS (aerobic granular sludge); SBR (sequencing batch reactor); GSBR (granulating sequencing batch reactor);WWTP (wastewater treatment plant); EGSB (expanded granular sludge bed); HRT (Hydraulic retention time); ML(V)SS-mixed liquor (volatile) suspended solids; VER (volumetric exchange ratio)

Table 2: Findings on aerobic granules formation with addition of cations (Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Fe_3O_4) for rapid granulation

Ref	Cations	Operational Conditions	Findings
[85]	Mg^{2+}	1) SBR: 10 L, working volume 2) VER: 50%; Temp: 25˚C; Cycle period: 4 h; settling time: 60 min 3) 10 mg/L of Mg^{2+} was inoculated in the reactor	1) AGS were firstly appeared on day 4; Full granulation in 18 days ;45% of granules had size > 0.6 mm on day 30; 2) Augmentation of Mg^{2+} have significantly decreased the granulation time, from 32 days to 18 days
[45]	Ca^{2+} Mg^{2+}	1) SBR: 12 L, working volume 2) VER: 75%; Temp: 24 ˚C; Airflow rate: 1.2 cm/s; Cycle period: 5 h; Inoculum: activated sludge from WWTP 3) Settling time reduced from 10 min to 1min 4) Two reactors used (R1 and R2); R1 was seeded with 40 mg/L Ca^{2+} and R2 was seeded with 40 mg/L Mg^{2+}	1) AGS initially appeared on 16^{th} cycles with addition of Ca^{2+} and appeared on 30^{th} cycles with addition of Mg^{2+} in 2) Full granulation achieved during 88^{th} cycles and 100^{th} cycles in R2 3) 60% of granules (1.3- 2 mm) observed in R1; In R2, 61% of granules (0.3 mm to 1.3 mm) 4) Addition of Ca^{2+} fastened the formation of granules
[83]	Ca^{2+} Mg^{2+}	1) SBR: 3 L, working volume 2) Temp: 25 ˚C; Cycle period: 5 h 3) Inoculum: Activated sludge from WWTP 4) Settling time reduced from 45 min to 15 days 5) Reactor (R2) was dosing with 25mg/L Ca^{2+} and reactor (R3) was dosing with 25mg/L Mg^{2+}	1) In R2, granules appeared on day 9 and achieved full granulation on day 16 2) In R3, granules were observed on day 14 and achieved full granulation rate on day 24 3) The granulation speed increased with the addition of Ca^{2+} compared to Mg^{2+}
[47]	Fe^{2+} Fe^{3+} Fe_3O_4	6) SBR: 2 L, working volume 7) VER: 50%; Airflow rate: 3 L/min; Temp: 13 ˚C; Cycle period: 2 h; HRT: 4 h 8) Inoculum: 2 g/L activated sludge 9) Ions were added into different reactors; Fe^{2+} (R2), Fe^{3+} (R3) and Fe_3O_4 (R4)	4) Granules appeared on day 11 with addition of Fe_3O_4, earlier than: Fe^{2+} (day 16) and Fe^{3+} (day 27) 5) On day 11, 0.27 mm granule were observed in R4 6) On 30^{th} day of operation 96% and 82% of granules in R4 and R2: > 2mm; only 12.5% of granules in R3 > 2mm 7) Fe_3O_4 accelerated the granule formation by increasing the abundance of zooglea, contributed to more secretion of EPS
[133]	Fe^{2+}	1) SBR: 1.30 L, working volume 2) VER: 50%; Temp: 25 ˚C; Cycle period: 4 h; HRT: 8 h; Settling time: reduced from 20 min to 3 min after 3 days; Inoculum: 400 mL seed sludge from sewage treatment plant; 5 mg/L Fe^{2+} constantly added into reactor 1 (R1) and 30 mg/L Fe^{2+} added into reactor 2 (R2) only in the first cycle	1) AGS appeared in R2 on day 9; in R1, AGS appeared on day 15 2) R1 achieved full granulation on day 36, while R2 required 48 days to be fully granulated 3) 73% of granules > 0.8 mm in R1; In R2, only 37% of granules > 0.8 mm

*Notes: AGS (aerobic granular sludge); SBR (sequencing batch reactor); WWTP (wastewater treatment plant); HRT (Hydraulic retention time); VER (volumetric exchange ratio); EPS (extracellular polymeric substances)

Table 3: Findings on aerobic granules formation with addition of static magnetic field and electric field for rapid granulation

Ref	Enhancers	Operational Conditions	Findings
[88]	48 mT static magnetic field	1) SBR: 3.5 L, working volume 2) VER: 50%; Temp: 22 ˚C; Airflow rate: 0.1 m^3 /h; Cycle period: 6 h; 3) Inoculum: activated sludge from secondary settling tank 4) Initial settling time: 9 min; reduced to 3 min on day 16 5) Magnetic field with intensity of 48 mT attached to the reactor	1) Granulation time decreased from 41 to 25 days 2) On day 18th, small granules visible in the reactor; 3) full granulation was achieved on day 25th 4) Granules had dense and compact bacterial structure that shows magnetic field could influence dominant bacterial of granules
[87]	15 mT static magnetic field	1) Schott bottles, h:230 mm; d: 101 mm 2) Airflow rate: 6.5 L/min; Temp: 24 ˚C; 3) Inoculum: activated sludge; dissolved oxygen: > 5 mg/L 4) 9 mT, 15 mT and 30 mT magnetic field were used	1) After 10 hours, % of aggregation achieved 90.4% under 15 mT intensity 2) After 30 hours, % of aggregation increased to more than 95% 3) At 15 mT intensity, surface hydrophobicity reached to 54% within 48 hours 4) Addition of magnetic field with 15 mT intensity have positively influence the initial state of aerobic granulation
[89]	20 mT static magnetic field	1) SBR: 3 L, working volume 2) Airflow rate: 2.5 cm/s; Cycle: 6 h; Settling time: 15 min 3) Inoculum: 1.5 L sludge from sewage STP 4) Reactor was operated with magnetic field at 20 mT intensity	1) On 7th day of operation, irregular sludge with diameter 0.2 mm appeared 2) On day 20th, small visible granules were clearly observed with size of 1 mm 3) After 90 days, granules become mature with average size of 6.5 mm and reached maximum to 8.9 mm 4) Addition of magnetic field was effective in formation of biogranules
[127]	Low intensity direct current (DC) electric field via reactive iron anode	1) SBR: 1.6 L, working volume; 2) Reactor was inserted with a pair of iron-titanium electrodes 3) VER: 37.5%; Airflow rate: 1.2 cm/s; cycle period: 4 h; settling time: decreased from 20 min to 5 min; HRT: 10.7 h 4) Inoculum: activated sludge from local WWTP 5) 1.0 V DC was constantly supplied to the reactor	1) Granules were observed after 20 days with diameter of 0.2mm 2) After 60 days, granules become mature and the size were stabilized at 1.23 –1.28 mm 3) Mature granules had structure of clear-cut, rigid, and compact 4) Addition of electric field accelerated the granulation rate as well as maintaining the granular structure

*Notes: SBR (sequencing batch reactor); WWTP (wastewater treatment plant); VER (volumetric exchange ratio); HRT (hydraulic retention time)

Table 4: Findings on aerobic granules formation with addition of granular and magnetic activated carbon for rapid granulation

Ref	Enhancers	Operational Conditions	Findings
[135]	0.2 mm granular activated carbon (GAC)	1) SBR: 4 L, working volume; VER: 50% 2) Airflow rate: 1.5 cm/s; Cycle period: 6 h; settling: 5 min 3) 0.2mm GAC was added into reactor which inoculated with activated sludge	1) Visible granules appeared after 14 days with size 2) Granules becomes mature after 39 days and the size reached 1.2 mm
[95]	0.2 mm granular activated carbon	1) SBR: 10 L, working volume 2) VER: 50%; Airflow rate:1.0 cm/s; Cycle period: 4 h; Settling time: 10 min; Inoculum: seed sludge from WWTP 3) 1000 mg/L GAC with size of 0.2 mm was inoculated in the reactor	1) After 10 days, microbial attachment was firstly observed and completed on day 26 2) After 43 days, GAC was completely covered and clear boundary between floc and inner core were observed 3) GAC (size 0.2 mm) served as nucleating agent that could facilitate initial microbial attachment
[93]	3.08 g/L magnetic activated carbon (MAC)	1) Schott bottle: 1 L; 500 mL working volume 2) Magnetic field intensity: 15 mT; Airflow rate: 1.5 cm/s 3) 1.0- 5.0 g/L magnetic activated carbon were inoculated in the bottle	1) At the best experimental condition of 3,000 mg/L sludge concentration and 3.08 g/L of MAC, a maximum of 56% SHb was obtained in 24 hr of aeration time. 2) MAC induce microbial attachment and increase aggregation
[94]	0.125-0.3 mm granular activated carbon (GAC)	1) SBR: 4 L, working volume 2) VER:50%; Temp: 25 ˚C; Airflow rate: 0.4 L/min; Cycle period: 4.8 h; Inoculum: 4 g/L seed sludge local STP 3) Reactor was inoculated with 14.5 g GAC (diameter: 0.125-0.300 mm)	1) No obvious granules appeared until day 20th 2) On day 21th onwards, size of granules increased from 0.1 mm to 0.5 mm; Full granulation was achieved on day 71th 3) Maximum size of granules reached 0.635 mm 4) GAC provided strong support medium to granules; granules able to reduce their compaction
[91]	0.22 mm granular activated carbon (GAC)	1) SBR: 2.4 L, working volume 2) Airflow rate: 2 L/min; Cycle period: 4 h; settling time: 30 min; HRT: 6 h; Influent COD: 200 mg/L 3) Inoculum: activated sludge from full scale STP 4) Consists of 2 operating phase: Phase I (first 30 days); Phase II (next 90 days) 5) 7.2g GAC size of 0.224 mm was added into the reactor	1) No granulation occurred in Phase I; sludge with size < 0.13 mm were observed 2) In Phase II, GAC-fed granules began to formed rapidly and small granules (diameter: 0.15 mm) appeared after 10 days in Phase II 3) Full granulation was achieved after 20 days in Phase II 4) Mature granules had a mean size of 0.6 mm
[92]	0.22 mm granular activated carbon (GAC)	1) Bioreactor: 200 mL; working volume 2) Airflow rate: 8 L/min; HRT: 12 h; Inoculum: nitrifying activated sludge cultivated from lab-sacale fermentor 3) 0.1 g GAC (size: 0.22 mm) were inoculated in the reactor	1) With addition of GAC, size of sludge increased from 0.18 to 0.27 mm after 12 days and granules appeared after 14 days 2) Mature granules had mean size of 0.36 mm 3) GAC shortened the granulation time from 42 days to 21 days

*Notes: AGS (aerobic granular sludge); SBR (sequencing batch reactor); WWTP (wastewater treatment plant); STP (sewage treatment plant); HRT (Hydraulic retention time); VER (volumetric exchange ratio)

Table 5: Findings on aerobic granules formation with addition of synthetic polymers for rapid granulation

Ref	Synthetic Polymer	Operational Conditions	Findings
[100]	PAC	1) SBR: 2 L, working volume 2) Airflow rate: 2 L/min; Cycle period: 6 h; 3) settling time: reduced from 15 min to 5 min on day 8 4) 500 mg/L PAC was added into the reactor for 8 days	1) Granules firstly observed on day 7th and becomes stable after 35th day. 2) On 30th day, 45% of granules were in range 1.0-2.5 mm; 10% of granules > 2.5 mm; 3) On 50th day, 10% of granules had size > 3.5mm; 4) PAC- fed granules decreased granulation time from 17 days to 7 days
[98]	PHB	1) SBR: 2L, working volume; 2) Temp: 25 ˚C; settling time: 5 or 15 min; HRT: 12 h; SRT < 10 days	1) Granulation was occurred on 80th day and granules becomes dominant on 100th day 2) The mature PHB-rich granules showed a regular morphology with diameter of 1.0–3.8 mm
[96]	PAM	1) CSTR: 5 L, working volume; 2) HRT: 4 d; airflow rate: 1 m/h; Inoculum: sludge from anaerobic pond of cassava starch treatment plant 3) PAM was inoculated in the reactor with dose 2 mg/g SS	1) After 120 days, 70% of granules had diameter of > 0.1 mm 2) 16% of granules were in range over 0.6 mm 3) Addition of PAM was suitable for initial stage of granulation
[97]	PAC	1) SBR: 2.4 L, working volume 2) VER: 50%; airflow rate: 2 L/min; cycle period: 6 h; settling time: reduced from 15 min to 5 min 3) Inoculum: 1.2 L activated sludge from local WWTP 4) 50 mL PAC with conc. 20g/L was added into reactor STF_{SBR} (8 days) and reactor LTF_{SBR} (40 days)	1) Granules firstly appeared on day 7th and day 8th in LTF_{SBR} and STF_{SBR} 2) full granulation was achieved after 15th and 16th days in both reactors. 3) In C_{SBR} (without PAC), no granules observed until day 21th and 4) full granulation was achieved on day 29th

*Notes: SBR (sequencing batch reactor); WWTP (wastewater treatment plant); CSTR (continuous stirred tank reactor); STF_{SBR} (short term feed SBR); LTF_{SBR} (long term feed SBR); C_{SBR} (control SBR); HRT (Hydraulic retention time); ML(V)SS-mixed liquor (volatile) suspended solids; VER (volumetric exchange ratio); PAC (poly aluminium chloride); PHB (poly hydroxybutyrate); PAM (polyacrylamide);

Table 6: Natural coagulant used for different wastewater treatment

Reference	Wastewater	Natural coagulant	Condition	Performance
[109]	Palm oil mill effluents	*Moringa Oleifera* seeds	pH: 4-9; Dosage: 500-6000mg/L;	COD removal: 52.5% ; SS removal: 99.2% ; settling velocity: 0.25 cm/min; SVI: 295 cm ^{3}/g
[103]	Synthetic wastewater	*Jatropha Curcas* seeds	pH: 3; Dosage: 120 mg/L;	Turbidity removal: 99%
[136]	Synthetic turbid water	Margaritarea Discoidea seeds	pH:3.94; Dosage: 10mL/L	Turbidity removal>90% Coagulation efficiency: 98%
[112]	Palm oil mill effluents	Rice starch	Dosage: 2 g/L; pH: 3;	TSS removal: 84.1%; COD removal: 17.4%
[116]	OSPW	*Opuntia ficus indica mucilage*	pH:7-8; Dosage: 1500 mg/L	Turbidity: 98%
[114]	Textile Wastewater	Plantago Major L	pH: 6.5; Dosage: 297.6 mg/L	Colour: 92.4%; COD: 81.6%
[113]	Landfill Leachate	Native Sago Trunk Starch	pH: 4; Dosage: 7000 mg/L	Colour: 94.7%; SS: 99.2%; Turbidity: 98.9%
[96]	Synthetic wastewater	Chitosan	Dosage: 13.0 mg/g VSS;	EPS production: 50 mg/g VSS; SVI: 36.1 ml/ g VSS
[108]	Highly turbid water	Chitosan	Chitosan: 5 mg/L; $AlCl_3$: 13.5 mg/L; pH:7	Turbidity Removal: 99.9%
[137]	Textile Wastewater	*PAFC-Starch-g-p (AM-DMDAAC)*	pH:7; Dosage: 0.2 mL/mg dye	Dye removal: 86%
[138]	Turbid water	Chitosan	pH:6.6-7.0; chitosan: 0.8 mg/L; PAC: 4 mg/L	Turbidity: 87% Residual Al: 0.07 mg/L;
[110]	Paper mill effluents	*Moringa Oleifera* seeds	pH: 6-8; Dosage: 150 mg/L;	COD removal: 97.28%; Turbidity : 96%
[107]	Palm oil mill effluents	Chitosan Mushroom	pH: 3; Dosage: 20 mg/L;	COD: 75%; BOD: 73%; TSS: 98%
[58]	Organic solvent	Cationic-Chitosan	Dosage: 2.4 mg gVS/S;	COD removal: >90%, granules size:> 2 mm;
[105]	Palm oil mill effluents	*Cicer arietinum*	pH: 6.69; Dosage: 2.6 g/L	Turbidity: 86%; COD: 56%; SS: 87%
[111]	Synthetic turbid water	*Papaya* Seeds	pH: 7; Dosage: 0.2 mg/L;	Turbidity: 100%
[139]	Synthetic kaolin	*Opuntia ficus indica*	pH:10; Dosage: 18 mg/L	Turbidity: 72%; coagulant efficiency:84%
[117]	Synthetic turbid water	*Sterculia foetida*	pH: 7.8; Dosage: 20 mg;	Turbidity: 97%
[115]	Landfill leachate	*Tamarindus Indica* Seeds (TIS)	pH:6; TIS Dosage: 2000 mg/L; PAC Dosage: 2750mg/L	COD: 67.4%; SS: 99.3%; Color: 97.3% with combination of PAC

**Notes: PAC (Polyaluminium chloride); NACI (Sodium chloride); COD (Chemical Oxygen Demand);TSS (total suspended solid); EPS(extracellular polymeric substance);BOD (biological oxygen demand; PACI (poly-aluminium chloride; NACI (sodium chloride; SS(suspended solid); $AlCl_3$: Aluminium Chloride; PAFC- Starch-g-p (AM-DMDAAC): (polyaluminum ferric chloride-starch graft copolymer with acrylamide and dimethyl diallyl ammonium chloride; OSPW: oil sands process-affected water*

Table 7: Comparison on the physical properties of aerobic granules developed using different types of enhancers

Reference	Type of enhancer	Description	System	Characteristic of biogranules							
				Structure	Ave. Dia. (mm)	Day granule was formed	*IC	Initial SVI_{30} (mL/g)	SVI_{30} (mL/g)	Settling velocity (m/h)	MLSS and MLVSS (g/l)
[14]	No enhancer	No enhancer	SBR	Irregular shape	< 0.2	day 133	_	_	_	_	_
[119]	No enhancer	No enhancer	SBR	Regular	1.5	day 49	_	84.7	22.3	33	MLSS decreased to 2.2 on the first 15 days and increased to 8.2 on days 75; MLVSS: 6.5
[123]	No enhancer	No enhancer	SBR	Compact	3.5-4	day 30	_	131	42	88	MLSS increased from 7.11 to 10.3; MLVSS: 8.5
[134]	No enhancer	No enhancer	GSBR	Compact with spherical shape	0.2	day 14	_	_	_	_	_
[31]	No enhancer	No enhancer	Flow system	Less dense granules	0.5	day 14	_	_	_	_	_
[23]	No enhancer	No enhancer	SBR	Smooth and compact	1.2-1.8	day 60	_	_	_	_	_
[13]	No enhancer	No enhancer	SBR	Smaller mature granules	0.6	day 44	_	40	38	18	MLSS increased from 2 .9 to 8; Ratio of MLVSS/MLSS > 90%
[79]	Biomass	*Pseudomonas veronii* strain B	GSBR	Compact	0.5	day 3	_	154	70	_	_
[73]	Biomass	Anaerobic granules	SBR	Compact and round shape with anaerobic patches	2.3	day 30	9.4	276.6	69	80	MLSS increased from 2.9 (initial period) to 7.3 (final period); MLVSS increased from 1.9 to 5.6 on day 66^{th}
[122]	Biomass	Anaerobic granules	SBR	Compact	2.5	day 35	11	218	61	42	MLSS increased from 3.14 to 7; Ratio of MLVSS/MLSS increased from 69% to 84%

Table 7: Comparison on the physical properties of aerobic granules developed using different types of enhancers (continue)

Reference	Type of enhancer	Description	System	Characteristic of biogranules							
				Structure	Ave. Dia. (mm)	Day granule was formed	*IC	Initial SVI (mL/g)	SVI_{30} (mL/g)	Settling velocity (m/h)	MLSS and MLVSS (g/l)
[75]	Biomass	10% crushed granules	SBR	Regular	1	day 40	_	_	< 100	10	MLSS increased from 3.4 to 5.8 and maintained at 3.0
[72]	Biomass	Aerobic granules	SBR	Unstable structure	1	day 57	_	80	28	_	MLSS increased from 2.6 to 3.414
[57]	Biomass	Dewatered sludge	SBR	Compact	2 - 4	day 2	99%	12.5	55	_	MLSS increased from 4 to 5.2 on day 20; MLVSS/MLSS ratio: 0.7
[127]	Electric field	Electric field	SBR	Compact and spherical	1.2-1.3	day 10	_	75	34	_	MLSS increased from 1.9 to 4.5; MLVSS/MLSS ratio: 0.72
[89]	Magnetic field	Static magnetic field	SBR	Dense and compact	6.5	day 20	_	_	_	92.54	_
[128]	Carbon sources	Glucose	SBR	Loose and fluffy	2.5	day 21-mature granule	_	_	_	_	_
[128]	Carbon sources	Glucose and acetate	SBR	Very compact	1	day 21	_	_	_	_	_
[125]	Carbon sources	Glucose	SBR	Regular and compact	0.46	day 35	99%	80.6	30	_	Initial MLSS: 5.958; increased to 8.509
[121]	Carbon sources	Acetate	SBR	Granular with irregular surface	1.5	14	_	198	33.7	35.7	_
[91]	Activated carbon	Granular activated carbon	SBR	Compact round shape with clear boundary	0.6	day 35	_	90	30	6	_

Table 7: Comparison on the physical properties of aerobic granules developed using different types of enhancers (continue)

Reference	Type of enhancer	Description	System	Characteristic of aerobic granules							
				Structure	**Ave. Dia. (mm)**	**Day granule was formed**	***IC**	**Initial SVI (mL/g)**	**SVI_{30} (mL/g)**	**Settling velocity (m/h)**	**MLSS and MLVSS (g/l)**
[94]	Activated carbon	Granular activated carbon	SBR	Small, Dense, compact and round shape	0.84	day 21	_	200	20	_	_
[100]	Synthetic polymer	PAC	SBR	Compact and round shape	3.2	day 7	_	176	38	_	Initial MLSS: 7.8, increased to 8.0 on day 50th
[98]	Synthetic polymer	PHB	SBR	Regular shape	1-3.8	day 56	_	150	60	_	Initial MLSS: 1.5, increased to 10 on day 150th
[83]	Cations	Magnesium ion- Mg^{2+}	SBR	Compact and round shape	1.2	day 14	_	195	37	41.80	_
[83]	Cations	Calcium ion- Ca^{2+}	SBR	Compact and round shape	1.5	day 9	_	195	28	48.60	_
[47]	Cations	Magnet powder- Fe^3O_4	SBR	Dense, large and compact structure	96% of granules > 2	day 11	_	221.78	28.5	49.68	Initial MLSS: 2, increased to 10.32
[47]	Cations	Ferric ion – Fe^{3+}	SBR	Dense, small and compact structure	12.5% granules > 2	day 27	_	221.78	60	18.72	Initial MLSS: 2, slightly increased to 2.96

*Notes: IC (Integrated coefficient); PAM: (Polyacrylamide); PAC: (Polyaluminium chloride); PHB: (Polyhydroxylbutane); SVI (sludge volume index); SBR (sequencing batch reactor);; GSBR (granulating sequencing batch reactor; ML(V)SS (mixed liquor (volatile) suspended solids);

Table 8: Main works on the removal performances of aerobic granules with and without addition of enhancers

Ref	Granules	Wastewater	Wastewater Characteristics (mg/L)	Removal performances
[73]	Anaerobic granules-fed Aerobic Granules	Textile wastewater	COD: 1270; Ammonium chloride: 160; Ammonia:38; Dipotassium phosphate: 580; Colour; 1020 ADMI	1) COD and ammonia removal achieved 71% and 67% at initial period and increased to 94% and 95% at the end of operation. 2) Colour removal increased from 25% during start-up to 62% at the end of operation
[128]	Glucose- fed Aerobic Granules; Glucose/ Acetate-fed Aerobic Granules	Synthetic wastewater	Ammonium chloride: 200; Dipotassium phosphate: 30; Calcium chloride: 30; Magnesium sulphide: 25; Iron (II) sulfate:20	1) COD removal achieved 96.1% with addition of glucose; 97.5% with addition of glucose and acetate 2) Nitrification & denitrification rate of granules was 8.03 and 2.73 with addition of glucose; glucose+ acetate achieved rate of 8.38 and 3.36 3) TN removal efficiencies were 74.6% with glucose and 83% with glucose + acetate 4) SND removal efficiencies were 34% with glucose and 40.1% with glucose + acetate
[75]	Crushed granules-fed Aerobic Granules	Domestic wastewater	Total COD:326; Soluble COD: 179; VFA: 21; ammonium:51; TP: 11; Phosphate: 9; TN: 67	1) Organic matter removal efficiencies reached 80% 2) The system achieved 85% and 94% nitrogen and phosphorus removal at the end of operations 3) SND increased over 50% when nitrite at low concentration and reduced to 20% on day 65 when nitrite at high concentration
[100]	PAC-fed Aerobic granules	Synthetic wastewater	Influent COD: 750; Glucose: 1L; Ammonium chloride: 200; Magnesium sulphide: 25; Iron (II) sulfate: 20;	1) Effluent of COD achieved 59 mg/l with removal of 92% 2) Ammonium conc. decreased from 38 mg/l to 17.6 mg/l after 10 days 3) Ammonium removal efficiency were between 64% 75%
[47]	Fe_3O_4- fed Aerobic Granules; Fe^{3+}- fed Aerobic Granules	Synthetic wastewater	COD: 1600; Ammonium chloride:75; Phosphorus: 15; Calcium chloride: 15; Magnesium sulphide: 12.5	1) COD and ammonium removal: 94.76% and 97.68%
[133]	Fe^{2+} - fed Aerobic Granules	Synthetic wastewater	COD:600; Monopotassium phosphate: 10; Ammonium chloride:100	1) High phosphorus removal was obtained at 92% 2) Sludge phosphorus content (45.6 mg/g-SS) with higher bioavailability (95%)

Table 8: Main works on the removal performances of aerobic granules with and without addition of enhancers (continue)

Ref	Biogranules	Wastewater	Wastewater Characteristics (mg/L)	Removal performances
[127]	Electric Field- fed Aerobic Granules	Synthetic wastewater	COD: 600 ; Ammonium Nitrogen: 60; Phosphate: 10; Magnesium ion: 12; Calcium ion: 20	1) COD removal efficiency achieved stable value of 97.12%; TP removal increased to 80.52%; TN removal: 68.05% and ammonia removal: 99% 2) Effluent concentration of nitrate was 19.99 mg/L, unable to meet environmental- friendly discharge value
[89]	Magnetic Field-fed Aerobic Granules	Pharmaceutical wastewater	*no data available*	1) COD removal increased from 64% to over 90% after 10 days of operations 2) TP removal increased to 99% on 50th day of operations 3) Orthophosphate removal was about 9% during start-up period and increased to 93%
[125]	Glucose- fed Aerobic Granules	Synthetic petroleum wastewater	Influent COD:600-900; Ammonium: 25-40; TN: 30-47;	1) COD removal efficiency achieved 95% as the conc. decreased from 625 mg/l to 30 mg/l; Oil removal achieved over 90%, oil content maintained below 25mg/l; On the first 40 days, ammonium and TN removal achieved 100% and 85%; Addition of 400 mg/L glucose after day 120 increased the TN and ammonium nearly 100%
[57]	Dewatered sludge-fed Aerobic Granules	Synthetic wastewater	COD:1000; TN:50; TP:6; Calcium chloride: 1.50; Iron(II) sulfate:0.24; Magnesium sulphide:0.27	1) COD removal achieved only 66.91% on day 2 of operation and increased over 90% 2) Ammonium removal rate achieved > 98%; concentration of NO2-N was lower than NO3-N
[123]	No addition of enhancer	Livestock wastewater	COD: 3600; BOD: 1750; TN:650;TP:380;TSS: 230	1) COD, TN and TP removal rate were slightly lower by 74%,73% and 70%.
[119]	No addition of enhancer	Rubber wastewater	COD: 1850; SS:270; TN: 278; AN: 49	1) COD removal rate: 96.5%; Ammonia removal rate: 95%; 2) Total nitrogen removal rate: 89.4%
[134]	No addition of enhancers	Landfill Leachate	COD: 4560; Ammonium: 945; NO_3^-: 0.3	1) COD removal efficiency achieved 70% 2) Ammonium removal efficiency achieved only 59%
[23]	No addition of enhancers	Slaughterhouse wastewater	COD: 1250 ± 150; Ammonia: 120 ± 20; TP: 30 ± 5	1) COD removal: 95.1%; Ammonia removal efficiency: 99.3%; 2) TP removal efficiency: 83.5%
[13]	No addition of enhancers	Real domestic wastewater	COD:900; Ammonium:54; TN: 70; PO_4^3 – P:8; Oil and grease: 280	1) COD removal efficiencies: 89%; Ammonium removal: 60% 2) PO_4^3 – P removal efficiencies: 76%
[140]	No addition of enhancers	Saline wastewater	Glucose: 1536; NH_4Cl: 240; Seawater crystal: 0% to 8%	1) COD removal decreased to 25% with increasing of salinity to 8% 2) NH_4^+ -N removal decreased to 21% as salinity increased to 8%

*Notes: TP (Total phosphorus); TN (Total nitrogen); NH4+-N (Ammonium nitrogen); COD (chemical oxygen demand); BOD (biological oxygen demand); TSS (total suspended solid); NO_2-N (nitrite); NO_3-N (nitrate); PO_4^{3-}-P (phosphate); VFA(volatile fatty acid); SND (simultaneous nitrification denitrification); Fe_3O_4 (magnetic powder); NH_4-Cl (Ammonium Chloride)