

# **DELETERIOUS EFFECTS OF SOLUBLE EXTRACELLULAR POLYMERIC SUBSTANCES ON POLYACRYLAMIDE DEMAND FOR CONDITIONING OF ANAEROBICALLY DIGESTED SLUDGE**

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## **Abstract**

High polyacrylamide (polymer) demand for conditioning of sludge, especially anaerobically digested sludge (ADS), is a major issue for the water industry. Currently, this problem is being investigated and the reasons for doing so are varied. It has been demonstrated that excess amounts of soluble extracellular polymeric substances (EPS) can lead to high polymer demand for conditioning. This study developed a simple and unique yet effective method for quantifying the contribution of soluble EPS to conditioning polymer demand. It did this by measuring absorbance at 191.5 nm wavelength of the supernatant derived from conditioned ADS. Experimental results confirmed that approximately 87 wt% of soluble EPS interacted with polyacrylamides during the conditioning process. Furthermore, they revealed that a specified amount of soluble EPS could not be removed by polymer flocculation despite high polymer dosage. This study concluded that about 86 wt% of the polyacrylamide used for conditioning was consumed solely by soluble EPS. These results confirm the important role of reducing this EPS fraction in ADS in order to curtail significant chemical costs for sludge conditioning and dewatering.

**KEYWORDS:** *Anaerobically digested sludge; extracellular polymeric substances; polyacrylamides; polymer demand; sludge conditioning*

## 1. Introduction

High polymer demand for dewatering is an intractable problem for the water industry worldwide. The industry has come to accept that its operations will have to bear expensive chemical costs associated with sludge conditioning and the expenses involved in transporting biosolids with excess water content. It was reported that 50–60% of annual operating costs of a wastewater treatment plant (WWTP) accounted for the sludge handling phase [1, 2, 3]. Consequently, it has posed difficult questions that all waste treatment facilities will have to deal with, the most important being: how can these significant costs of sludge treatment be reduced? It is estimated that increasing the sludge solids content from 2% to 30% can help reduce 90% of the sludge volume, leading to a considerable saving in transport costs [4]. However, in order to achieve this dewatering outcome, a large amount of expensive polyacrylamides is required for sludge conditioning. In the case of Sydney Water, about AUS\$2.5 million per year was spent solely on polyacrylamides for conditioning and dewatering (information provided by a project run by Sydney Water). A thorough understanding of polyacrylamide consumption during conditioning is vitally important for minimizing the amount of chemicals set aside for dewatering.

It is believed that understanding the mechanisms of bio-flocculation can help to optimize sludge conditioning and dewatering. Extracellular polymeric substances (EPS) produced by microbes have been demonstrated as being important components in the bio-flocculation of microbial cells [5, 6]. Behaviors of EPS in flocculation and dewatering of activated sludge together with EPS characteristics have been discussed comprehensively in several other studies [7, 8, 9, 10, 11]. EPS may contain up to 95% of moisture and are closely linked to bound water which is difficult for mechanical dewatering to release [12, 13, 14, 15]. EPS can be found in two forms,

namely soluble and bound EPS, which can be further subdivided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). Effects of soluble EPS, LB-EPS and TB-EPS on conditioning and dewatering have been investigated by a number of studies. It has been documented that TB-EPS exert positive impacts on bio-flocculation [16] while LB-EPS are found to wield a negative influence on sludge flocculation [17] and settleability [18]. Nevertheless, the dissolved biopolymer or soluble EPS fraction is often highlighted as an important factor to determine polymer demand for dewatering [19, 20].

Anaerobically digested sludge (ADS) is often observed as comprising poorer bio-flocculation ability and dewaterability when compared to waste activated sludge (WAS) [21, 22, 23]. Deficiency in natural bio-flocculation requires a larger dose of synthetic conditioners such as polyacrylamides for conditioning to supplement poor bio-flocculation. This enables sludge flocs to have the strength to withstand the high shear of mechanical dewatering and, consequently, improve dewatering performance. The selection of suitable types and dosages of synthetic conditioners for dewatering has mainly been a trial and error process [24]. A number of studies have set out to investigate and identify the components of sludge that consumed the large proportion of polymer demand during conditioning. Colloidal particles have been reported in many analyses as the main consumers of conditioners for dewatering [24, 25, 26, 27, 28, 29].

Recent studies have proved that the high polymer demand for conditioning of digested sludge is attributed to the increase in soluble EPS released during digestion, especially when anaerobic digestion is used [19, 20, 30]. However, how soluble EPS in ADS interact with the conditioning polymer and how much of this EPS fraction contributes to the polymer demand for dewatering are not yet well understood. Houghton et al. [31] stated there is an optimum EPS content for sludge dewatering. It means that concentrations of EPS which are too large or too small can result in destabilization of bio-flocs. EPS act as natural glue-like substances that adhere bio-flocs together. However, an excess amount of these negatively charged particles could not only

lead to destabilization of the floc matrix but also retain water [29]. Recent studies claimed that the addition of support media (such as filler and biochar) to anaerobic digestion so that microorganisms are immobilized, not only enhances sludge digestibility but also improves the dewaterability of digested sludge [32, 33]. Moreover, since it has been proved that microorganism immobilization increases the degradation rate of dissolved organics, it may be possible to decrease the polymer demand for conditioning. However, more research is needed to confirm this.

This study was mainly focused on quantifying the contribution of soluble EPS to polymer demand for conditioning of anaerobically digested sludge. With this new knowledge, the study can identify feasible approaches to either reduce or optimize the amount of soluble EPS released during anaerobic digestion that could favor both sludge flocculation and dewatering processes. This, ultimately, will help to substantially cut both chemical and biosolids transportation costs in WWTPs.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Sludge sample

Anaerobically digested sludge (ADS) was collected from anaerobic digesters located at the Cronulla wastewater treatment plant (WWTP) run by Sydney Water, Australia. The plant treats 53000 m<sup>3</sup>/day of domestic wastewater and produces 19000 tonnes of biosolids each year. In the sludge treatment process, the Cronulla plant uses two-stage anaerobic digestion (AD) for sludge stabilization and biogas production, while cationic polyacrylamide is employed for conditioning and centrifuges serve the purpose of dewatering.

ADS samples were collected over several periods from an anaerobic digester's sampling point (before being conditioned and dewatered) at Cronulla (from June 2016 to January 2018).

These samples were then immediately transported to the laboratory and stored at 4°C. Sludge samples were naturally warmed to room temperature (20 – 25 °C) before the commencement of all experiments and analyses. The general characteristics of ADS are listed in Table 1, which summarizes the average values of 6 different sampling times.

**Table 1**

### **2.1.2. Conditioning chemical for dewatering**

This study used cationic polyacrylamide FO 3801 (SNF), which is currently utilized at Cronulla WWTP, for conditioning experiments. FO 3801 is a linear, very high charge density and ultra-high molecular weight polymer [34]. The polymer solution for conditioning was prepared by dissolving the polymer powder in tap water at a concentration of 0.35% w/v (currently used at the WWTP). The polymer solution was prepared using a bench-scale agitator (3-blade impeller, Heidolph RZR 2020). Polymer mixing time and aging time both lasted 30 minutes. The polymer solution was then used within two days of the experiments commencing to achieve representative and best performance.

## **2.2. Experimental methods**

### **2.2.1. EPS extraction protocol**

EPS fractions including soluble EPS, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) were extracted using a method proposed by Higgins et al. [35]. Briefly, 50 ml of the ADS sample was centrifuged at 3000xg for 15 minutes at 5°C. The supernatant was collected as soluble EPS. After that, the bottom sediment was resuspended to a volume of 100 ml using a buffer solution (pH = 7) consisting of NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> at the molar ratio of 1:1.6 (1N HCl was used to adjust the pH of the buffer solution to 7). The suspension was mixed at 1500 rpm for 10

minutes using a 3-blade impeller and then centrifuged at 3000xg for 15 minutes at 5°C. The bulk solution was collected as LB-EPS and for the next stage, the pellet was resuspended to a volume of 100 ml using 1N NaOH. Then the suspension was mixed at about 500 rpm for 2 hours using a magnetic stirrer and eventually centrifuged at 3000xg for 15 minutes at 5°C. The bulk solution was collected as TB-EPS. The collected solutions of soluble EPS, LB-EPS and TB-EPS were filtered through a 2.7 µm filter paper (Whatman filter paper No. 542). Selecting the filter paper pore size was based on work done by Higgins et al. [19]. The filtrate samples were used for EPS analysis. In this study, EPS concentration was calculated as the total amount of protein (PN) and polysaccharides (PS).

### **2.2.2. Evaluating the contribution of soluble EPS to polymer demand for conditioning**

The conditioning tests were carried out for both soluble EPS (or the supernatant extracted as noted in sub-section 2.2.1), which was called test A, and the whole ADS (as-received sludge), which was referred to as test B (see Figure 1 below). These tests were designed for the purpose of calculating how much polyacrylamide was consumed by only soluble EPS.

#### **Figure 1**

Different concentrations of polymer FO3801 solution were added to the solution of soluble EPS in test A and the whole ADS in test B. The polymer concentration (in mg) of polyacrylamide per gram of the whole ADS (mg/g TS) solid content and their equivalent in mg polyacrylamide per litre of as-received sludge (mg/l) are presented in Table 2. The mixtures of the supernatant and polymer solution were rapidly mixed at 1000 rpm for 10 seconds using a bench-scale agitator. Subsequently, the conditioned mixtures were centrifuged at 3000xg for 5 minutes and the conditioned mixture's bulk solution was taken for EPS, zeta potential and absorbance measurement.

**Table 2**

### **2.2.3. Measurement of excess polymer concentration in the supernatant (absorbance measurement)**

The excess polyacrylamide content in bulk solutions derived from the conditioning tests with both tests A and B were determined by measuring the absorbance (Abs) of the bulk solution as previously reported by Gibbons and Örmeci [36] and Al Momani and Örmeci [37]. These studies have shown that polymer concentration in water samples can be accurately determined by measuring their absorbance at 191.5 nm wavelength. In this study, the measurement was used for samples from both tests A and B. This method was applied to determine the contribution of soluble EPS to polymer demand for conditioning by establishing a relationship between absorbance and polymer dosage for conditioning. The relationship curve is divided into three ranges (as illustrated in Figure 2). The optimum polymer dose (OPD) in each case was the dose that led to the minimum value of Abs.

**Figure 2**

In the underdose range, the polyacrylamide added to sludge firstly reacts with suspended solids as well as soluble EPS. As the polymer dose (PD) increased, the absorbance values fell due to the removal of suspended particles in the supernatant. Then, the cationic polyacrylamide molecules were adsorbed on the sludge particles' negative surface. Approaching the OPD, the surfaces of the sludge particles were almost completely covered by the polymer molecules; the adsorption decreased due to the limited number of available adsorption sites. At the OPD point, the absorbance value would be very minimal. In the overdose range, when all the adsorption sites were occupied, the excess polymer added would enter the supernatant. This could be detected by an increase in the Abs of the supernatant. Consequently, when PD increased the Abs also

increased. In other words, at the overdose range, higher excess polymer content led to the supernatant having a higher Abs value. A relatively linear relationship was observed to exist between Abs and PD at the overdose range. This correlation could be useful in determining excess polymer content in the supernatant.

#### **2.2.4. Analytical methods**

All chemical analyses were carried out in duplicate using chemicals purchased from commercial sources and used as received. Proteins were measured by the Peterson modification of the Lowry method [38] using bovine serum albumin (Sigma-Aldrich) as the standard. Polysaccharides were determined by the Phenol-Sulphuric method [39] using glucose as the standard. Zeta potential of the sludge particles was measured using a Malvern Instrument (ZetaSizer Nano ZS-90). A pH meter (Hana, model HI 9025C) served to measure the pH level. The total solids content was analyzed following Standard Method 2540B [40].

UV-Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu) was utilized for measuring the absorbance of the samples at a wavelength of 191.5 nm. The recommended working range was between 0 and 1 absorbance unit (AU) [36, 37]. The supernatant of ADS had a strong yellowish color, which interfered with the Abs measurements and as a result, samples were diluted. The samples were diluted with DI water at a ratio of 1:80 (optimized by reducing the sample Abs to the recommended working range (0 – 1 AU) of the instrument).

### **3. Results and discussion**

#### **3.1. Characterization of EPS compositions in ADS**



### 3.1.1. Spatial distribution of EPS

Based on the experimental results, the total amount of EPS in ADS was  $280 \pm 44$  mg/g TS, which accounted for  $28 \pm 4.4$  wt% of ADS total solids content. It has been reported that the total amount of EPS extracted from sludge can vary depending on wastewater treatment systems as well as extraction methods. It is therefore difficult to compare the EPS concentration of different sludge types. Liao et al. [41] stated that the EPS composition is one of the main governing factors of bio-flocculation and not the quantity of EPS alone.

#### Figure 3

Figure 3 presents the distribution of EPS fractions of ADS. According to the results, the EPS were distributed mainly in the inner layer of the sludge flocs, with LB-EPS and TB-EPS accounting for about 70 wt% (or  $198.2 \pm 38.3$  mg/g TS) and 10 wt% (or  $23.4 \pm 3.1$  mg/g TS) of total EPS content, respectively. In comparison, fewer EPS were present in the outer layer, with soluble EPS accounting for about 20 wt% (or  $58.6 \pm 8.6$  mg/g TS) of total EPS content. In other words, the amount of TB-EPS far outweighed those of the other two fractions.

In the present study, Sydney Water's Cronulla WWTP Water used a polymer dose of 14–19 mg/g TS for dewatering. This polymer dose was significantly higher than those of other WWTPs (approximately 8 mg/g TS on average) operated by Sydney Water, and these do not utilize anaerobic digestion prior to dewatering. The study attributed this high dose of conditioning polymer to the substantial increase in soluble EPS content of sludge in the facilities using anaerobic digestion.

#### Figure 4

It can be seen from Figure 4 that anaerobic digestion resulted in a considerable release of EPS into the solution. As compared to ADS, WAS from the other two WWTPs operated by Sydney Water only needed about 6–8 mg/g TS of polymer to achieve similar dewatering performance.

Although the other two fractions of EPS also consume cationic polymer during conditioning, soluble EPS are believed to be mainly responsible for the polymer requirement for dewatering of the digested sludge.

### **3.1.2. Protein and polysaccharides**

Protein (PN) and polysaccharides (PS) are the two main components of EPS [7]. The interaction between PN and PS is one of the most important forces that help EPS form the floc matrix, along with hydrophobic interactions, hydrogen bonding and ionic interactions [42]. As a result, investigating the relationship between these two EPS constituents is essential to understand the flocculation mechanisms.

It can be noticed in Figure 3 that, no matter when referring to soluble EPS, LB-EPS or TB-EPS, PN content was predominant when compared to PS content, with total PN accounting for almost 80 wt% of total EPS content (or  $222 \pm 37$  mg/g TS – Table 1). This agreed with a number of other studies examining anaerobically digested sludge [19, 20, 23, 43]. Specifically, in this study, PN content in TB-EPS (TB-PN) constituted more than 55 wt% of total EPS (or  $156.6 \pm 31.7$  mg/g TS – Figure 3). One possible reason for the excess amount of PN in digested sludge could be due to the fact that PS (especially ones with low molecular weights) are more easily digested than PN [44]. TB-PN is believed to help enhance sludge dewaterability and settleability because of its hydrophobic characteristics [42, 45, 46]. In the meantime, soluble PN contents have been demonstrated to contribute to polymer demand for conditioning [19, 20, 30]. In this study, soluble PN content was the second largest amount, accounting for 17 wt% of total EPS (or  $47.9 \pm 8.3$  mg/g TS – Figure 3).

On the other hand, the total PS content, accounting for 20 wt% of total EPS content ( $58 \pm 14$  mg/g TS – Table 1), has been demonstrated as contributing to anionic and hydrophilic (high affinity for water) properties of EPS [47, 48]. The highly negative ZP of ADS in this study ( $-30.2$

$\pm 2.2$  mV – Table 1) could primarily be due to the extracellular PS. The effects of PS on sludge conditioning and dewatering have been proved to be mostly negative [7]. In addition, soluble PS ( $10.7 \pm 0.8$  mg/g TS – Figure 3) have also been considered as one of the major determinants of polymer demand for conditioning [19, 20, 30].

### **3.2. Removal of soluble EPS during conditioning**

#### **3.2.1. Soluble EPS analysis**

Figure 5 illustrates the residual soluble EPS contents at different polymer doses for both tests A and B. When the polymer dose increased, the soluble EPS content decreased until the polymer dose of 14 mg/g TS was reached. From that dose onwards, the residual soluble EPS content remained constant at about 9.2 mg soluble EPS/g TS. This observation was for both conditioning tests A and B (Figure 5a). It indicates that, regardless of the excess amount of polyacrylamide added, a certain amount of soluble EPS could not be removed by polymer flocculation during conditioning. In this study, about 87 wt% of the total soluble EPS was flocculated by the polyacrylamide used.

#### **Figure 5**

Figure 5b presents the percentage of soluble EPS removed in tests A and B at different polymer dosages. After a certain amount of EPS was removed during conditioning (in this study, a maximum 87 wt% of soluble EPS removed during conditioning), there was no more reaction between the EPS and the polyacrylamide, even with a further increase in the conditioner dosage (Figure 5b). In other words, after reaching the highest level of removal of soluble EPS, excess polymer added went into the supernatant; this is supported by the results reported in sub-section 3.3. This could furthermore be due to the effect of repulsive forces when adding an enormous amount of cationic, high molecular weight polyacrylamide to the sludge.

A slightly higher percentage of soluble EPS was removed in test A with the supernatant as compared to that in test B. In the case of test A, the highest removal of soluble EPS was achieved at a polymer dose of 12 mg/g TS. On the other hand, in test B, the highest EPS removal was obtained at a polymer dose of 14 mg/g TS. This difference between the two tests in terms of soluble EPS could be explained by un-flocculated LB-EPS as LB-EPS being converted into soluble EPS during an intense stirring process. It was assumed that TB-EPS are not easily released into solution during conditioning and dewatering.

### **Figure 6**

Figure 6 presents the residual soluble PN and PS contents in both tests A and B. About 86 wt% of the total removed soluble EPS in both tests was made up of PN for all the polymer doses studied. At the dose of 12 mg/g TS in test A and 14 mg/g TS in test B, more than 90 wt% of soluble PN and 74 wt% of soluble PS was flocculated during conditioning. From those polymer doses onward, the removal percentage of soluble PN and PS remained nearly constant with the increase in the polymer dosage beyond 12 mg/g TS. These results could suggest that the currently used polyacrylamide at the WWTP studied was more effective in removing soluble PN than soluble PS. It could be due to the fact that PN plays an important role in polymer bridging by virtue of its higher affinity with cations [49].

#### **3.2.2. Zeta potential analysis**

Figure 7 shows that the ZP values of conditioned sludge flocs from conditioning tests A and B increased with the elevation in polymer dosages. The ZP values of conditioned supernatant in test A were higher than those in test B. However, for both tests, the changes in ZP values divided the conditioning polymer dosages into three distinct ranges and these are explained in more detail immediately below.

#### **Polymer dose of 0–8 mg/g TS**

ZP values of samples in both conditioning tests increased only marginally from -27.3 mV (at the dose of 0 mg/g TS) to about -24 mV in test A and to -26 mV in test B (at the dose of 8 mg/g TS). This range can be considered as the underdose range where the polymer dose was not enough to neutralize the soluble substances. As a result, negatively charged particles were present predominantly in the supernatant, leading to highly negative ZP. As can be seen in Figure 7, the residual soluble EPS content at the dose of 8 mg/g TS in both tests A and B was high (17.6 and 26.5 mg soluble EPS/g TS, respectively). This could also suggest that all polymer particles added were consumed by soluble EPS and the precipitates may have been of subcolloidal size which would not have been adsorbed on the sludge particles.

**Figure 7**

#### **Polymer dose of 8–18 mg/g TS**

A sharp increase in ZP values was observed for both conditioning tests A and B, from about -25 mV (at the dose of 8 mg/g TS) to about 23 mV (at the dose of 18 mg/g TS). Unlike the lower range of polymer dose (0 – 8 mg/g TS) where ZP values of the two tests were quite similar to each other, in this range, ZP values in test A were higher than those in test B. Specifically, from the dose of 8 mg/g TS, ZP in test A quickly increased to 0 mV at the dose of 14 mg/g TS, while ‘zero’ charge point in test B was obtained only at the dose of 16 mg/g TS. This could be explained by the presence of LB-EPS in test B which consumed more polymer for flocculation to reach the point of neutralization as compared to only soluble EPS in test A. Based on the charge neutralization mechanism, the polymer dose of 16 mg/g TS could be regarded as the OPD for conditioning. Nevertheless, the charge neutralization does not need to be completely achieved during sludge conditioning since effective flocculation is attained by both charge neutralization and polymer bridging formation concurrently. Therefore, the ‘zero’ surface charge point could possibly be considered as an overdose already [20]. As a result, the dose of 14 mg/g TS with ZP of -11.2 mV

could be regarded as the OPD, which in fact agrees with the suggestion from the results of residual soluble EPS content in the conditioned supernatant.

### **Polymer dose of 18 mg/g TS onwards**

The ZP values of both tests were positive and increased marginally with a further increase in the polymer dose. The trend of this range was similar to the first polymer dose range results. This range was considered as the overdose range of polymer conditioning. It is observed for both tests A and B that, from the dose of 14 to 24 mg/g TS, the amount of residual soluble EPS stopped decreasing and remained constant (Figure 5a) regardless of the increase in polymer dose and ZP values (up to 28 mV at the dose of 24 mg/g TS – Figure 7). This indicated an excess amount of cationic polymer in the supernatant, which resulted in overdose conditioning. As well, high positive ZP values of the conditioned supernatant at high polymer dosage could be attributed to excluded volume effect. Excluded volume effect refers to the concept that one part of a long chain molecule cannot occupy space that is already occupied by another part of the same molecule [50]. In other words, the excluded volume of a long chain polymer is the volume that is inaccessible to other polymer chains in the system as a result of the first chain's presence. This phenomenon prevents the excess polyacrylamides added during conditioning from reacting with sludge particles either through charge neutralization or bridging formation. The evidence for this effect will be shown in sub-section 3.2.3.

### **3.2.3. Flocculation performance of polyacrylamide during conditioning**

Figure 8 presents the calculated amounts of soluble EPS removed by a unit mass of conditioning polyacrylamide at different dosages. It shows that when increasing the dosage of conditioner, the amount of soluble EPS removed by 1 mg of polyacrylamide decreased in both tests A and B. It means adding an excess amount of polyacrylamide to the sludge led to competition among the polyacrylamide particles when they approached the surface of sludge particles due to

the aforementioned effect, which is excluded volume. This competition, consequently, resulted in the polyacrylamide displaying poorer flocculation performance, which explains the disadvantage of excess or overdose conditioning.

### **Figure 8**

FO 3801 (the currently used polyacrylamide at Cronulla WWTP) is a very high charge density and ultra-high molecular weight polymer. Thus, as a consequence of the excluded volume phenomenon, flocculation performance of each FO 3801 molecule with sludge particles may be limited in the case of high polymer dose conditioning.

### **3.3. Measurement of excess polymer content in the supernatant**

Figure 9 depicts the relationship between the sample Abs values and polymer doses for both tests A and B. A wide range of polymer dosages was selected to cover the underdose, optimal polymer demand (OPD) and overdose ranges. Similar patterns were observed in both conditioning tests. In the underdose range, sample Abs sharply decreased with increasing polymer dosage. The dose that led to the minimum value of Abs was considered as OPD (12 mg/g TS in test A and 14 mg/g TS in test B). In the overdose range, sample Abs rose slowly with the increase in polymer dose, resulting in a linear relationship. However, some differences between the two tests were still evident. Specifically, at the dose of 8 mg/g TS, Abs value in test B was much higher than that in test A. A possible reason for this difference could be attributed to un-flocculated LB-EPS which can be released into the solution phase due to the intense mixing during conditioning.

### **Figure 9**

As compared to test A (Figure 9a), OPD of that in test B was higher (Figure 9b) due to the consumption of conditioner for not only soluble EPS but also LB-EPS. It suggests that 12 out of 14 mg of polyacrylamide/g TS or 86% of conditioning dosage was used for the flocculation of soluble EPS. In other words, a large amount of conditioning polyacrylamide was consumed by soluble EPS.

Cronulla WWTP, on the sampling days, used 14 mg/g TS of polymer FO3801 for sludge conditioning, which was the OPD (according to this study's findings) for its effective sludge conditioning. As a result, only a small amount of polyacrylamide remained in the centrate. The Abs value of the full-scale centrate (collected from Cronulla WWTP on the same sampling days) was 0.360, which was quite similar to that of the lab-scale centrate (0.368). This confirms that the Abs measurement method is a suitable alternative method for measuring the excess polyacrylamide concentration in the centrate.

#### 4. Conclusions

The results articulated in this study show that there was always a certain amount of soluble EPS which could not be removed by polyacrylamide flocculation despite the excess amount of conditioner added. About 87 wt% (maximum) of the total soluble EPS content of ADS was removed by flocculation with polyacrylamide. Also, Abs measurement results show that about 86 wt% of polyacrylamide used for conditioning was consumed solely by soluble EPS. This demonstrates the need to find an alternative polymer that can play the main role in sludge conditioning and not for the consumption of soluble EPS. Doing so can reduce the significant chemical costs for sludge conditioning in the WWTPs. Moreover, with the new knowledge developed in this study, the water industry can now set concrete targets in operating anaerobic digesters so that the contents of soluble EPS are minimized and optimized. It will result not only in the reduction of polyacrylamide demand for dewatering but also much more efficient anaerobic



digestion. In this study, the method of quantifying the contribution of soluble EPS to polymer demand for conditioning was studied for sludge dewatering using centrifuges. Further research is needed to validate the method with other dewatering systems such as filter press.

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## Table captions

**Table 1.** Characteristics of anaerobically digested sludge from Cronulla WWTP.

**Table 2.** Polyacrylamide dose range used in tests A and B.

**Table 1** Characteristics of anaerobically digested sludge from Cronulla WWTP.

pH	TS <sup>a</sup>	ZP <sup>b</sup>	TPN <sup>c</sup>	TPS <sup>d</sup>
	(g/l)	(mV)	(mg/g TS)	(mg/g TS)
7.5 ± 0.3	19.8 ± 2.0	-30.2 ± 2.2	222 ± 37	58 ± 14

<sup>a</sup> TS: Total solids content;

<sup>b</sup> ZP: Zeta potential;

<sup>c,d</sup> TPN, TPS: Total Protein and Total Polysaccharides of all EPS fractions.



**Table 2** Polyacrylamide dose range used in tests A and B.

<b>mg/g TS</b>	4	8	12	14	16	18	20	24
<b>mg/l</b>	69	138	207	250	276	310	345	414

## Figure captions

**Figure 1.** Procedure of conditioning tests A and B.

**Figure 2.** Relationship curve between absorbance of the supernatant and polymer dosage for conditioning (adopted from a Sydney Water project).

**Figure 3.** Characterization of EPS fractions in ADS from Cronulla WWTP. Error bars represent the standard deviations of PN or PS contents of ADS at 6 sampling times.

**Figure 4.** Soluble EPS content of the sludge fed to dewatering equipment (before polymer addition) in three WWTPs operated by Sydney Water (Cronulla WWTP with ADS, WWTPs A and B (without anaerobic digestion) with waste activated sludge (WAS) A and B, respectively). Error bars represent the standard deviations of soluble EPS content (Data on characteristics of WAS A and B is adopted from a previous Sydney Water project).

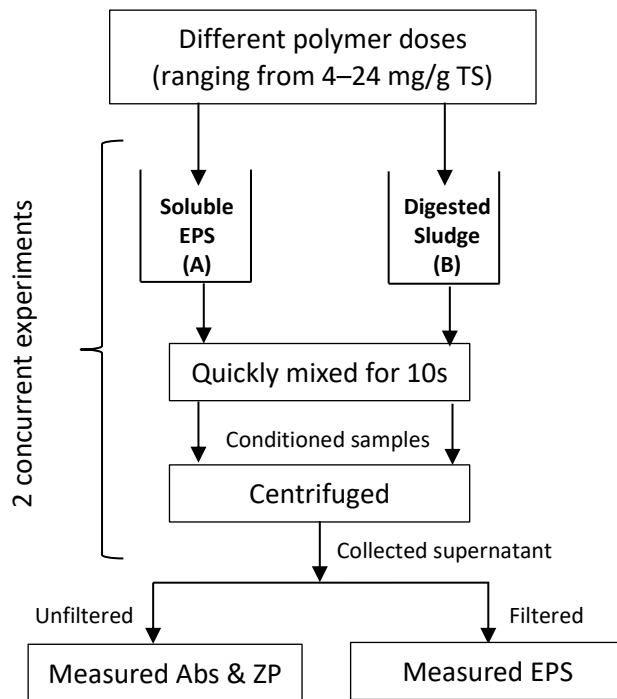
**Figure 5.** (a) Residual soluble EPS content and (b) removal percentage of soluble EPS at different polymer dosages in tests A and B.

**Figure 6.** Residual contents of soluble protein and polysaccharides at different polymer dosages in (a) test A and (b) test B.

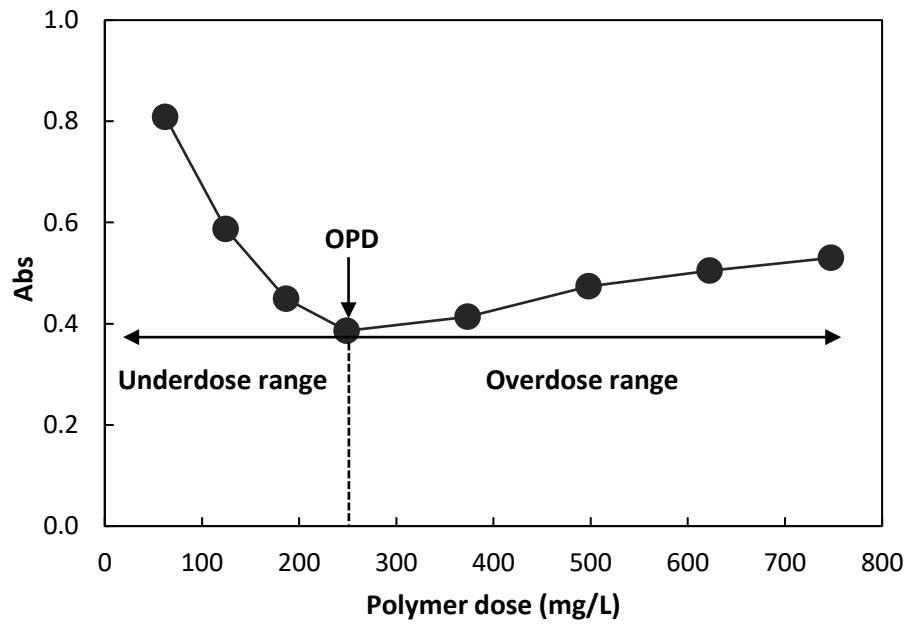
**Figure 7.** Zeta potential at different polymer dosages in tests A and B.

**Figure 8.** Total soluble EPS content removed by 1 mg of polyacrylamide at different conditioning dosages in tests A and B.

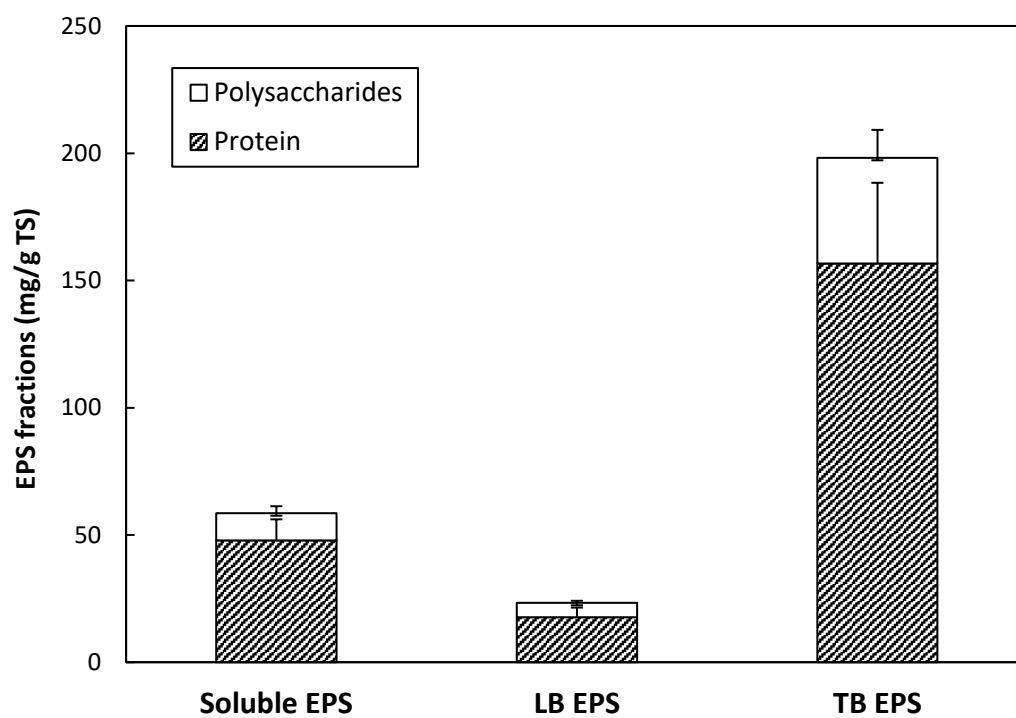
**Figure 9.** Relationship curve of sample absorbance and polymer dose in (a) test A and (b) test B (at a dilution ratio of 1:80).



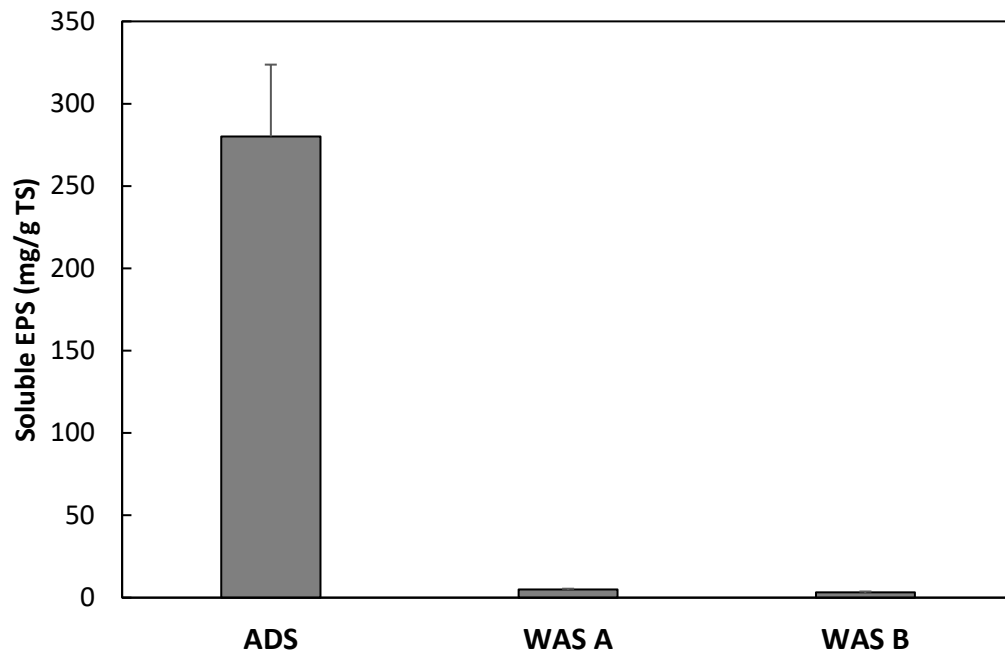
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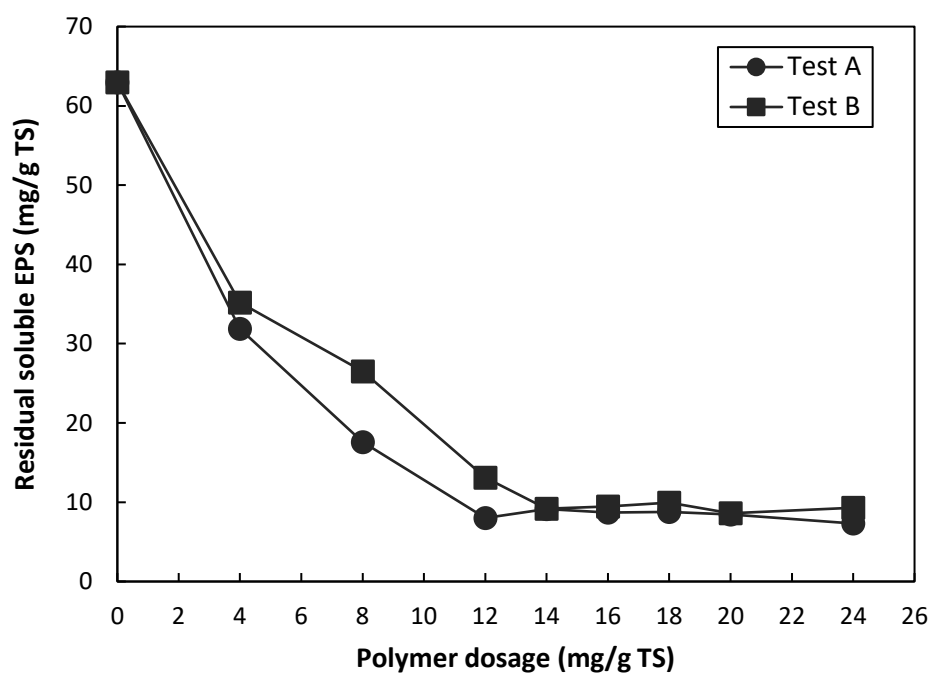
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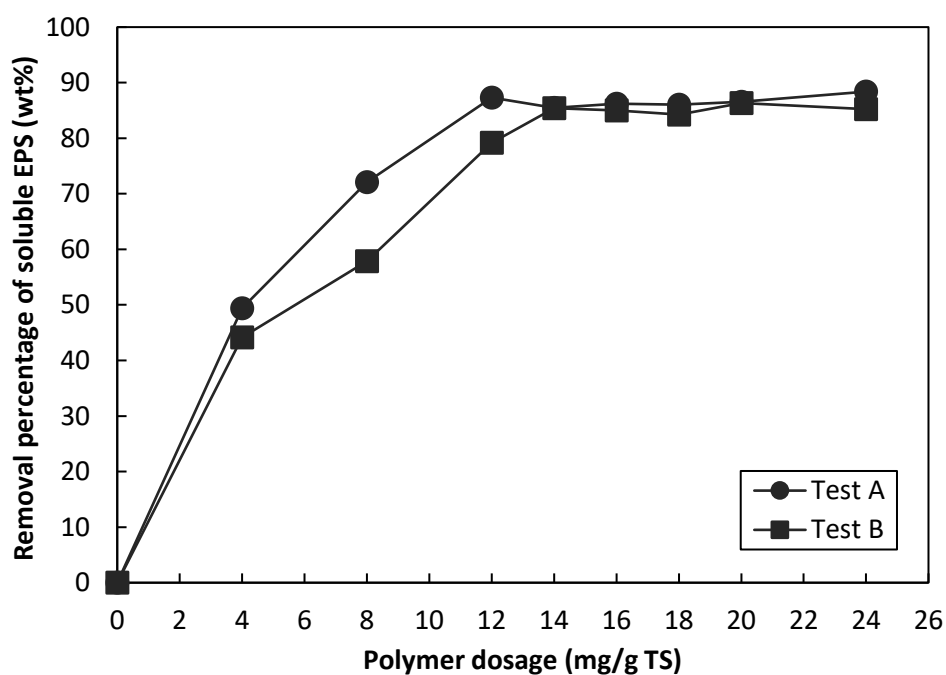
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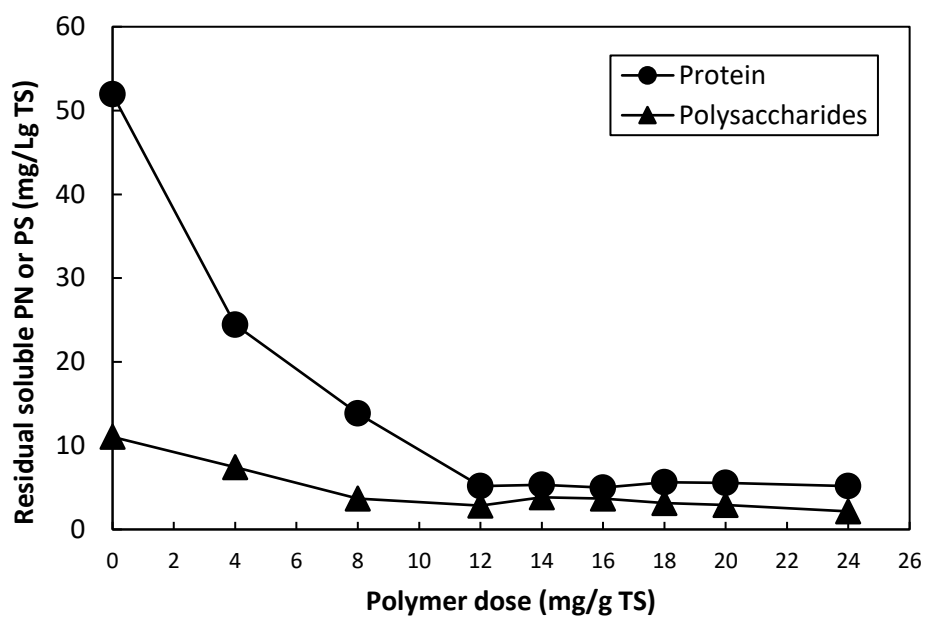


(a)

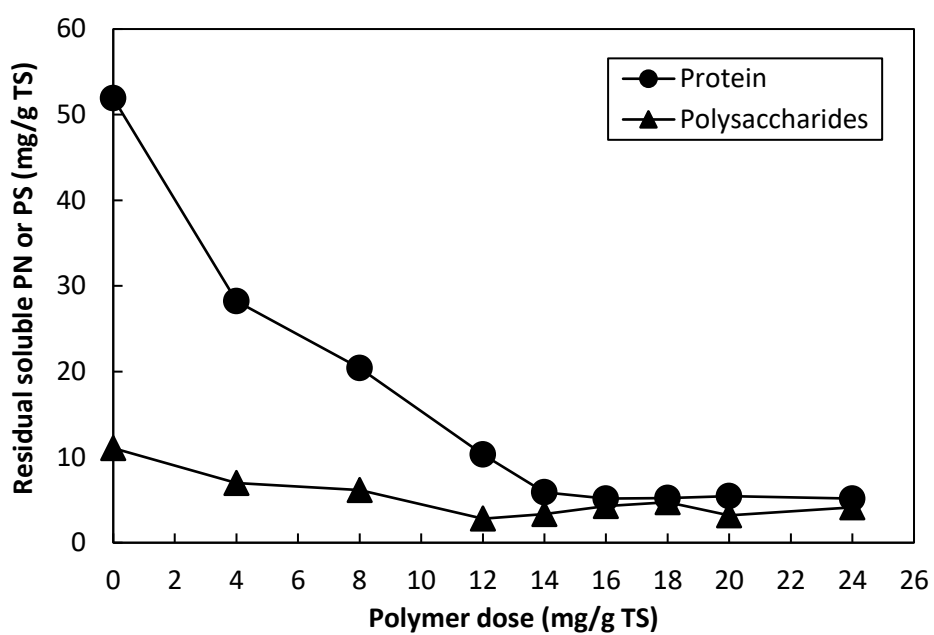


(b)

**Figure 5** (a) Residual soluble EPS content and (b) removal percentage of soluble EPS at different polymer dosages in tests A and B.



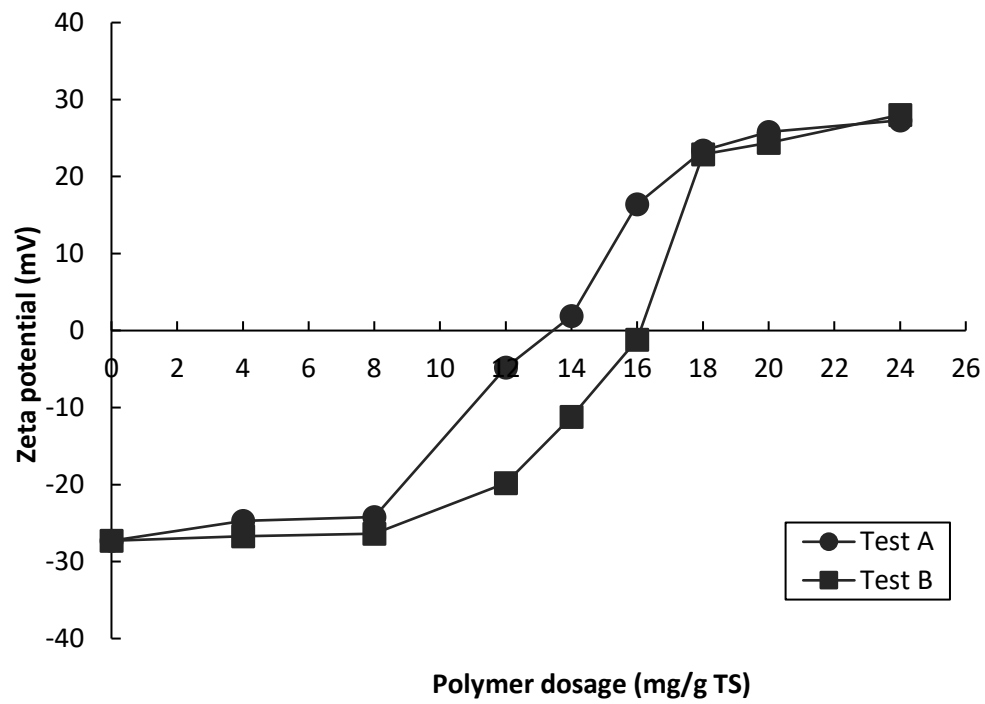
(a)



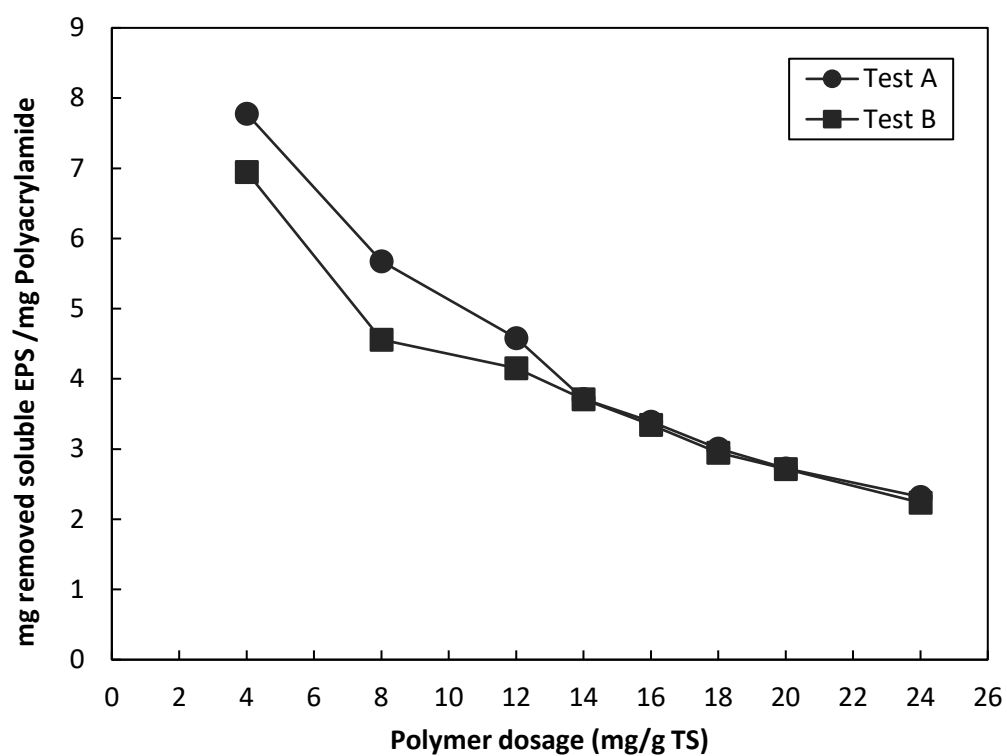
(b)

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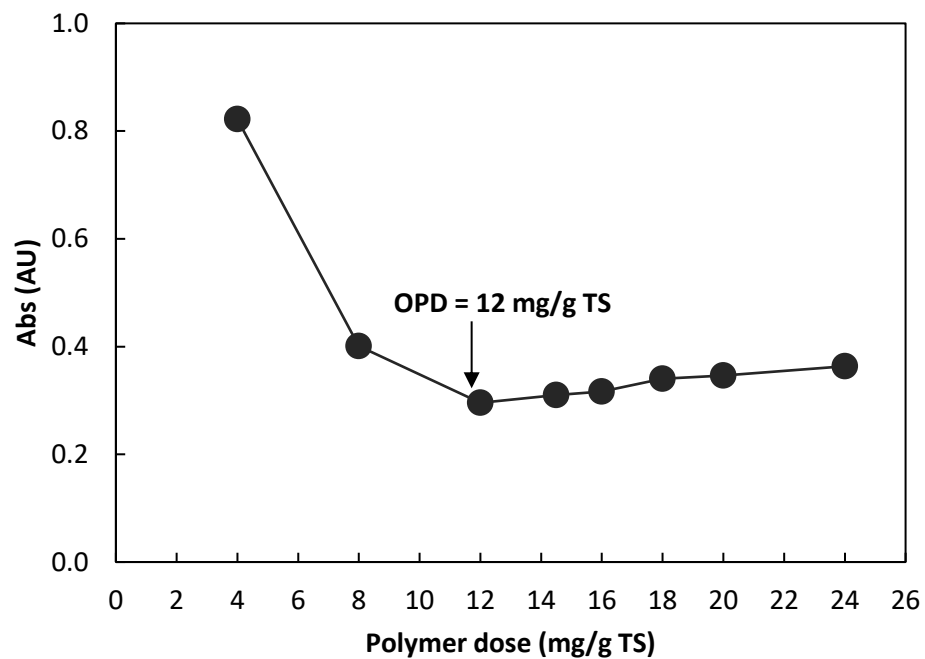




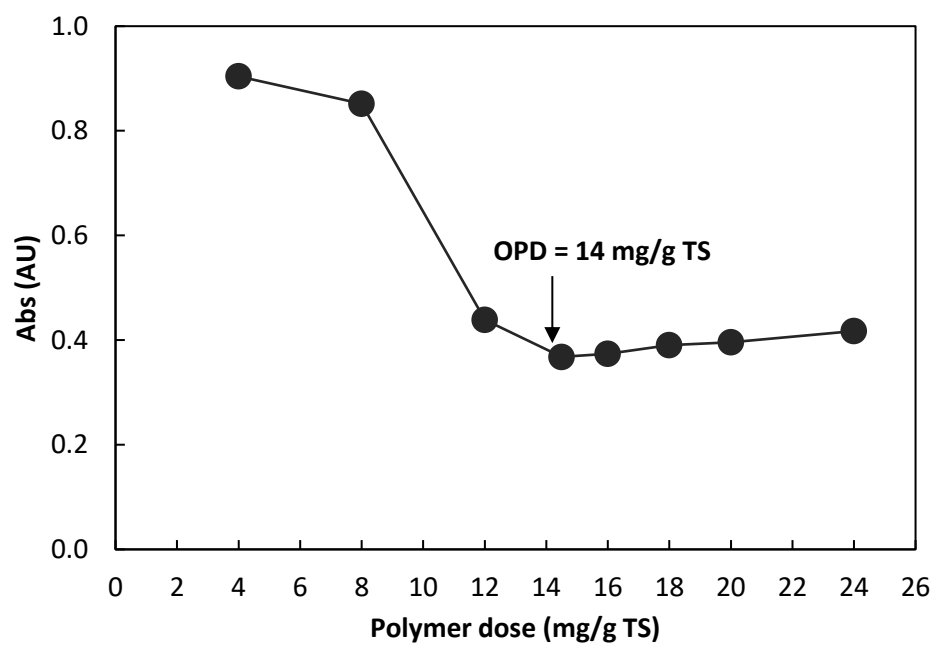
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(a)



(b)

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