

# Influence of Seed Sludge Concentration in Aerobic Granulation Process: A Focus on the Extracellular Polymeric Substances (EPS) Production and Settling Properties

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KEYWORDS	ABSTRACT
Mixed liquor suspended solid (MLSS) Aerobic granulation Extracellular polymeric substances (EPS) Settling velocity Microbial aggregates	To addressing the sludge concentration issues, aerobic granulation process has been introduced to enhance the biological wastewater treatment. This study aims to compare the influence of different sludge concentration on the EPS production, sludge settling properties and to investigate the relationship between these two parameters during aerobic granulation process. Sludge samples with different concentration were used as the seed sludge in lab scale bioreactor which continuously operated in SBR operation. EPS extraction and settling properties were monitored during the granulation process. Finding shows that, EPS ratio produced on high MLSS sludge was 1.42, while low MLSS was 1.25. In addition, the good settling properties was shown in high MLSS sludge with the velocity of 0.28cm/s compared to low MLSS with only 0.072cm/s. In conclusion, the diversity of microbial communities in high MLSS sludge contributes to increased EPS production, which facilitates the formation of larger flocculant sludge that enhances the settling properties.

## 1. INTRODUCTION

In sewage wastewater treatment, activated sludge exists at varying concentrations. These concentrations consists of suspended solid forming a mixture with incoming domestic wastewater which known as mixed liquor suspended solids (MLSS)[1]. The concentration of MLSS plays the crucial role in the performance of biological treatment systems. The excessively high concentration levels of MLSS can cause sludge bulking, making it difficult for sludge to settle, while low MLSS level leads to insufficient biomass resulting the ineffective of pollutant degradation process [1][2]. Therefore, by addressing these issues, the introduction of aerobic granular sludge (AGS) technology has significantly improved the biological wastewater treatment efficiency. AGS forms dense sludge flocs through the microbial aggregation process, which enhances settling performance and ensures high efficiency in pollutant removal[3][4].



The major component of microbial aggregation are the microorganisms and extracellular polymeric substances (EPS)[5]. EPS is a slimy like structure released by the microorganisms which mainly consists of polysaccharides and protein[6]. The matrix of EPS properties is significant in maintaining the flocculation ability and sludge settleability in the biological treatment system[7]. Therefore, the aim of this study is to compare the EPS production, which significantly influences the settling properties, between low and high MLSS seed sludge in the aerobic granulation process. There are three objectives involved in this study: (i) to measure the EPS production. (ii) to observe the settling velocity (SV) and (iii) to investigate the relationship between the EPS production and settling velocity in AGS process.

## 2. EXPERIMENTAL PROCEDURE

The seed sludge sample was collected at aeration tank in Indah Water Konsertium (IWK) domestic sewage treatment plant as illustrated in Table 1.

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Table 1 Seed sludge sampling location

IWK Bintong, Perlis	IWK Taman Anggerik, Baling, Kedah
	
Low MLSS: 267.7mg/l	High MLSS: 4286mg/l

Two identical lab scale bio-reactor (working volume = 500ml) was setup with 250ml of seed sludge. The bioreactor was operated 24hours continuously as fill and draw via SBR operation for 28days. For each 48 hours the settling velocity was monitored and recorded as  $\text{cms}^{-1}$ . Then sludge samples were collected for extraction of two main EPS component. Total carbohydrate (TC) was analysis by using phenol-sulphuric digestion while protein by using Bradford Assay.

### 3. RESULTS AND DISCUSSION

#### a) EPS Production

The composition of protein and carbohydrates was represented in EPS ratio (Protein/Carbohydrate). Microbes utilized the carbohydrates as the carbon source

to produce protein as the main component in the biofilm formation[8]. Figure 1 illustrated the EPS ratio produced in low MLSS and high MLSS sludge during aerobic granulation process.

The similar finding shown in both sludge concentration where the EPS ratio increases from day 0 to day 28. The increment indicates the growth of microbial aggregates which reforming the loose structure into larger and denser sludge as the sludge age throughout the process[9]. Besides, the EPS ratio in low MLSS sludge initially recorded as 0.54 then continuously increasing up to the highest value at 1.25 on day 28. However, in high MLSS sludge the ratio initially recorded as 0.69 then also shows the continuously increment until reach the highest value at 1.42 on day 28.

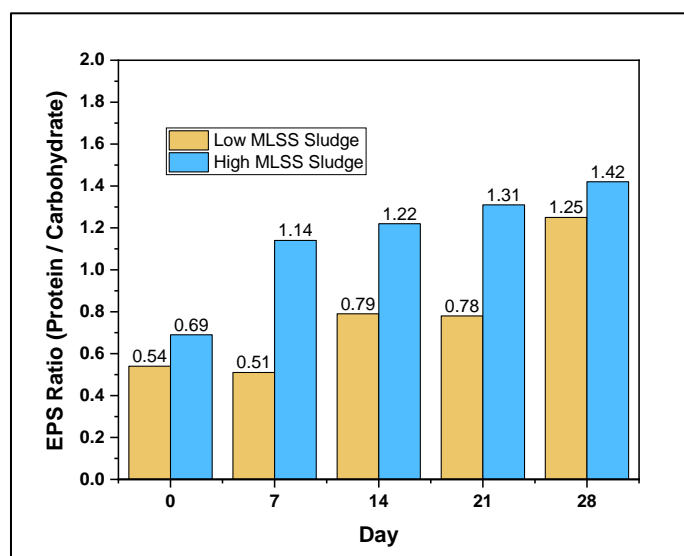
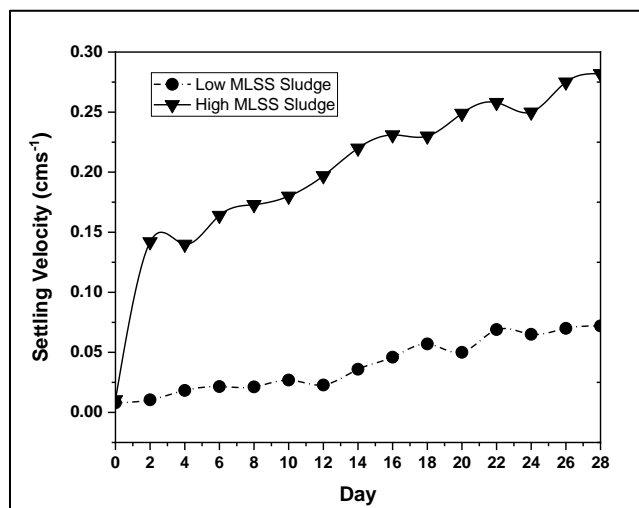


Figure 1. EPS ratio on low MLSS sludge and high MLSS sludge.

So, the result shows the high MLSS produced higher ratio of EPS compared to the low MLSS sludge which addressing that high MLSS sludge composed of divers species of microorganism which promotes the biosynthesis of EPS on the cells to the solid surface[10]. This is also supported by [11] which have proven that, the high diversity of microbial colony in the environment were essential in producing a high productivity of sludge bio-flocculation.

#### b) Settling Properties

Settling velocity (SV) is significant in solid-liquid separation in any wastewater treatment process. Figure 2 shows the SV on the different sludge concentration. The SV shows similar trends for both sludge which continuously increasing from day 0 to day 28. However, the highest settling for high MLSS sludge recorded was 0.282 cm/s while low MLSS sludge was 0.072cm/s which indicates the high MLSS sludge give the higher settling compared to low MLSS sludge.



**Figure 2.** Settling velocity (SV) on low MLSS sludge and high MLSS sludge.

The rapid settling velocity in high MLSS sludge addressing the direct consequence of sludge flocculation from the aggregated microbial cells[12]. On the other hand, low MLSS sludge exhibit lower settling due to the formation for fluffy flocs which mainly composed of filamentous bacteria. This is also supported by [13] which stated that, the overgrowth of filamentous bacteria will affect the stability of sludge biomass which leads to the deterioration of microbial aggregates thus resulting in poor settling properties.

EPS production does play the significant role in the sludge settling properties. The more EPS matrix produced on the microbial aggregates the larger sludge flocs will be developed. This situation creates larger and denser bio granules formation which attributes to high in settling properties[14]. Furthermore, sludge with high MLSS concentration is the best selection of seed sludge in developing bio granules via biological wastewater treatment.

#### 4. CONCLUSION

Overall, high MLSS sludge exhibited a higher EPS ratio compared to low MLSS sludge, suggesting a more diverse microbial community that supports biofilm synthesis. Additionally, high MLSS sludge also demonstrated the

good settling properties which attributes to the formation of larger and denser sludge flocs. As the conclusion, high production of EPS plays a significant role in promoting the microbial aggregation into sludge flocs, thereby enhancing sludge settling properties.

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