



Modified centrifugal technique for determining polymer demand and achievable dry solids content in the dewatering of anaerobically digested sludge

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Received 20 November 2015; Accepted 17 February 2016

ABSTRACT

This study aims to characterize anaerobically digested sludge (ADS) and correlate the sludge characteristics in terms of soluble organic compounds with polymer demand (PD) during sludge conditioning. The PD required to achieve maximum dewatering of the ADS studied is in the range of 8–10 kg polymer/dry ton. The commonly used capillary suction time parameter to evaluate the solid–liquid separation ability was not a reliable indicator for assessing dewatering. Instead, in this study, a modified centrifugal technique proposed by Higgins (Higgins MCT) was used to assess the maximum achievable dry solids content of the biosolids cake. The Higgins MCT is readily obtained using a bench-scale centrifuge equipped with a modified centrifuge bucket. Using the Higgins MCT, the maximum dry solids contents obtained from conditioned ADS was 30 wt%. These values were comparable to the dry solids content obtained from the same sludge at full-scale level. Our results suggest Higgins MCT is suitable for assessing the final dry solids content and simulating the dewatering process.

Keywords: Capillary suction time; Optimal polymer dose; Modified centrifugal technique; Sludge characteristics

1. Introduction

Sludge management is an important step in wastewater treatment. The sludge from primary and

secondary sedimentation in a wastewater treatment plant (WWTP) contains a significant amount of organic matter. Prior to disposal or beneficial reuse, sewage sludge must be treated by either aerobic or anaerobic digestion to reduce detrimental undesirable effects of waste sludge on environment. The digested

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sludge is then dewatered to remove as much water as possible to minimize subsequent costs of transportation or any further processing [1,2]. It was reported that 30–50% of annual treatment operating costs attributed to sludge dewatering stage [3,4]. Sewage sludge in general, and anaerobically digested sludge (ADS) in particular, possess a highly complex nature with wide variation in their physical, chemical, and biological characteristics [5,6]. Therefore, it is necessary to characterize key sludge properties in detail to evaluate their effects on conditioning and dewatering [7]. Novak et al. [8] found that soluble biopolymers, mainly protein and polysaccharides, released to the supernatant during digestion, can neutralize a portion of the polymers used for conditioning, resulting in a high polymer demand (PD). In other words, the polymer used for conditioning would have to satisfy the PD of the liquid phase prior to the polymer being available to flocculate the sludge particles.

Previous studies have focused mostly on optimizing the conditioning regimes, such as polymer type, optimal polymer demand (OPD), mixing intensity, and yet have largely overlooked the effect of dewatering devices on conditioned sludge [9]. Different dewatering devices, such as belt presses and centrifuges, have different operations and shear intensity, which greatly affects the efficiency of sludge dewatering. As a result, it is not feasible to use only one dewaterability indicator for all dewatering processes [10]. Additionally, traditional dewatering indicators such as specific resistance to filtration (SRF) and capillary suction time (CST) have been mainly used for evaluating filterability of sludge [11,12]. There seems to be no reliable test properly reflecting the solid–liquid separation ability of sludge.

SRF and CST are the two most common techniques currently used for assessing sludge filterability [11] or dewaterability rates [12]. The SRF developed by Coackley and Jones [13] is relatively independent of suspended solids (SS) concentration at values higher than 0.5%, and as a result, SRF could be applied for sludges with different solids content [14]. However, SRF only measures the kinetics of dewatering and cannot determine the final dryness of the cake. The filtration compression cell test, one of the modifications of original SRF test, was proved to be effective in predicting the final cake solids content of the belt filter presses [15]. In comparison, CST measurement developed by Baskerville and Gale [16] is a popular, fast, and versatile technique [17]. However, the CST test can only be used to determine the most favourable flocculation condition rather than sludge dewatering.

In addition, the CST test cannot be used to predict the final cake dry solids content. Since charge neutralization is considered to be one of the major mechanisms governing flocculation during polymer conditioning [17], the reduction in the negative zeta potential (ZP) values of conditioned sludge compared with the unconditioned sludge were possibly used to estimate the PD.

The crucial role of dewatering is to maximize the dry solids. Industrial centrifuges achieve dewatered cakes with dry solids content typically in the range of 26–30%, while dry solids content of less than 22% is considered as constituting ineffective dewaterability [18]. Conventional methods for measuring dewaterability barely resemble the actual sludge dewatering processes [19]. SRF and CST are measured in the absence of a “force” that drives the water out of the sludge. CST measures dewaterability under gravity and SRF does it under mild vacuum [17]. Meanwhile full-scale dewatering operates at high force to extract water from the sludge flocs. One should therefore establish a method that is able to not only estimate the final cake solids concentration but also simulate the real dewatering process.

It has been found that the stresses imparted to sludge during dewatering have a significant impact on dewatering efficiency in terms of cake solids content. In a previous study, Higgins et al. [20] utilized Gt value in determining the effect of shear stress or mixing intensity on OPD using a calibrated lab-scale mixer. In this case, G is the velocity gradient (s^{-1}) and t is time of mixing (s). Using this dimensionless parameter that quantifies mixing, they determined the stresses of dewatering devices applied on sludge using shear stress as equivalent. However, similar to CST tests, the final cake solids achievable at full-scale cannot be predicted with their bench-scale method [21]. Recently, Higgins et al. [22] have developed the Higgins modified centrifugal technique (Higgins MCT) to overcome difficulties associated with assessing sludge dewaterability using a centrifuge. The method proposed by Higgins et al. [22] is further evaluated in this study. The centrifugal force applied on the sludge cake is measured using a dimensionless parameter gt , which is the product of relative centrifugal force or g (which is related to centrifuge rotating speed and rotor radius) and centrifugation time t (s). The modified centrifugal measurement which proved to be suitable in estimating the final cake concentration as well as simulating the real dewatering process could help overcome the difficulties and shortcomings that traditional techniques have encountered.

The aims of this study are as follows:

- (1) Characterize the ADS obtained from a WWTP.
- (2) Determine correlations of sludge characteristics, such as soluble protein and polysaccharides, with PD and sludge dewaterability.
- (3) Establish whether PD can be reduced whilst improving dewatering performance.
- (4) Establish whether the laboratory scale Higgins MCT can be used to predict the performance of full scale centrifuges.

2. Materials and methods

2.1. Materials

2.1.1. Sludge

ADS was obtained from the Wollongong WWTP, New South Wales, Australia. The plant treats about 45,000 m³ wastewater/d (with a population equivalent of 200,000 people). The average concentrations of COD, NH₃-N, PO₄³⁻-P in the influent wastewater are 113, 68, and 47 mg/L, respectively. This sludge is a blend of anaerobically digested primary and waste activated sludge. It was collected from a sampling point before polymer conditioning at the plant as it enters the centrifuge. Samples at five different times (from September 2013 to March 2014) were collected to characterize the physical and chemical parameters. Parameters measured were: temperature, pH, ZP, total solids (TS) content, volatile solids (VS) content, soluble COD, soluble protein (sP), and polysaccharides (sPS). The dewatered cake and centrate were also collected at the outlet of the centrifuge to evaluate the sludge dewatering efficiency at the plant and centrate quality. This was done by determining cake solids content and SS, respectively.

2.1.2. Polymer

Zetag8165, currently used in Wollongong WWTP, was used in this study. This is a very high molecular weight and medium-high charge cationic polyacrylamide-based polymer. High-charge cationic polyacrylamide is used in the Wollongong WWTP as it is appropriate for the ADS (with negative charge surface). A stock polymer solution was prepared by dissolving Zetag8165 in distilled water at a concentration of 0.1% w/v.

2.2. Experimental methods

2.2.1. Conditioning test

Experiments were carried out by transferring 500 mL of sludge sample into 1 L beakers. Different

pre-determined amounts of the stock polymer solution were mixed with the sludge using a bench-scale agitator. Conditioned sludge was used for the CST tests (to determine OPD and optimal mixing intensity) as well as ZP measurement (to determine charge neutralization point) and modified centrifugal tests. Each test was done in duplicate and the average value is reported.

2.2.2. CST test

Although CST test is not a reliable indicator for dewaterability, it has still been used popularly in many previous studies to determine OPD by virtue of its quick and simple technique. However, the OPD determined by CST method (OPD_{CST}) may not lead to best dewatering efficiency due to the method's previously mentioned shortcomings. As a result, in the present study, CST test was utilized together with the new method (MCT test) so that those shortcomings could be overcome.

For OPD determination, CST values of conditioned sludge with different polymer doses were measured and the dose that resulted in the lowest value of CST method was defined as optimum. For optimal mixing intensity determination, after mixing at a pre-determined mixing time between 30–300 s and a mixing speed of 100–500 rpm, conditioned sludge samples were used for the CST tests to identify mixing condition that led to the shortest CST.

CST was determined using 304B Portable CST Unit, Triton Electronics Ltd, UK using Whatman paper No. 17 (which is a standard grade of chromatography paper). Details on the procedure are given elsewhere [23].

2.2.3. ZP test for determining the OPD

ZP was measured using Malvern Instrument (ZetaSizer Nano ZS-90). ZP values of both unconditioned and conditioned sludge were measured after these sludge samples were diluted 50 times. For conditioned samples, sludge flocs were first shaken to break them into small particles and the supernatant was taken 10 min later for measurement. Polymer dose leading to 0 mV of ZP (OPD_{ZP}) was considered to be the charge neutralization point of the conditioning process.

2.2.4. Analytical methods

The ADS sample was centrifuged at 3,000 rpm for 15 min and then the supernatant was filtered using

Whatman paper No. 542 to measure soluble COD, protein, and polysaccharides. The selection of filter paper was based on the study by Higgins et al. [20]. Soluble COD was analysed using Hatch COD vials while soluble protein and polysaccharides were measured using modified Lowry et al. [24] and phenol–sulphuric methods [25], respectively. TS, SS, and VS were measured following Standard methods 2540B, 2540D and 2540E [26], respectively. Temperature and pH of sludge before conditioning were measured by pH meter (Hana, model HI 9025C).

2.2.5. Higgins modified centrifugal technique

A bench-scale centrifuge was modified to ensure that the dewatered cake is kept separate from the centrate. This modified centrifuge was used to determine cake solids content of ADS before and after conditioning. This method was developed by Higgins et al. [22].

In the present study, the centrifuge tubes were modified as shown in Fig. 1. A support was provided to hold the filter paper (Whatman paper No. 4) about half way from the bottom of the centrifuge tube. The sludge sample was placed right on the filter paper and the centrifuge was operated at different gt values (100,000–1,100,000). After centrifuging, the corresponding cake solids were measured. Plots of gt value vs. cake solids content (%) were made and compared at different polymer doses.

Values of gt were determined by the following formula:

$$gt = g \times t \quad (1)$$

Here g is the relative centrifugal force which is related to centrifuge rotating speed (revolutions per minute, RPM) and rotor radius (cm) by the following equation:

$$g = (1.118 \times 10^{-5})RS^2 \quad (2)$$

where t is centrifugation time (s). Table 1 displays the conversion between centrifuge rotating speed and g for 7 cm of rotor radius of the lab-scale centrifuge used in the study.

3. Results and discussion

3.1. Sludge characterization

The average values of the main parameters of five ADS samples collected from Wollongong WWTP are summarized in Table 2. It shows that this sludge sample could represent a typical ADS of Wollongong WWTP, with TS about 25 g/L ($\approx 2.5\%$) and VS/TS around 60%.

The CST value of unconditioned ADS was relatively high ($1,481 \pm 156$ s) in comparison to that of undigested sludge such as waste activated sludge with CST only 70 ± 11 s. This indicates high PD for conditioning and possibly poor dewaterability [23]. This finding is consistent with previous studies which showed that both aerobic and anaerobic digestions deteriorate the sludge conditioning and dewatering [8,27,28]. The ZP at pH 7.5 was -29.6 ± 0.9 mV, which is considered responsible for hydration and electrostatic repulsion preventing the particles naturally forming flocs [29].



Fig. 1. Modified centrifuge tube before and after test. The photo on the left shows the dewatered cake formed above the centrate in the modified centrifugal test.

concurrently. Therefore, even “zero” or low negative surface charge could possibly be considered as overdosing already. Results from different sampling times demonstrate that ZP of -5 mV is enough to obtain dewatered cake of high TS and centrate of low SS.

3.2. Effects of sludge properties on sludge conditioning and dewatering

3.2.1. Correlations of TS content and VS content with CST and OPD

TS content has been considered to be an important parameter that is often used for calculating the amount of conditioning polymer for a given sludge. The original concept of conditioning referred to neutralization of the surface charge of sludge particles using oppositely charged conditioners, which primarily decides the PD for conditioning, until the idea of soluble biopolymers emerged [8,20,27,31]. The polymer dosage is often expressed on a mass basis, commonly expressed as kilograms of polymer per ton of dry solids (kg/t DS), which is practically for the purpose of estimating the cost of conditioning process as well as of comparing different sludge types.

Our results with five samples show that correlations of TS with both CST ($R^2 = 0.15$ —Table 3) and OPD_{CST} ($R^2 = 0.00$ —Table 3) were insignificant. VS has better relationships with CST ($R^2 = 0.38$ —Table 3) and OPD_{CST} ($R^2 = 0.32$ —Table 3) compared to TS. This may be because VS is a parameter representative of the organic matter content, and therefore it has an impact on sludge conditioning and dewatering. However, low correlation coefficient could be due to the fact that VS consists of many different types of organic matter but not all of them require PD.

The experiment results show insignificant relationships of TS and VS with both CST and OPD_{CST} . This demonstrates that PD determined merely on TS may not be accurate, leading to overdosed or under-dosed conditioning of sludge. Therefore, the calculation of polymer needed should consider other influencing factors, including sludge composition, rather than TS alone.

3.2.2. Correlations of soluble biopolymers with CST and OPD_{CST}

Among the parameters studied, soluble biopolymers correlated the best with CST both separately ($R^2 = 0.43$ for sP; $R^2 = 0.63$ for sPS—Table 3) and together ($R^2 = 0.46$ —Table 3), with CST increasing with higher soluble biopolymer concentration. This implies that soluble biopolymers hinder sludge dewaterability.

Novak et al. [8] found that soluble biopolymers, mainly protein and PS are responsible for the excessive PD for conditioning. Similar results were obtained in this study as both soluble protein and PS had good correlations with OPD_{CST} ($R^2 = 0.90$ for protein and $R^2 = 0.97$ for PS—Table 3). These results confirm that soluble biocolloid concentration can be used as an important factor in determining as well as predicting the optimal PD for sludge conditioning.

Since both soluble biopolymers contribute to the PD, correlation could improve when considering these two components together [20]. The data displayed a stronger correlation ($R^2 = 0.92$ —Table 3) for OPD_{CST} compared to protein or PS individually. The relationship between OPD_{CST} and the ratio sP/sPS was also good ($R^2 = 0.83$ —Table 3).

Table 3
Correlations of ADS characteristics with CST and OPD_{CST}

| Sludge characteristics | Correlations with CST | | Correlations with OPD_{CST} | |
|------------------------|------------------------|------------------------------------|-------------------------------|------------------------------------|
| | Correlation equations | Correlation coefficients (R^2) | Correlation equations | Correlation coefficients (R^2) |
| ZP | $y = -88.2x - 1,127$ | 0.25 | $y = -0.7x - 13.2$ | 0.17 |
| TS | $y = 378.6x + 485.3$ | 0.15 | $y = -0.2x + 8.1$ | 0.00 |
| VS | $y = 1747.6x - 1263.2$ | 0.38 | $y = 9.9x - 7.8$ | 0.32 |
| VS/TS | $y = 332.2x + 1274.8$ | 0.02 | $y = 5.1x + 4.2$ | 0.05 |
| sCOD | $y = 0.1x + 1308.6$ | 0.02 | $y = 0.004x + 3.3$ | 0.71 |
| sP | $y = 1.2x + 1211.9$ | 0.43 | $y = 0.01x + 4.1$ | 0.90 |
| sPS | $y = 9.6x + 785.95$ | 0.63 | $y = 0.1x - 0.02$ | 0.97 |
| sP+sPS | $y = 1.04x + 1162.3$ | 0.46 | $y = 0.01x + 3.5$ | 0.92 |
| sP/sPS | $y = 118.4x + 1108.9$ | 0.25 | $y = 1.9x + 1.6$ | 0.83 |

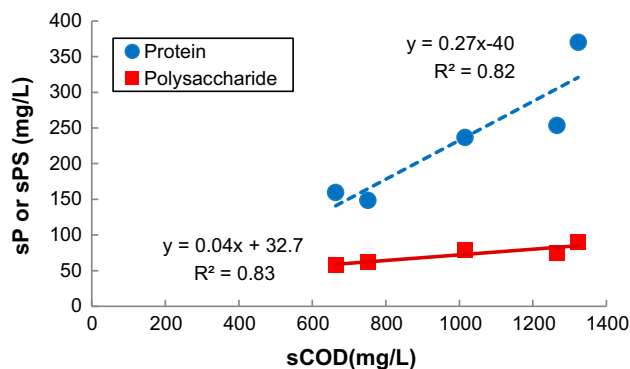


Fig. 2. Relationships between soluble COD and soluble protein and polysaccharides.

3.2.3. Soluble COD as a surrogate measure of soluble biopolymers

Since soluble protein and polysaccharides analyses are not typically used for field measurements due to the specific equipment and reagents requirements, Higgins et al. [20] suggested that soluble COD could be used as a good surrogate parameter. This is supported by the good correlation between OPD_{CST} and soluble COD (with $R^2 = 0.71$ —Table 3) as shown in Fig. 2. In addition, linear relationships of soluble COD with soluble biopolymers were also observed (with $R^2 = 0.82$ for protein and $R^2 = 0.83$ for PS—Fig. 2). This suggests that soluble COD could be used as a substitute to soluble protein and PS in OPD_{CST} determination.

3.2.4. Correlations of ZP with CST and OPD_{CST}

It has been known that one of the main mechanisms of coagulation/flocculation is charge neutralization [32]. However, the results showed a weak negative linear trend ($R^2 = 0.25$ —Table 3) between ZP and CST. This could imply that the more negative ZP, the less effective the sludge dewatering. Similarly, a weak correlation of ZP and OPD_{CST} was observed as

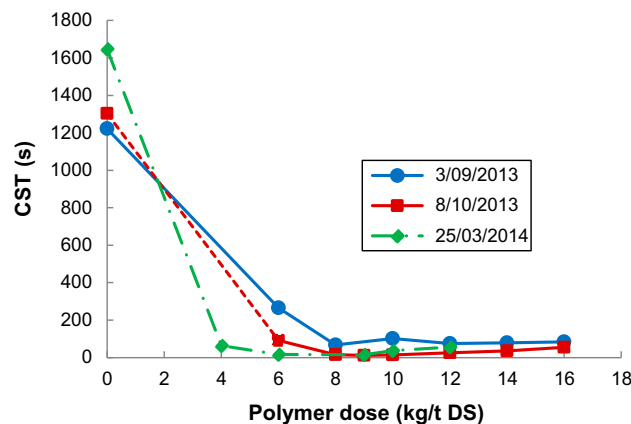


Fig. 3. OPD_{CST} on different sampling days.

shown in Table 3 ($R^2 = 0.17$). However, this technique can provide useful indirect information in determining the PD according to charge neutralization [33].

3.3. Comparisons of different indicators for sludge conditioning and dewatering

3.3.1. Capillary suction time (CST)

The initial results show that mixing speed of 200 rpm and mixing time of 1 min led to the lowest CST value for conditioned sludge at the same polymer doses of the WWTP on the sampling days. Thus, these conditions were used in the subsequent conditioning experiments. Table 4 presents values of OPD_{CST} during different sampling times. It was observed that the CST values rapidly decreased with increasing polymer until a dose of 6–9 kg/t DS and remained almost constant afterwards (Fig. 3). Thus, this dose range was taken as OPD_{CST} for the sludge used. This value was much lower (about 50%) than the currently used polymer dose at the WWTP. It is also understandable that plant operators tend to add extra polymer to ensure that the solids capture is maximized. Hence there will always be more than what is “theoretically” needed.

Table 4

Comparison of OPDs determined by different indicators and polymer dose currently used at the WWTP studied

| Sampling dates | OPD_{CST} (kg/t DS) | OPD_{ZP} (kg/t DS) | Polymer dose at the WWTP (kg/t DS) |
|-------------------|-----------------------|----------------------|------------------------------------|
| 03 September 2013 | 8 | – | 12 |
| 08 October 2013 | 9 | 14 | 12 |
| 04 December 2013 | 6 | 9 | 12 |
| 07 February 2014 | 6 | 10 | 9 ^a |
| 25 March 2014 | 6 | 10 | 9 ^a |

^aPolymer dose at the WWTP was reduced from 12 to 9 kg/t DS since 1/2014.

3.3.2. ZP—Charge neutralization

In comparison with CST tests in determining OPD, using charge neutralization to determine the optimum of polymer dose may result in overdosed conditioning. Results reported in Table 4 show that OPD indicated by ZP (OPD_{ZP}) was about 1.5 times greater than that indicated by CST. This may be due to the fact that charge neutralization is not the only flocculation mechanism. Polymer bridge formation also plays a role in achieving efficient flocculation.

3.3.3. Higgins modified centrifugal technique (Higgins MCT)—A new centrifuge based laboratory scale sludge dewatering

3.3.3.1. Effects of centrifugal intensity (gt) on solids cake content. A modified lab-scale centrifuge device was studied to overcome the difficulties encountered by the traditionally used dewaterability indicators that do not mimic full-scale centrifuges. Effects of centrifugal speed (rpm) and time on sludge dewatering efficiency as individual parameters were also investigated. Fig. 4 displays a graph of cake solids vs. centrifugal time (t) for different g values (Fig. 4(a)) and a graph of cake solids vs. centrifugal speed (g) for different t (Fig. 4(b)). These two graphs reveal a similar trend in that higher g and longer t led to better solids cake content. However, it could be observed that higher g resulted in more significant improvement in solids cake than longer t . This could explain why high speed centrifuges are preferable at WWTPs. Besides, longer t requires bigger size centrifuges.

In fact, operation of centrifuges is characterized by both centrifugal speed and time. Therefore, it is necessary to consider the effect of these parameters together

to fully reflect the centrifugal intensity as well as properly evaluate the efficiency of sludge dewatering by centrifuge.

Fig. 5 presents the plots of cake solids content of unconditioned and conditioned sludge at different gt values. The results show that the increased gt values result in the improvement in cake solids, which implies that the higher the dewatering intensity, the better the dewatering properties in terms of cake solids. However, beyond a certain value of gt , the percentage of cake solids content remained almost the same despite the increase in intensity. Besides, it is noted that without conditioning, the maximum cake solids achievable by centrifuge was only about 16% (Fig. 5(a)). After conditioning with the same polymer dose used at WWTP (12 kg/t DS), the cake solids increased to around 30 wt% (Fig. 5(b)), which was similar to the dewatered cake concentration observed at the WWTP. This demonstrates that the modified centrifugal measurement can simulate the full-scale centrifuge operation and represent the real dewatering performance which was reported to be about 27–29%. In addition, one could notice from Fig. 5 that the maximum cake solids achievable without conditioning (Fig. 5(a)) can be obtained with much lower dewatering intensity after conditioning (Fig. 5(b)). It confirms the need for polymer conditioning treatment prior to dewatering.

3.3.3.2. OPD determination by Higgins MCT. In order to establish relationships between PD and dewatering, modified centrifugal test was carried out at different polymer doses, including the dosage currently used at WWTP and OPD_{CST} (Fig. 6). There was not much difference in DS values obtained at the two polymer doses (Fig. 6(a) and (b)). This means that half of the

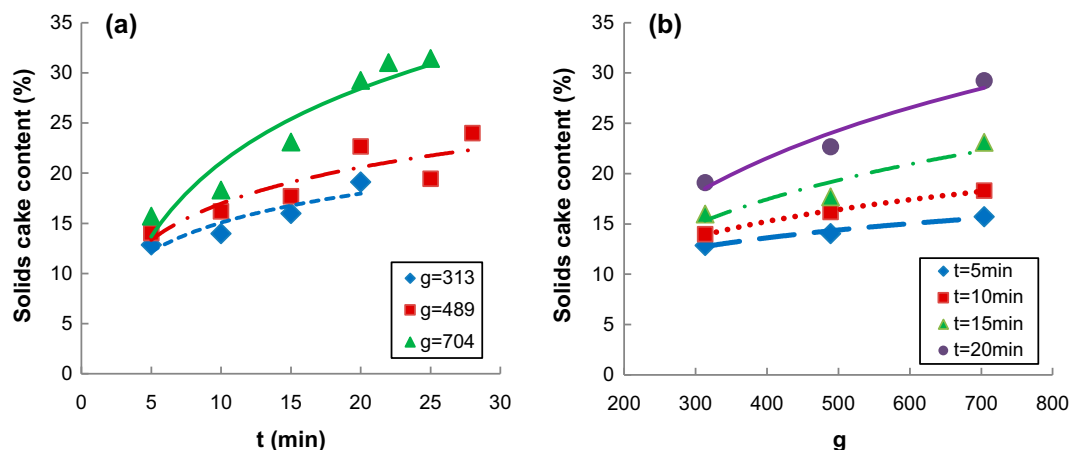


Fig. 4. Effects of (a) centrifugal speed and (b) centrifugal time on solids cake content.

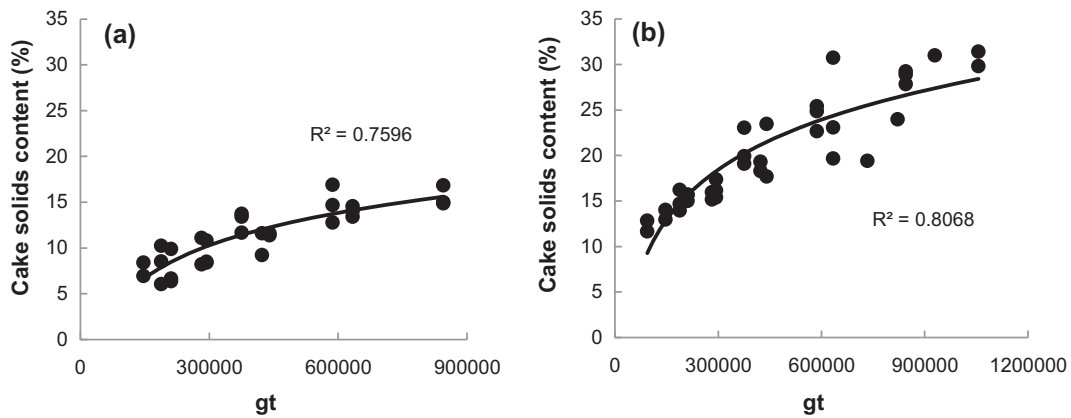


Fig. 5. Cake solids content of ADS (a) without conditioning and (b) with conditioning after centrifuge as a function of *gt* values.

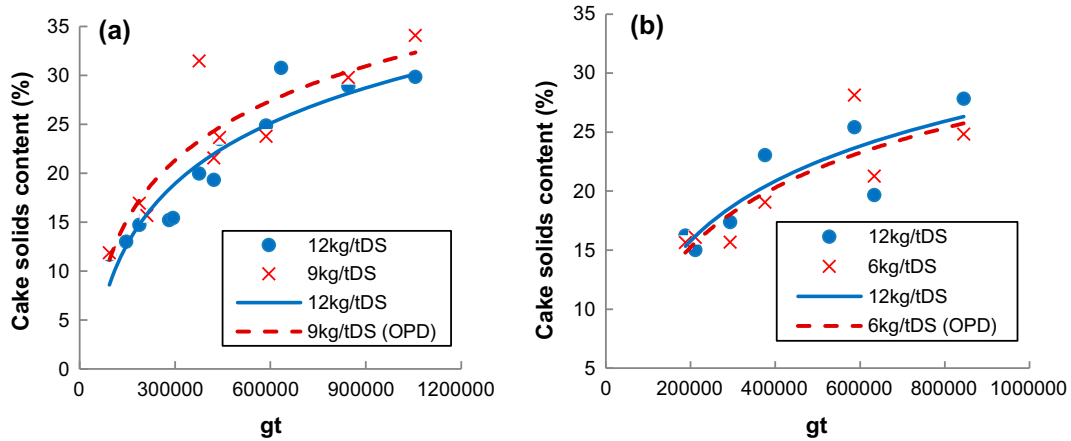


Fig. 6. Effect of *gt* values of centrifuge on cake solids of conditioned sludge on (a) 8 October 2013 and (b) 4 December 2013.

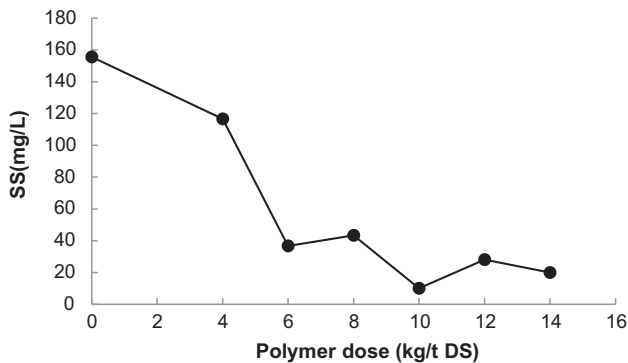


Fig. 7. Effect of polymer dose on SS in the filtrate from the modified centrifugal tests.

used polymer dose at the WWTP could be used to obtain the same cake solids. This results in a significant decrease in the PD. Thus, Higgins MCT can potentially be a representative and more accurate method to estimate the OPD because it determines the cake solids content of the biosolids cake. However, this outcome needs to be validated.

3.3.3.3. *Centrate quality as a controlling parameter of sludge conditioning and dewatering.* The reduction in the polymer dose can lead to a reverse effect on the sludge centrate quality (Sydney Water 2013). Thus, the centrate from modified centrifugal test was collected and its SS was measured in order to evaluate the effect of polymer dose on the SS in centrate. The variation of SS based on polymer dose is presented in Fig. 7.

Fig. 7 shows that SS in the centrate reached its lowest value at a polymer dose of 10 kg/t DS. The reduction in polymer dose from 10 to 8 and 6 kg/t DS led to almost similar values of SS in the centrate (43 and 37 mg/L). However, these values were only slightly higher than that of polymer dose of 12 kg/t DS (28 mg/L). Thus, a lower dose can be applied in the WWTP even in terms of centrate quality. This result is similar to the previous report by Higgins et al. [20] who found that polymer dose of 9.1 kg/t DS is the OPD for the comparable ADS (soluble COD, soluble protein and polysaccharides of 1,048, 285 and 51 mg/L, respectively).

4. Conclusions

The following conclusions can be made based on the results of this experimental investigation:

- (1) There were good correlations between soluble biopolymers and OPD_{CST} , which highlights the major role of soluble biopolymers in deciding conditioning PD. Also, insignificant relationships were observed when sludge characteristics were related to CST.
- (2) ZP could give useful indirect information in determining the PD based on charge neutralization. However, the results from the present study show that using charge neutralization as an OPD indicator may lead to overdosed conditioning since effective flocculation could be achieved both by charge neutralization and polymer-bridging formation. More experiments are necessary to determine the effect of charge neutralization on cake solids content.
- (3) According to CST and Higgins MCT, lower polymer doses (8–10 kg/ton DS) were suitable for conditioning of ADS from the WWTP. Nevertheless, field tests are necessary to confirm this finding.
- (4) Higgins modified centrifugal technique (Higgins MCT) can be successfully used to evaluate the dewatering of ADS with and without conditioning by estimating the maximum solids cake achievable by the centrifuge. However, this study investigated only samples of ADS. Therefore, further studies for other sludge types that are also dewatered by centrifugation are required to prove the universality of this technique.

Acknowledgements

Funding for this study was provided by both Sydney Water and TRILITY. The authors thank the collaboration of Matthew Higgins (mhiggins@bucknell.edu) to test the MCT developed by him.

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