

© 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>  
The definitive publisher version is available online at <https://doi.org/10.1016/j.seppur.2020.116557>

**EFFECTS OF EXTRACELLULAR POLYMERIC SUBSTANCE  
FRACTIONS ON POLYACRYLAMIDE DEMAND AND DEWATERING  
PERFORMANCE OF DIGESTED SLUDGES**

Vu Hien Phuong To<sup>a</sup>, Tien Vinh Nguyen<sup>a,\*</sup>, Heriberto Bustamante<sup>b,\*</sup>, Saravanamuthu

Vigneswaran<sup>a</sup>

<sup>a</sup> *University of Technology, Sydney, 15 Broadway, Ultimo NSW 2007, Australia*

<sup>b</sup> *Sydney Water, 1 Smith Street, Parramatta NSW 2150, Australia*

\* Corresponding authors

Tien Vinh Nguyen, Email: Tien.Nguyen@uts.edu.au

Heriberto Bustamante, Email: Heri.Bustamante@sydneywater.com.au

## 1 **Abstract**

2 High polymer demand in sludge conditioning is an intractable aspect of the water industry.  
3 This study investigated the effects of extracellular polymeric substances (EPS) fractions on  
4 polyacrylamide demand for conditioning and dewatering performance. Specifically, it  
5 examined aerobically and anaerobically digested sludges from seven full-scale wastewater  
6 treatment plants (WWTPs). Our study successfully quantified the contributions of soluble EPS  
7 to polyacrylamide demand during conditioning and explained the role of tightly bound EPS  
8 (TB-EPS) in determining the digested sludges' dewatering performance. Results show that the  
9 concentrations of soluble EPS in the sludges varied between 92 – 1148 mg/L. Experimental  
10 results also demonstrated that between 25% - 80% of polyacrylamides used for conditioning  
11 were wasted in “parasitic” reactions with soluble EPS. The residual cationic polyacrylamide  
12 left in solution, after the parasitic reactions, was substantial and varied between 35 – 254 mg/L.  
13 Despite this outcome, the zeta potential values of dewatered sludge cakes remained negative,  
14 i.e. between -24 – -35 mV. These indicated that the residual soluble cationic polyacrylamides  
15 would not have been absorbed on the negatively charged sludge particles. This explained the  
16 relatively poor performance of the dewatering stage in the treatment plants studied.  
17 Furthermore the results suggested the TB-EPS attached to the sludge particles would be  
18 responsible for the poor dewatering. We postulated that the TB-EPS would gelify and  
19 immobilize the water surrounding the sludge particles. Our study suggested that new and more  
20 effective polymers for conditioning are needed to both: (i) reduce polymer demand; and (ii)  
21 improve the dewatering performance.

22 *Keywords:* Anaerobically digested sludge; aerobically digested sludge; soluble extracellular  
23 polymeric substances; conditioning polyacrylamide demand; tightly bound extracellular  
24 polymeric substances; dewatering performance

## 25 **1. Introduction**

26 The water industry spends hundreds of millions of dollars per year on polyacrylamides for  
27 conditioning and dewatering of wastewater sludge. Polyacrylamide demand can reach up to 20  
28 – 25 kg polymer/dry tonne of biosolids [1]. However, the high level of polyacrylamide demand  
29 does not guarantee an equally high dewatering performance. Sydney Water is in charge of 25  
30 wastewater treatment plants (WWTPs) of which, typically, the cake solids content varies on  
31 average between 19 – 22 wt%.

32 Substantial amounts of research have been carried out over the years in an attempt to better  
33 understand the roles of extracellular polymeric substances (EPS), which mainly comprise  
34 polysaccharides and proteins, on polyacrylamide demand for conditioning and dewatering.  
35 However, any progress that has been made to date is still limited, and this makes the job of  
36 industry specialists more difficult to do. Besides, most of the studies on sludge characterisation  
37 and its impacts on conditioning and dewatering have been carried on undigested waste  
38 activated sludge (WAS). The main hypothesis for the high polymer demand for dewatering is  
39 the presence of biocolloidal materials in solution. Furthermore, Higgins et al. [1] postulated  
40 that the bio-colloids were dislodged fragments of “bio-flocs” of which particle size was below  
41 a mean size of 4.2  $\mu\text{m}$ . Also, working with undigested WAS, Erikson [2] established that  
42 cationic polymers were mainly consumed in neutralization of biopolymers and flocculation of  
43 colloids and, to a lesser extent, in rebuilding floc fragments and improving existing flocs.

44 Excess amounts of highly charged extracellular polymeric substances (EPS) have  
45 resulted in compromising efficient sludge dewatering due to their stable gel-like suspended  
46 structure that holds water [3-5]. The dissolved biopolymers or soluble EPS are often  
47 highlighted as an important factor to determine polymer demand for dewatering and  
48 deterioration of sludge properties [1, 6-8]. The impacts of soluble EPS on conditioning and

49 dewatering differ markedly for different sludge types. When compared to activated sludge,  
50 digested sludge has encountered more serious problems with dewatering because digestion  
51 conditions result in dramatic increases in soluble EPS, especially anaerobic digestion [1, 9, 10].  
52 Novak et al. [8] reported that soluble protein was the most important in identifying conditioning  
53 demand for anaerobically digested sludge. Meanwhile, for aerobically digested sludge, soluble  
54 polysaccharides were the most fundamental. However, Houghton and Stephenson [11] stated  
55 there is an optimum EPS content for sludge dewatering. This means that concentrations of EPS  
56 which are too large or too small can result in destabilization of bio-flocs.

57         Recently, To et al. [12] developed a simple, yet unique experimental design that have  
58 demonstrated that around 87% of the cationic polyacrylamides used in a WWTP was removed  
59 by precipitation with soluble anionic EPS present excessively in anaerobically digested sludges  
60 (ADS). The findings reported by To et al. [12] has opened a new line of research that would  
61 better link academic research to industry needs in regards to understanding what is needed to  
62 reduce polymers chemicals costs and also on dewatering performance.

63         In comparison to soluble EPS, little research has been done on the effects of other EPS  
64 fractions on conditioning and dewatering. It has been documented that TB-EPS exert positive  
65 impacts on bio-flocculation [13] while LB-EPS are found to wield a negative influence on  
66 sludge flocculation [14] and settleability [15].

67         High polyacrylamide demand in sludge conditioning is a common feature of the water  
68 industry. The reason for the modest dewatering performance after conditioning with high  
69 concentrations of high molecular polyacrylamides still remains, to a large extent, unknown.  
70 Yet, answers to these questions are of great importance to the industry in its attempts to: firstly,  
71 reduce significant chemical costs; and secondly, minimize the expenses involved in  
72 transporting biosolids for beneficial reuse applications.

73 Therefore, the objectives of this paper were two-fold:

- 74 (i) Quantify the wastage of cationic polyacrylamides in side reactions with EPS that  
75 contribute to high polymer demand during conditioning of anaerobically and  
76 aerobically digested sludge from several WWTPs
- 77 (ii) Establish the role of different EPS components that limit the dewatering performance  
78 of all sludges studied. .

79

## 80 **2. Materials and methods**

### 81 *2.1. Materials*

#### 82 *2.1.1. Sludge samples*

83 Anaerobically digested sludge (ADS) and aerobically digested sludge (AEDS) samples were  
84 collected from five of Sydney Water's WWTPs that employ anaerobic digestion methods  
85 (Cronulla, West Camden, Glenfield, Warriewood, and Malabar) and two WWTPs using  
86 aerobic digestion (Winmalee and Penrith), respectively. The selected WWTPs use cationic  
87 polyacrylamides for conditioning and centrifuges for dewatering. Details of different types of  
88 sludge being either anaerobically digested sludge (ADS) or aerobically digested sludge  
89 (AEDS) in the selected WWTPs are shown in Table A1 in the Appendix.

90 The sludge samples were collected from June 2016 to September 2018. The samples were  
91 immediately transferred to the laboratory for characterizing their physical and chemical  
92 parameters (pH, zeta potential (ZP), total solids (TS) content, EPS fractions) on the same  
93 sampling days. Samples used for conditioning tests were stored at 4°C (in order to minimize  
94 the microbial activity). Sludge samples were allowed to reach room temperature (20 – 25°C)  
95 before all experiments and analyses were run. General characteristics (TS, pH) of digested  
96 sludge are displayed in Table A2 of the Appendix.

97 *2.1.2. Conditioning chemicals for dewatering*

98 This study utilized cationic polyacrylamides that are currently used by the WWTPs.  
 99 Characteristics of the polyacrylamides are summarized in Table 1. A polyacrylamide solution  
 100 for conditioning was prepared by dissolving the polymer powder in tap water at the same  
 101 concentrations used in the respective WWTPs. The polyacrylamide solutions were prepared  
 102 using a bench-scale agitator (3-blade impeller, Heidolph RZR 2020). Both polymer mixing  
 103 time and aging time were 30 minutes. The polymer solution was used within two days of the  
 104 experiments to minimize degradation and ensure maximum performance.

105 **Table 1**

106 Characteristics of the cationic polyacrylamides used by the respective WWTPs that were  
 107 studied in this research. (Note that all the cationic polyacrylamides used in the WWTPs were  
 108 linear polymers).

<b>Sludge types</b>	<b>Polyacrylamides used in respective WWTPs</b>	<b>Molecular weight</b>	<b>Cationic charge density</b>	<b>Concentration of stock polymer solution used at respective WWTPs (%)</b>
<b>ADS1</b>	FO 3801	Ultra-high	Very high	0.3
<b>ADS2</b>	Zetag 8185	Ultra-high	High	0.25
<b>ADS3</b>	Zetag 8185	Ultra-high	High	0.45
<b>ADS4</b>	Zetag 8165	Ultra-high	High	0.2
<b>ADS5</b>	FO 4190	Ultra-high	Low	0.2
<b>AEDS1</b>	FO 4490	Very high	High	0.3
<b>AEDS2</b>	FO 4290	Very high	Medium	0.4

109

110

111 2.2. *Experimental methods*

112 2.2.2. *EPS extraction protocol*

113 Three EPS fractions, namely soluble EPS, loosely bound EPS (LB-EPS) and tightly bound  
114 EPS (TB-EPS), were extracted from the studied digested sludges using the method proposed  
115 by Higgins et al. [16]. Briefly, 50 ml of ADS or AEDS sludge sample was centrifuged at  
116 3000xg for 15 minutes at 5°C. The supernatant was collected as soluble EPS. After that, the  
117 sediment was resuspended to a volume of 100 ml using a buffer solution (pH = 7) consisting  
118 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. The suspension was mixed at 1500 rpm  
119 for 10 minutes using the 3-blade impeller and then centrifuged at 3000xg for 15 minutes at 5°C.  
120 The bulk solution was collected as LB-EPS.

121 In the next stage, the sediment (after centrifugation) was resuspended to a volume of 100 ml  
122 using 1N NaOH. The suspension was mixed at about 500 rpm for 2 hours using a magnetic  
123 stirrer and centrifuged at 3000xg for 15 minutes at 5°C. The bulk solution was collected as TB-  
124 EPS. The collected solutions of soluble EPS, LB-EPS and TB-EPS were filtered through a 2.7  
125 µm filter paper (Whatman filter paper No. 542). Selection of the filter paper pore size was  
126 based on a study conducted by Higgins et al. [1]. Furthermore, the centrate samples were used  
127 for EPS analysis. In this study, the determined EPS concentration would correspond to the total  
128 amount of protein (PN) and polysaccharides (PS). The protocol is systematically illustrated in  
129 Fig. A1 in the Appendix. In this study, the unit mg/L was used for soluble EPS and LB-EPS  
130 while the unit mg/g TS was used for TB-EPS. The reasons for employing different units for  
131 different components of EPS are explained below:

- 132 - The unit for soluble EPS and LB-EPS is mg/L (mg of EPS per litre of liquid sludge)  
133 which is because these EPS fractions are dissolved (soluble EPS) or partially dissolved  
134 (LB-EPS) cellular components present in the surrounding liquor phase of bio-flocs.



135 These components of sludge actively react in solution with soluble polyacrylamide  
136 particles during conditioning.

137 - The concentration of TB-EPS expressed as mg/g TS was because the species were not  
138 dissolved but were forming parts of the sludge solids. It was also used to compare the  
139 TB-EPS of the different sludge types which have different TS contents.

140

### 141 *2.2.3. Evaluation of the contribution of EPS fractions to polymer demand for conditioning*

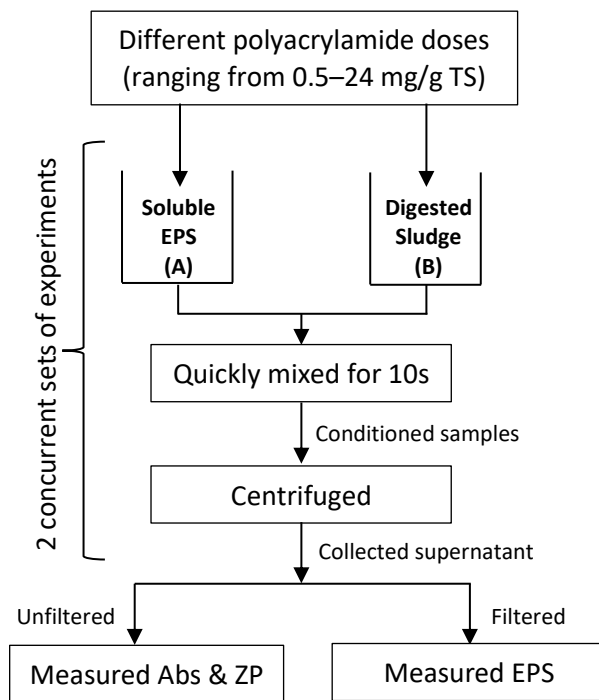
142 We have designed a new, simple, yet unique experiment that has allowed us to quantify the  
143 fate of cationic polyacrylamides when contacted with digested sludges prior to dewatering [9].  
144 The rationale to develop the experiment was based on the high cationic polyacrylamide doses,  
145 expressed in mg/L or mg/g TS, for the conditioning of the estimated relatively low specific  
146 surface area of solids in sludges. Here, mg/L means mg of polyacrylamide (powder) needed  
147 for conditioning of 1 litre of liquid sludge. mg/g TS means mg polyacrylamide (powder) needed  
148 for conditioning of 1 gram of total dry solids of sludge. If TS of a certain sludge is a g/L (g of  
149 dry solid per litre of liquid sludge), the calculation from mg/L to mg/g TS is as follows:

$$150 \quad \text{mg/L} = \text{mg/g TS} \quad (\text{Eq. 1})$$

151 Two sets of conditioning tests were carried out concurrently for both soluble EPS (extracted  
152 in Stage 1 – Fig. A1), called test A, and the whole digested sludge (as-received sludge), referred  
153 to as test B. These sets of experiments were designed to calculate how much cationic  
154 polyacrylamide was consumed by the soluble EPS only. The remainder of the polyacrylamide  
155 was utilized for flocculation of LB-EPS, TB-EPS and sludge particles.

156 Different concentrations of polyacrylamide solution were added to the solution of soluble  
157 EPS and the whole digested sludge. The polyacrylamide concentration was expressed in mg/g  
158 TS (mg of polyacrylamide per g of the total solids content of digested sludge). The

159 polyacrylamide doses ranged from 0.5 – 24 mg/g TS. The mixtures were rapidly mixed at 1000  
 160 rpm for 10 seconds using the 3-blade impeller. Conditioned mixtures were then centrifuged at  
 161 3000xg for 5 minutes and supernatants of the conditioned mixtures were taken for EPS, zeta  
 162 potential and absorbance measurements. Fig. 1 below illustrates the procedure for the  
 163 conditioning tests.



164

165 **Fig. 1.** Schematics of the experimental procedure used to quantify the precipitation of the  
 166 cationic polyacrylamides with the soluble EPS.

167

168 *2.2.4. Procedure to quantify cationic polyacrylamide precipitation*

169 Excess polyacrylamide content in the sludge supernatant from the conditioning tests was  
 170 determined by measuring the absorbance at 191.5 nm (Abs) of the supernatant collected after  
 171 centrifuging conditioned samples [17, 18]. In the present study, the measurement was used for  
 172 samples from both tests A and B. This method was applied to determine the contribution of  
 173 soluble EPS to polymer demand for conditioning. It was done by establishing a relationship

174 between absorbance and polyacrylamide dosage for conditioning. The relationship curve is  
175 presented in Fig. A2 in the Appendix.

176 The relationship curve is divided into three ranges: underdose, optimal dose, and overdose.  
177 The optimum polymer dose (OPD) in each case was the dose that led to the minimum value of  
178 Abs. OPD in test A (OPD<sub>A</sub>) was the amount of polyacrylamide consumed by soluble EPS while  
179 OPD in test B (OPD<sub>B</sub>) was the polyacrylamide demand for conditioning of digested sludge. In  
180 this study, OPD<sub>A</sub> and OPD<sub>B</sub> were considered as the doses that resulted in no un-precipitated  
181 polyacrylamide remaining in the solution after conditioning in tests A and B. The difference in  
182 the OPD values of tests A and B was considered to be the polyacrylamide fraction used for  
183 flocculation of LB-EPS, TB-EPS and sludge particles. The proportion of conditioning  
184 polyacrylamide consumed by soluble EPS was calculated as OPD<sub>A</sub>/OPD<sub>B</sub>. Details for the  
185 procedure have been documented in a recent study [12].

186

#### 187 *2.2.4. Analytical methods*

188 All chemical analyses were carried out in duplicate using chemicals purchased from  
189 commercial sources and used as received. Proteins were measured by the Peterson modification  
190 of the Lowry method [19] using bovine serum albumin (Sigma-Aldrich) as the standard.  
191 Polysaccharides were determined by the Phenol-Sulphuric method [20] using glucose as the  
192 standard. Zeta potential of the sludge particles was measured using the Malvern Instrument  
193 (ZetaSizer Nano ZS-90). pH was measured with a pH meter (Hana, model HI 9025C). The  
194 total solids content was analyzed following Standard Method 2540B [21].

195 UV-vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu) served to measure the  
196 absorbance of the samples at a wavelength of 191.5 nm. The recommended working range was  
197 between 0 and 1 absorbance unit (AU) [17, 18]. The supernatant of ADS had a strong yellowish  
198 color, which interfered with the Abs measurements; as a result, the samples were diluted. These

199 samples were diluted with DI water at an optimal ratio (optimized by reducing the sample Abs  
200 to the recommended working range (0 – 1 AU) of the instrument).

201

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

#### 203 *3.1. General characterization of EPS compositions of different digested sludges*

204 Fig. 2 depicts the EPS composition profiles of digested sludges subjected to analysis. As  
205 noted here the total amounts of EPS in different ADS and AEDS differed, with ADS ( $299 \pm 40$   
206 mg/g TS) slightly higher than AEDS ( $252 \pm 24$  mg/g TS). The concentrations and compositions  
207 of the EPS depended on the digestion processes employed at WWTPs. Thus, ADS consistently  
208 produced higher concentrations of soluble EPS compared with AEDS. The causes for the  
209 variability in both quantity and compositions of the EPS with the upstream processes to the  
210 digestion stage are outside the scope of this study.

211 Regarding the distribution of EPS fractions, a similar pattern was observed for both ADS  
212 and AEDS, with TB-EPS content far outweighing LB-EPS and soluble EPS contents. TB-EPS  
213 contents in ADS were around 75 wt% of total EPS while those in AEDS were almost 97 wt%  
214 of total EPS (Fig. 2d). One reason for the smaller ratio of TB-EPS in ADS compared with  
215 AEDS is that  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ ,  $\text{S}^{2-}$ , and  $\text{HCO}_3^-$  produced during anaerobic digestion, bind  
216 multivalent cations (such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Fe}^{2+}$ ) to form precipitates (such as  $\text{MgNH}_4\text{PO}_4$ ,  
217  $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$ ,  $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ , and  $\text{FeS}_2$ ). This action reduces the concentration of  
218 multivalent cations which consequently may cause TB-EPS to become soluble or LB-EPS [22].  
219 Yu et al. [13] stated that TB-EPS have positive impacts on bio-flocculation. However, this  
220 would not guarantee more efficient dewatering.

221 ADS in this study had larger amounts of soluble EPS (up to 20 times higher – Fig. 2a) and  
222 LB-EPS (up to 8 times higher – Fig. 2b) as compared to those in AEDS. This may be due to

223 the effect of the hydrolysis process of anaerobic digestion where complex organic compounds  
224 in sludge break down into soluble substances. It has been proved that an increase in soluble  
225 EPS content creates more polyacrylamide demand for conditioning [1, 6, 8]. Sheng et al. [23]  
226 claimed that excessive amounts of LB-EPS can result in a weaker aggregation structure and  
227 consequently, poor bio-flocculation. Experimental results also indicate that a higher dose of  
228 polyacrylamide is needed for conditioning of ADS in comparison with AEDS. The soluble EPS  
229 in the ADS was consistently higher (between 281.7 and 1147.5 mg/L) compared with the  
230 concentration of soluble EPS in AEDS (91.2 to 98.6 mg/L).

231

232

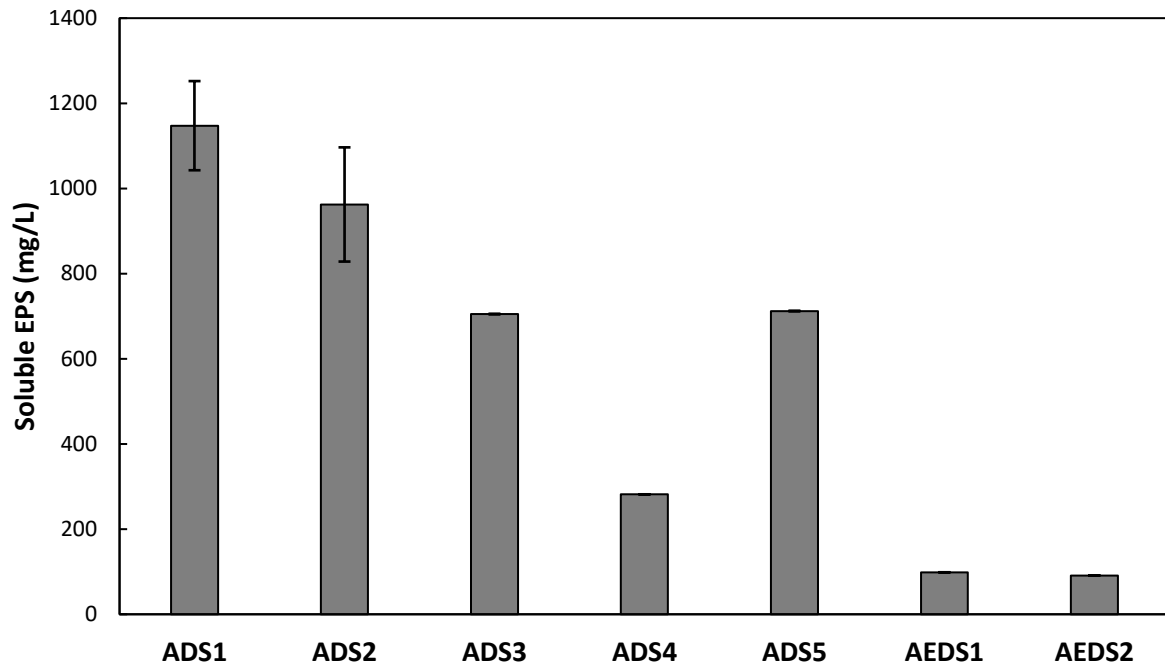
233

234

235

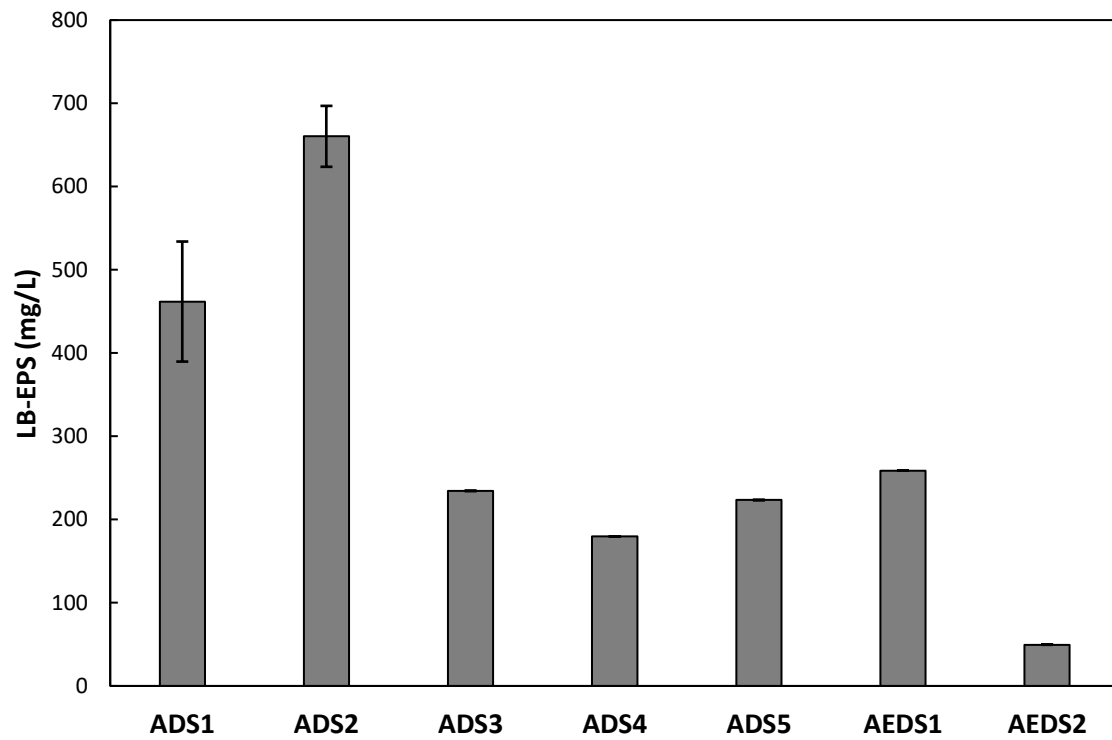
236

237



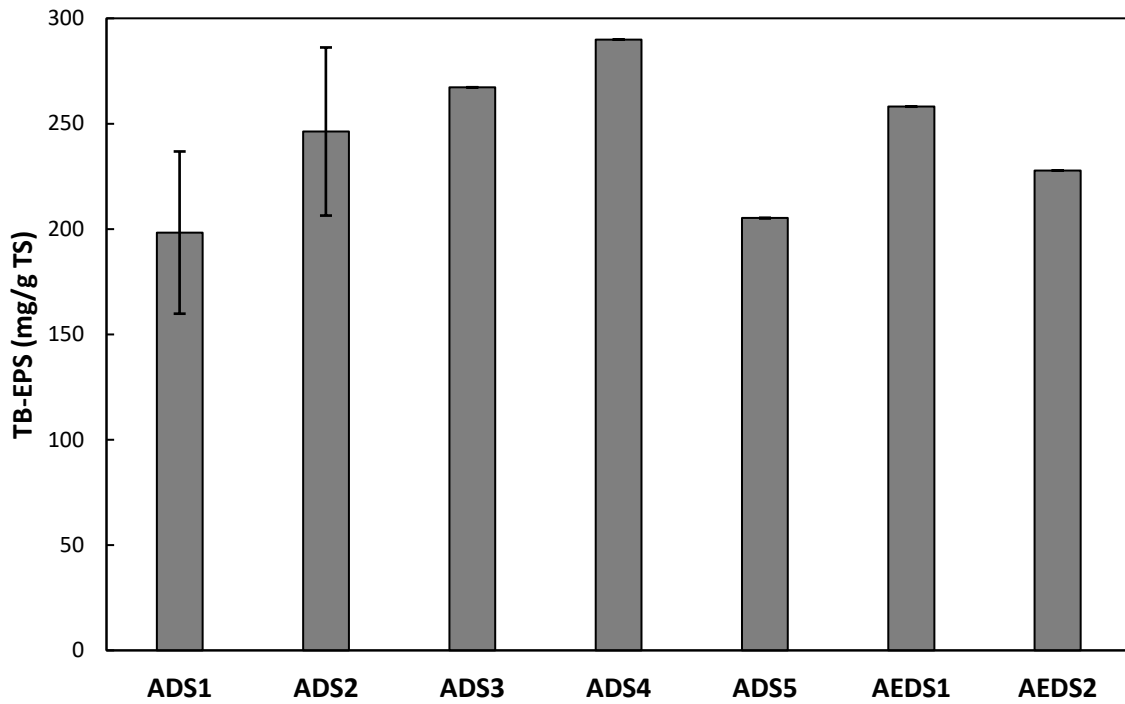
238

239 (a)



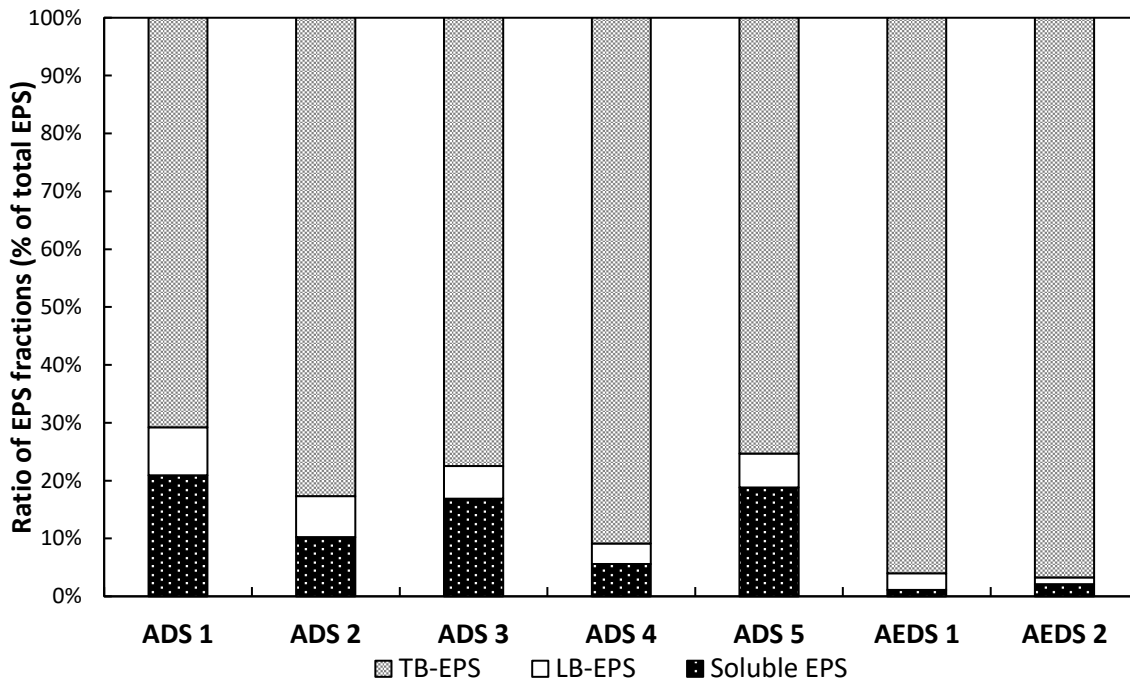
240

241 (b)



242

243 (c)



244

245 (d)

246 **Fig. 2.** (a) Concentration of soluble EPS, (b) concentration of LB-EPS, (c) concentration of  
 247 TB-EPS and (d) EPS fractions percentage. The concentrations of TB-EPS were expressed in  
 248 mg/g TS since TB-EPS are strongly attached to sludge particles.

249 *3.2. Conditioning tests with whole digested sludge and supernatant*

250 Table 2 summarizes the calculated percentage of conditioning polyacrylamides consumed  
 251 by only soluble EPS, which ranged from 25% to 86%. The experimental results demonstrated  
 252 that the parasitic reaction between cationic polyacrylamides and soluble EPS described in To  
 253 et al. [12] also occurred in other six WWTPs that used both anaerobic and anaerobic digestion.  
 254 Results indicate that higher consumption of conditioning polyacrylamides by soluble EPS was  
 255 measured for ADS (40 – 86% of the cationic polyacrylamide dosed) in comparison with AEDS  
 256 (25 – 33%).

257 **Table 2**

258 Percentage of cationic polyacrylamide precipitated by soluble EPS only during conditioning  
 259 (calculation from both tests A and B).

<b>Sludge types<sup>a</sup></b>	<b>% of conditioning polymer precipitated by soluble EPS</b>	<b>Concentration of soluble EPS (mg/L)</b>	<b>OPD<sub>A</sub> mg/L (mg/g TS)</b>	<b>OPD<sub>B</sub> mg/L (mg/g TS)</b>
<b>ADS1</b>	86	1148	215 (12)	250 (14)
<b>ADS2</b>	75	963	187 (6)	249 (8)
<b>ADS3</b>	71	705	86 (7)	121 (10)
<b>ADS4</b>	43	282	48 (3)	111 (7)
<b>ADS5</b>	67	712	56 (4)	83 (6)
<b>AEDS1</b>	33	99	33 (1)	100 (3)
<b>AEDS2</b>	25	91	9 (0.5)	36 (2)

260 <sup>a</sup> Sludge types refer to the upstream processes where each sludge was subjected to either aerobic or  
 261 anaerobic digestion.



262 Table 3 presents the calculated amounts of polyacrylamides (in mg/L and mg/g TS)  
 263 remaining in the solution phase after reacting with soluble EPS in test A when using  
 264 polyacrylamide dosages currently utilized for full-scale conditioning in this study's WWTPs.  
 265 Our results confirmed that a significant amount of "un-precipitated" polyacrylamide remained  
 266 in the liquid phase. Furthermore results show that the amount of "un-precipitated"  
 267 polyacrylamide available for conditioning was much smaller for ADS when compared to  
 268 AEDS. This was attributed to the much smaller amount of soluble EPS in AEDS in contrast to  
 269 that concerning ADS (Table 3).

270 **Table 3**

271 Concentration of polyacrylamide remaining in the solution after the reaction with soluble EPS  
 272 (Test A).

<b>Sludge types</b>	<b>Concentration of polyacrylamide currently used in the WWTPs studied expressed in mg/L and mg/g TS</b>	<b>OPDA mg/L (mg/g TS)</b>	<b>Concentration of un-precipitated polyacrylamide after reaction with soluble EPS in test A mg/L (mg/g TS)</b>
<b>ADS1</b>	250 (14)	215 (12)	35 (2)
<b>ADS2</b>	250 (8)	187 (6)	63 (2)
<b>ADS3</b>	193 (16)	86 (7)	107 (9)
<b>ADS4</b>	111 (7)	48 (3)	63 (4)
<b>ADS5</b>	56 (4)	56 (4)	0
<b>AEDS1</b>	183 (6)	33 (1)	150 (5)
<b>AEDS2</b>	339 (19)	9 (0.5)	330 (18.5)

273

274 *3.3. Zeta potential analysis and full-scale cake solids contents*

275 The results shown in Table 4 indicated that zeta potential values of sludge before and after  
276 conditioning with cationic polyacrylamides were negatively charged. Depending on the  
277 WWTP, the cationic polyacrylamide dosed varied between 4 – 19 mg/g TS (Table 3). Based on  
278 this experimental finding, despite the large excess of cationic polyacrylamide left in solution  
279 following the parasitic precipitation reaction, the zeta potential values of the dewatered cake  
280 remained negative for all cases. This meant that the residual un-precipitated cationic  
281 polyacrylamides would not have been adsorbed on the conditioned sludge particles prior to  
282 dewatering.

283 From Table 4, it is clear that ZP values of dewatered cake were much more negative as  
284 compared to those of sludge before conditioning for all sludge samples. We have hypothesized  
285 that, prior to conditioning, the outer layers which were soluble EPS and TB-EPS contributed  
286 mainly to the sludge particles' surface charges. After conditioning where soluble EPS and,  
287 possibly, LB-EPS were removed by cationic polyacrylamides, TB-EPS which appeared in  
288 much larger amounts compared to other EPS components (Fig. 2) contributed mainly to ZP of  
289 sludge samples.

290

291

292

293

294

295

296

297

298

299

300 **Table 4**

301 Zeta potential values of both ADS and AEDS before cationic polyacrylamide addition and their  
 302 respective biosolids cakes collected from their respective WWTPs after dewatering.

Sludge types	Zeta potential (mV)	
	Digested sludge before conditioning with cationic polyacrylamide	Dewatered cake after sludge is conditioned with the respective cationic polyacrylamide and centrifuged
ADS1	-30.2 ± 2.2	-35.3 ± 3.4
ADS2	-26.3 ± 0.8	-31.3 ± 3.0
ADS3	-22.7 ± 0.6	-24.3 ± 2.7
ADS4	-22.4 ± 0.5	-26 ± 1.3
ADS5	-20 ± 1.1	-28.6 ± 1.3
AEDS1	-22.3 ± 3.1	-28.7 ± 0.5
AEDS2	-17.7 ± 1.9	-23.5 ± 2.8

303

304 Table 5 shows that, regardless if a high dose of polyacrylamide was used for conditioning  
 305 of digested sludges, dewatering performances in most WWTPs studied were relatively poor (20  
 306 wt% on average). Although the sludge samples were collected from different WWTPs with  
 307 different processes upstream digestion, the TB-EPS contents of all sludge samples varied from  
 308 198-290 mg/g TS (Table 5). Generally, there was not much difference in TB-EPS between ADS  
 309 (about 241 ± 40 mg/g TS) and AEDS (about 243 ± 21 mg/g TS) as compared to soluble EPS  
 310 and LB-EPS. These results indicate that: firstly, the TB-EPS would determine the effectiveness  
 311 of dewatering; and secondly, there would be a relationship between this EPS fraction and  
 312 dewatering performance of digested sludge. This could be due to the differences in the chemical  
 313 natures of TB-EPS. EPS, which include soluble EPS, LB-EPS, and TB-EPS, are mainly

314 composed of proteins, carbohydrates, humic substances, nucleic acids, uronic acids and lipids  
315 [24-29]. These components possess a sticky fibrillar character, which is believed to strengthen  
316 sludge flocs and is subsequently important in high shear dewatering). It provides them with a  
317 pseudoplastic nature [30]. However, it is believed that these fibrillar EPS interact with water in  
318 a similar way as gels do [3]; the result is that EPS can retain up to 95% of moisture. EPS are  
319 also closely linked to bound water which is difficult for mechanical dewatering to release [31].  
320 Yu et al. [13] investigated the chemical characteristics of different EPS fractions. Based on their  
321 results, the protein was mainly found in TB-EPS (97.5–98.3%) while polysaccharides were  
322 distributed uniformly in different EPS fractions. They also found that while divalent cations  
323 were mainly allocated in soluble EPS (over 48.5%), trivalent cations were detected mainly in  
324 TB-EPS (over 97.5%). These chemical natures may help TB-EPS become closely attached to  
325 the surfaces of sludge particles when compared to other fractions of EPS. In effect, it means  
326 that they will not easily react to polyacrylamide during conditioning.

327

328

329

330

331

332

333

334

335

336

337

338 **Table 5**

339 Relationship between TB-EPS and full-scale dewatering performance (in terms of wt% cake  
340 solids content) obtained at the respective WWTPs.

<b>Sludge types</b>	<b>TB-EPS of digested sludge mg/g TS</b>	<b>Concentration of polyacrylamide currently used in the WWTPs studied mg/L (mg/g TS)</b>	<b>Cake solid contents of the obtained by centrifugation at the full-scale plant <sup>a</sup> wt%</b>
<b>ADS1</b>	198	250 (14)	21.1
<b>ADS2</b>	246	250 (8)	20.5
<b>ADS3</b>	267	193 (16)	21.6
<b>ADS4</b>	290	111 (7)	23.9
<b>ADS5</b>	205	56 (4)	28.6
<b>AEDS1</b>	258	183 (6)	20.6
<b>AEDS2</b>	228	339 (19)	18.3

341 <sup>a</sup> Full-scale dewatered cake solids contents obtained at the respective WWTPs by centrifugation after  
342 conditioning with cationic polyacrylamides.

343

344 *3.4. Discussion*

345 *3.4.1. Contribution of soluble EPS to polyacrylamide demand for conditioning of digested*  
346 *sludges*

347 Seven WWTPs were investigated in this study which had different upstream wastewater and  
348 sludge treatment configurations. Thus, both AEDS and ADS samples used in this study had  
349 different characteristics. Regardless of the diverse and varying sources of samples collected and

350 analysed, the parasitic precipitation reactions between polyacrylamides and soluble EPS during  
351 conditioning were observed for all sludge samples, with ADS being more severe than AEDS.

352 In the case of ADS studied, despite the high consumption of the polyacrylamide through the  
353 reaction with soluble EPS, there should be enough polyacrylamide to flocculate the sludge  
354 particles (see Table 3). In practice, ADS with high soluble EPS content requires a larger dose  
355 of polyacrylamides for conditioning. However, most of the polyacrylamide was consumed by  
356 soluble EPS during the sludge conditioning, especially ADS (67 – 86%). The experimental  
357 results indicated that by reducing the soluble EPS during anaerobic digestion, the amount of  
358 polyacrylamide consumed by parasitic reactions could be reduced.

359 In the case of AEDS studied, because of the low content of soluble EPS, a relatively small  
360 amount of the cationic polyacrylamides demand would be sufficient for conditioning (see Table  
361 3). These AEDS WWTPs also required large amounts of polyacrylamide for conditioning and  
362 this resulted in poor dewatering performance. Although the precipitations of cationic  
363 polyacrylamide by the soluble EPS in AEDS were relatively low (Table 3), the concentrations  
364 of the TB-EPS in AEDS samples were high (Table 5). This explains why the dewatering of  
365 AEDS was still low as the un-precipitated polyacrylamides were unable to “penetrate” the  
366 gelified water coating around the sludge particles.

367

368 *3.4.2. Mechanisms of the precipitation reaction between cationic polyacrylamides and soluble*  
369 *EPS*

370 Polyelectrolyte complexes are formed when macromolecules of opposite charge are allowed  
371 to interact. Depending on a variety of factors, it may cause the system to separate into a dilute  
372 phase and a concentrated complex phase, or it may result in a more-or-less compact precipitate  
373 or gel [32, 33]. At this stage, we propose that the parasitic reactions between cationic  
374 polyacrylamides and negatively charged EPS are governed by the same principles that control

375 the formation of “polyelectrolyte complexes”. A substantial body of research in the literature  
376 indicates that polyelectrolyte complexes have a wide variety of industrial applications, such as  
377 pharmaceuticals, cosmetics, paper and others [32-34].

378 The interaction between oppositely charged polyelectrolytes is, however, more complex than  
379 “positive-attract-negative”. The molecular structure of the polyelectrolytes in terms of polar  
380 and non-polar hydrophobic components also plays a role in determining whether a solid  
381 precipitate or a liquid coacervate is formed [35]. Furthermore, research in model polymeric  
382 systems shows that ionic strength plays a role in the formation of the complex polyelectrolytes.  
383 Increases in ionic strength from  $1 \times 10^{-4}$  M to  $1 \times 10^{-3}$  M favored the formation of the complex.  
384 However, higher ionic strengths reduced the formation of the polyelectrolyte complex [36]. The  
385 ionic strength would screen the oppositely charged by electrostatic interactions between the  
386 interacting oppositely charged organic groups. Therefore, any decline in electrostatic  
387 interactions between opposite charges would result in a decrease in the formation of the  
388 polyelectrolyte complex.

389 In this study, the precipitation of the cationic polyacrylamide with the soluble anionic EPS  
390 was observed at ionic strengths of  $1.7 - 7.0 \times 10^{-2}$  M, which are higher than in the case of the  
391 model system studied by De Vasconcelos et al. [36]. Such difference may be due to the much  
392 more complex spatial conformation of the soluble EPS. This illustrates the need to carry out  
393 further research to understand the structure of the complex EPS in sludges. A critical step is to  
394 develop or select conditioning polymers that are unreactive with the ubiquitous soluble EPS,  
395 which would significantly reduce the chemical costs for the water industry.

396

#### 397 *3.4.3. Contribution of TB-EPS to dewatering of digested sludges*

398 Zeta potential value of the digested sludges before conditioning was negative due to EPS.  
399 EPS are mainly semi-digested proteins and polysaccharides that contain carboxylic, phosphate

400 and other anionic groups which are fully ionized at natural pH. The zeta potential of digested  
401 sludges that made contact with larger doses of cationic polyacrylamides were still highly  
402 negative. This demonstrates that the cationic polyacrylamides would firstly react with the  
403 negatively charged soluble EPS and colloidal materials before reaching the sludge particles.

404 It is well established that protein denaturation is a prerequisite for gelation [37]. Proteins  
405 present in both wastewater and sludge during the digestion process would only be partially  
406 degraded. As a result, when most of the water is removed during dewatering, the partially  
407 degraded proteins and polysaccharides present in the sludge will form gels that hold water.  
408 Thus, the cationic polyacrylamides would only have a minimal effect on removing water from  
409 the gel formed by the TB-EPS around the sludge particles. This led to inferior flocculation  
410 performance which was later replicated in the poorer dewatering performance (see Table 5).

411 It is suggested that the TB-EPS (which only varied between 19 - 29 wt% of total sludge  
412 solids content) gelify water molecules and prevent the cationic polyacrylamides from  
413 flocculating the sludge particles. We know this because the biosolids dewatered cakes were all  
414 negatively charged (see Table 4). Our findings suggest that conditioning polyacrylamides for  
415 sludge dewatering should have “anti-gelling” properties in order to effectively flocculate the  
416 digested sludge. Lin et al. [38] have demonstrated that by destroying the structure of EPS with  
417 the enzyme lysozyme it is possible to release bound water. As result, sludge dewatering  
418 performance increased, with cake solids content up to 42 wt%.

419

#### 420 **4. Conclusions**

421 The present study has developed a universal methodology for measuring how  
422 polyacrylamide is consumed during conditioning, and for identifying new and potentially  
423 effective conditioners for dewatering. The new knowledge developed here is fundamental to  
424 finally understanding: firstly, high polyacrylamide demand in conditioning and dewatering; and  
425 secondly, helping the water industry to reduce significant chemical costs.



426 Our study can explain why high conditioning polyacrylamide demand occurs and attributes  
427 it to parasitic reactions between soluble EPS and ultra-high molecular weight cationic  
428 polyacrylamides. The experimental results showed that digested sludges with higher soluble  
429 EPS contents required larger amounts of conditioning polyacrylamides as well as higher  
430 fractions of polyacrylamide that were wasted in reacting with soluble EPS. In the case of ADS,  
431 40 – 86% of polyacrylamide was not used for flocculation of sludge particles while only 25 –  
432 33% of polyacrylamide would be wasted in the conditioning of AEDS.

433 Despite the large amounts of polyacrylamide utilized for conditioning in the WWTPs  
434 studied, their full-scale dewatering performance was poor, in fact between 18 – 20% (with  
435 ADS5 being the exception). Our results showed that the cationic polyacrylamide used had not  
436 interacted with the negatively charged sludge particles after reacting with soluble EPS. We  
437 proposed that the water in the sludge particles was “gelified” due to the large amount of TB-  
438 EPS that ranged between 200 and 250 mg/g TS.

439 In summary, polyacrylamide demand for the purpose of conditioning depends on the  
440 concentration of soluble EPS. Meanwhile, dewatering performance using cationic  
441 polyacrylamides is dependent on the TB-EPS around the sludge particles. We believe that any  
442 improvement in polymers for dewatering will need to meet three main targets, namely: (i) be  
443 unaffected by EPS which would reduce the industry’s chemical costs; (ii) can improve  
444 dewatering despite the large proportions of tightly bound EPS; and (iii) have no effect on the  
445 quality of biosolids and therefore not seriously compromising their advantageous reuse options.

446

## 447 **Acknowledgment**

448 This collaborative research project was supported by Sydney Water. We also thank the  
449 Faculty of Engineering and Information Technology, University of Technology Sydney for a  
450 PhD studentship.

451 **References**

- 452 [1] Higgins, M.J., Y.C. Chen, and S.N. Murthy, *Understanding factors affecting polymer*  
453 *demand for conditioning and dewatering*. 2006, Water Environment Research  
454 Foundation.
- 455 [2] Eriksson, L., *Conditioning of Biological Sludges with Cationic Poly-Electrolytes*.  
456 Water Science and Technology, 1987. **19**(5-6): p. 859-868.
- 457 [3] Keiding, K., L. Wybrandt, and P.H. Nielsen, *Remember the water-a comment on EPS*  
458 *colligative properties*. Water Science and Technology, 2001. **43**(6): p. 17-23.
- 459 [4] Neyens, E., J. Baeyens, and R. Dewil, *Advanced sludge treatment affects extracellular*  
460 *polymeric substances to improve activated sludge dewatering*. Journal of hazardous  
461 materials, 2004. **106**(2-3): p. 83-92.
- 462 [5] Mowla, D., H.N. Tran, and D.G. Allen, *A review of the properties of biosludge and its*  
463 *relevance to enhanced dewatering processes*. Biomass and Bioenergy, 2013. **58**(0): p.  
464 365-378.
- 465 [6] To, V.H.P., et al., *Modified centrifugal technique for determining polymer demand and*  
466 *achievable dry solids content in the dewatering of anaerobically digested sludge*.  
467 Desalination and Water Treatment, 2016. **57**(53): p. 25509-25519.
- 468 [7] Murthy, S.N. and J.T. Novak, *Factors affecting floc properties during aerobic*  
469 *digestion: implications for dewatering*. Water Environment Research, 1999. **71**(2): p.  
470 197-202.
- 471 [8] Novak, J.T., M.E. Sadler, and S.N. Murthy, *Mechanisms of floc destruction during*  
472 *anaerobic and aerobic digestion and the effect on conditioning and dewatering of*  
473 *biosolids*. Water Research, 2003. **37**(13): p. 3136-3144.

- 474 [9] To, V.H.P., et al., *Novel methodologies for determining a suitable polymer for effective*  
475 *sludge dewatering*. Journal of environmental chemical engineering, 2018. **6**(4): p.  
476 4206-4214.
- 477 [10] Lü, F., et al., *Dewaterability of anaerobic digestate from food waste: Relationship with*  
478 *extracellular polymeric substances*. Chemical Engineering Journal, 2015. **262**: p. 932-  
479 938.
- 480 [11] Houghton, J.I. and T. Stephenson, *Effect of influent organic content on digested sludge*  
481 *extracellular polymer content and dewaterability*. Water Research, 2002. **36**(14): p.  
482 3620-3628.
- 483 [12] To, V.H.P., et al., *Deleterious effects of soluble extracellular polymeric substances on*  
484 *polyacrylamide demand for conditioning of anaerobically digested sludge*. Journal of  
485 Environmental Chemical Engineering, 2019. **7**(2): p. 102941.
- 486 [13] Yu, G., P. He, and L. Shao, *Characteristics of extracellular polymeric substances (EPS)*  
487 *fractions from excess sludges and their effects on bioflocculability*. Bioresource  
488 Technology, 2009. **100**(13): p. 3193-3198.
- 489 [14] Li, X.Y. and S.F. Yang, *Influence of loosely bound extracellular polymeric substances*  
490 *(EPS) on the flocculation, sedimentation and dewaterability of activated sludge*. Water  
491 research, 2007. **41**(5): p. 1022-1030.
- 492 [15] Yang, S.-f. and X.-y. Li, *Influences of extracellular polymeric substances (EPS) on the*  
493 *characteristics of activated sludge under non-steady-state conditions*. Process  
494 Biochemistry, 2009. **44**(1): p. 91-96.
- 495 [16] Higgins, M.J., et al., *Role of protein, amino acids, and enzyme activity on odor*  
496 *production from anaerobically digested and dewatered biosolids*. Water Environment  
497 Research, 2008. **80**(2): p. 127-135.

- 498 [17] Gibbons, M.K. and B. Örmeci, *Quantification of polymer concentration in water using*  
499 *UV-Vis spectroscopy*. Journal of Water Supply: Research and Technology-Aqua, 2013.  
500 **62**(4): p. 205-213.
- 501 [18] Al Momani, F. and B. Örmeci, *In-line and real-time measurement of polymer*  
502 *concentration in water and wastewater*. Journal of Chemical Environmental  
503 Engineering, 2014.
- 504 [19] Peterson, G.L., *A simplification of the protein assay method of Lowry et al. which is*  
505 *more generally applicable*. Analytical biochemistry, 1977. **83**(2): p. 346-356.
- 506 [20] Dubois, M., et al., *Colorimetric method for determination of sugars and related*  
507 *substances*. Analytical chemistry, 1956. **28**(3): p. 350-356.
- 508 [21] APHA, *Standard methods for the examination of water and wastewater*. Vol. 21. 1995:  
509 American public health association Washington, DC.
- 510 [22] Wang, M., et al., *Kinetics of nutrient removal and expression of extracellular polymeric*  
511 *substances of the microalgae, Chlorella sp. and Micractinium sp., in wastewater*  
512 *treatment*. Bioresource technology, 2014. **154**: p. 131-137.
- 513 [23] Sheng, G., H. Yu, and X. Li, *Extracellular polymeric substances (EPS) of microbial*  
514 *aggregates in biological wastewater treatment systems: a review*. Biotechnology  
515 advances, 2010. **28**(6): p. 882-894.
- 516 [24] Goodwin, J.A.S. and C.F. Forster, *A further examination into the composition of*  
517 *activated sludge surfaces in relation to their settlement characteristics*. Water research,  
518 1985. **19**(4): p. 527-533.
- 519 [25] Fang, H.H.P. and X.S. Jia, *Extraction of extracellular polymer from anaerobic sludges*.  
520 Biotechnology techniques, 1996. **10**(11): p. 803-808.
- 521 [26] Nielsen, P.H. and A. Jahn, *Extraction of EPS*, in *Microbial extracellular polymeric*  
522 *substances*. 1999, Springer. p. 49-72.

- 523 [27] Liu, H. and H.H.P. Fang, *Extraction of extracellular polymeric substances (EPS) of*  
524 *sludges*. Journal of biotechnology, 2002. **95**(3): p. 249-256.
- 525 [28] Raszka, A., M. Chorvatova, and J. Wanner, *The role and significance of extracellular*  
526 *polymers in activated sludge. Part I: Literature review*. Acta hydrochimica et  
527 hydrobiologica, 2006. **34**(5): p. 411-424.
- 528 [29] Cloete, T.E. and D.J. Oosthuizen, *The role of extracellular exopolymers in the removal*  
529 *of phosphorus from activated sludge*. Water Research, 2001. **35**(15): p. 3595-3598.
- 530 [30] De Schryver, P., et al., *The basics of bio-flocs technology: the added value for*  
531 *aquaculture*. Aquaculture, 2008. **277**(3-4): p. 125-137.
- 532 [31] Xiang, Y., et al., *Extraction of sludge protein enhanced by electron beam irradiation*  
533 *and calcium oxide*. Journal of environmental chemical engineering, 2018. **6**(5): p. 6290-  
534 6296.
- 535 [32] Dakhara, S. and C. Anajwala, *Polyelectrolyte complex: A pharmaceutical review*.  
536 Systematic Reviews in Pharmacy, 2010. **1**(2): p. 121.
- 537 [33] Van der Gucht, J., et al., *Polyelectrolyte complexes: bulk phases and colloidal systems*.  
538 Journal of colloid and interface science, 2011. **361**(2): p. 407-422.
- 539 [34] Ankerfors, C., *Polyelectrolyte complexes: their preparation, adsorption behaviour and*  
540 *effect on paper properties*. 2008, KTH.
- 541 [35] Jha, P., et al., *pH and salt effects on the associative phase separation of oppositely*  
542 *charged polyelectrolytes*. Polymers, 2014. **6**(5): p. 1414-1436.
- 543 [36] De Vasconcelos, C.L., et al., *Effect of molecular weight and ionic strength on the*  
544 *formation of polyelectrolyte complexes based on poly (methacrylic acid) and chitosan*.  
545 Biomacromolecules, 2006. **7**(4): p. 1245-1252.
- 546 [37] Nishinari, K., H. Zhang, and S. Ikeda, *Hydrocolloid gels of polysaccharides and*  
547 *proteins*. Current opinion in colloid & interface science, 2000. **5**(3-4): p. 195-201.

548 [38] Lin, F., et al., *Effect of extracellular polymeric substances (EPS) conditioned by*  
549 *combined lysozyme and cationic polyacrylamide on the dewatering performance of*  
550 *activated sludge*. Chemosphere, 2019. **235**: p. 679-689.

551

552

553 **Appendix**

554 **Table A1**

555 Information on digestion processes of the WWTPs studied.

<b>WWTPs</b>	<b>Sludge types</b>	<b>Digestion process</b>
<b>Cronulla</b>	<b>ADS1</b>	Two-phase (acid phase and gas phase) mesophilic anaerobic digestion of mixed sludge (waste activated sludge (WAS) & primary sludge (PS))  SRT <sup>a</sup> = 22.5 days
<b>West Camden</b>	<b>ADS2</b>	Two-phase mesophilic anaerobic digestion of mixed sludge (WAS & PS)  SRT = 46.5 days
<b>Glenfield</b>	<b>ADS3</b>	One-stage mesophilic anaerobic digestion of mixed sludge (WAS & PS)  SRT = 18.5 days
<b>Warriewood</b>	<b>ADS4</b>	One-stage mesophilic anaerobic digestion of mixed sludge (WAS & PS)  SRT = 19 days
<b>Malabar</b>	<b>ADS5</b>	One-stage mesophilic anaerobic digestion of PS  SRT = 26 days
<b>Winmalee</b>	<b>AEDS1</b>	Aerobic digestion of WAS  SRT = 22.5 days
<b>Penrith</b>	<b>AEDS2</b>	Aerobic digestion of WAS  SRT = 4.5 days

556 <sup>a</sup> SRT: Sludge Retention Time

557

558

559 **Table A2**

560 General characteristics (TS, pH) of 7 digested sludge studied.

<b>Sludge types</b>	<b>TS (%)</b>	<b>pH</b>
<b>ADS1</b>	2.0	7.6
<b>ADS2</b>	3.2	7.5
<b>ADS3</b>	1.2	7.1
<b>ADS4</b>	1.6	7.3
<b>ADS5</b>	1.4	7.1
<b>AEDS1</b>	3.4	7.2
<b>AEDS2</b>	1.8	6.6

561

562

563

564

565

566

567

568

569

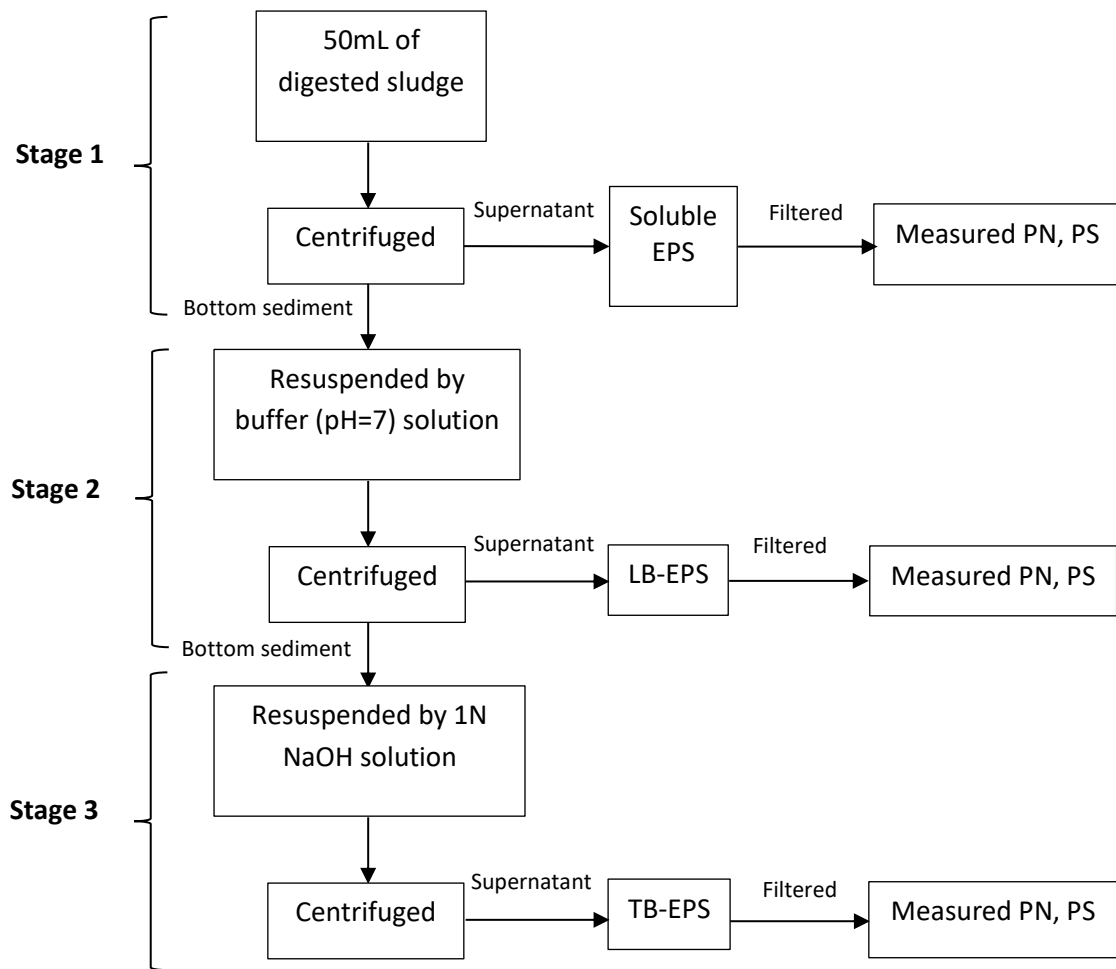
570

571

572

573





574

575

576 **Fig. A1.** EPS extraction protocol.

577

578

579

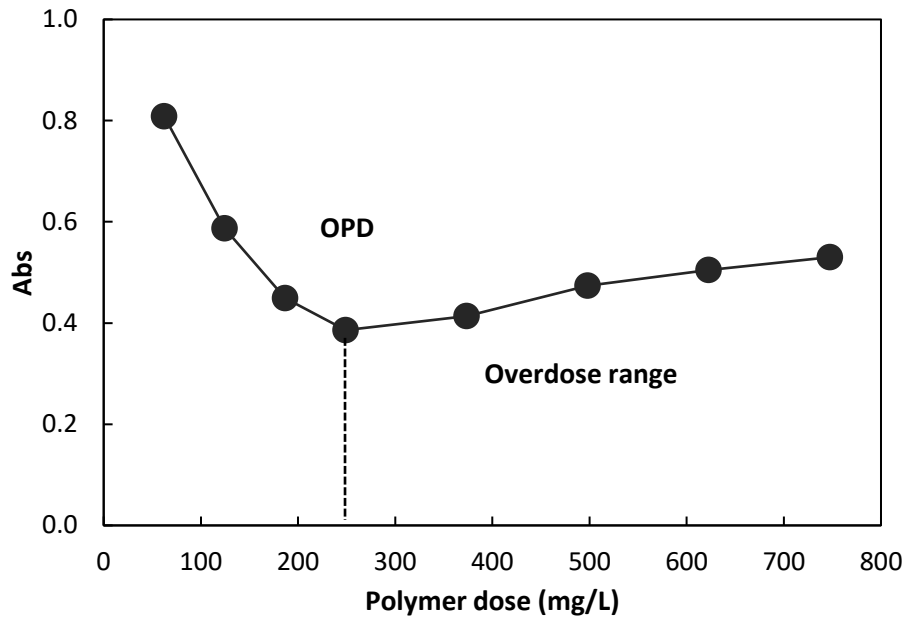
580

581

582

583

584



585

586 **Fig. A2.** Relationship curve between absorbance of the supernatant and polymer dosage for  
587 conditioning (adopted from a project involving Sydney Water).