

**EFFECT OF INFLUENT ORGANIC CONTENT ON DIGESTED SLUDGE
EXTRACELLULAR POLYMER CONTENT AND DEWATERABILITY**

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Abstract- The organic composition of the feed sludge to six laboratory-scale anaerobic digesters was adjusted by substituting a proportion of the primary sludge for glucose or propionic acid solution. Substitution with glucose caused an increase in the microbial extracellular polymer content of the digested sludge, which altered the particle size distribution of the sludge and increased the sludge dewaterability. Substitution with propionic acid gave similar results to the control. The relationship between the digested sludge extracted extracellular polymer yield and sludge filtrability determined using the capillary suction time test was significant at the $P = 0.01$ level. The level of extracellular polymer for optimum sludge dewaterability was calculated as 17.2 mg g^{-1} SS. The protein and carbohydrate composition of the extracted polymer did not appear to affect the sludge dewaterability. Results present will allow predictions to be made into the effect that changes in the influent sewage composition has on essential downstream processes.

key words- anaerobic digestion; dewaterability; extracellular polymer; organic amendment; particle size distribution; sludge

INTRODUCTION

Anaerobic digestion is currently the most common process used for sludge minimisation and stabilisation (Hudson and Lowe, 1996), with 50 % of the sludge produced in the European Union being treated in this way (Hall, 1995). The process of anaerobic digestion occurs in a number of discrete stages, the main ones being hydrolysis, acidogenesis and methanogenesis. Each stage is dependent on a different bacterial population, with all of the separate bacterial species living together in direct or indirect symbiotic associations (Gujer and Zehnder, 1983). Prior to disposal digested sludge is generally dewatered, giving a further reduction in the sludge volume and thereby decreasing disposal costs. The reported impact of anaerobic digestion on sludge dewaterability to date is mixed, with some studies indicating that digestion generally improves sludge dewaterability, (Rudolfs and Heukelekian, 1934; Brooks, 1970; Lawler *et al.*, 1986) whilst other studies have reported that digestion degrades sludge dewaterability (Pearson and Buswell, 1931; Karr and Keinath, 1978; Yan *et al.*, 1987; Houghton *et al.*, 2000a).

During anaerobic digestion organic debris is broken down resulting in a change in the particle size distribution (Karr and Keinath, 1978; Lawler *et al.*, 1986; Nellenschulte and Kayser, 1997). The sludge particle size distribution has been shown to be one of the key factors in controlling sludge dewaterability (Karr and Keinath, 1978). Reported differences in the affect of digestion on sludge dewaterability may therefore be related

to the biodegradability of the sludge used and differences in digester efficiency affecting the particle size.

Another parameter that can have an impact on the sludge dewaterability is the sludge microbial extracellular polymer (ECP) content (Ryssov-Nielsen, 1975; Novak *et al.*, 1977; Kang *et al.*, 1989). Microbial ECP is an organic polymer produced by bacterial cells and is frequently responsible for binding cells and other particulate matter together (Wingender *et al.*, 1999). Microbial ECP is highly hydrated and may contain up to 99 % water by weight (Costerton *et al.*, 1981) thereby retaining a high level of water in the sludge. By binding cells and particulate matter together it also changes the particle size distribution of the sludge, which again affects the dewatering process.

Investigations into the effect of microbial ECP on digested sludge dewaterability have been limited to date. Extraction and analysis of ECP from sludge samples obtained from both full-scale (Houghton *et al.*, 2000a) and laboratory-scale (Houghton *et al.*, 2000b) anaerobic digesters has indicated a positive correlation between increasing ECP yield and sludge becoming more difficult to dewater, but the relationship was not always significant at the $P = 0.05$ level.

Poxon and Darby, (1997) found no simple relationship between the quantity of ECP present and sludge dewaterability, though in this case the level of ECP present was quantified using an *in situ* dye adsorption method and the sludge dewaterability calculated using a filterability index based on the capillary suction time (CST) test. Any trend that was present appeared to be dependent on the digester feed composition, with increased quantities of ECP associated with high carbohydrate feed. In this case, the composition of the ECP produced was not investigated. The same trend was also noticed when different individual substrates were used as feed for anaerobic digestion, with glucose consistently producing a sludge with higher levels of extractable microbial

products than acetate, butyrate or propionate (Noguera *et al.*, 1994; Jia *et al.*, 1996; Kuo *et al.*, 1996). The impact on the sludge dewaterability was not determined in any of these studies.

The composition of the ECP also altered with the type of substrate, with ECP extracted from digested sludge fed glucose containing more protein and carbohydrate than the other substrates (Jia *et al.*, 1996). These results indicate that the acidogenic bacteria that utilise glucose in the first stage of the digestion process produce more ECP than the acetogenic bacteria that utilise butyrate or propionate, or the methanogenic bacteria that utilise acetate.

By adjusting the organic composition of the feed sludge to a series of laboratory-scale anaerobic digesters, the aim of this paper was to determine the impact that these alterations had on the digested sludge in terms of microbial ECP yield and composition, particle size distribution and sludge dewaterability. Simultaneous evaluation of each of these sludge parameters will allow predications to be made into the effect that changes in the influent sewage composition has on essential downstream processes.

MATERIALS AND METHODS

Digester feed

The digester feed consisted of either 100 % primary sludge or a mixture of primary sludge and a known quantity of either glucose or propionic acid. Settled primary sludge was collected from Saffron Walden municipal wastewater treatment works (Anglian Water plc., UK). After collection, the sludge was divided into 0.5 and 1.0 l aliquots and blended (Phillips Type: HR2845/AM) for 1 min, before being stored at -20 °C. Before

use, the sludge was defrosted for 24 h at room temperature, followed by heating to ~ 35 °C in a water bath. The mean COD of the collected primary sludge was 67.6 g l⁻¹. Glucose and propionic acid solutions (0.45 M and 0.67 M respectively) containing the same COD as the primary sludge were prepared and used as a substitute for a proportion of the primary sludge. The initial quantity of glucose or propionic acid used in the feed was 10 % by volume. This was increased by a further 5 % by volume every other day until a final volume of 40 % was reached by day 13, after which the feed composition remained constant until day 40. Seed sludge for the digesters was obtained from Milton Keynes municipal wastewater treatment works (Anglian Water plc., UK).

Experimental set-up

Six laboratory scale anaerobic digesters were constructed from 5 l Quickfit[®] fermentation vessels as detailed previously (Houghton *et al.*, 2000b). The digesters were operated as matched pairs using the following feed composition: 100 % primary sludge; primary sludge with glucose; primary sludge with propionic acid. Operating temperature of the digesters was 35 °C at a hydraulic retention time (HRT) of 20 d, with sludge wasted/fed once per day. The digesters were operated for 2 full HRT before being dismantled and the sludge analysed.

Extracellular polymer extraction

Extraction of ECP was carried out using a heat extraction process similar to that of Morgan *et al.* (1990). Samples of sludge (500 ml) were rinsed and then re-suspended in 0.25-strength Ringer's solution, heated to 80 °C for 1 h and cooled. After cooling to room temperature, the polymer was separated from the solid material by centrifugation (1800 G for 20 min followed by 5000 G for 20 min). The extracted polymer was

removed from the supernatant using solvent (3 vol acetone:1 vol ethanol) precipitation, overnight at 4 °C, followed by centrifugation at 5000 G for 20 min. The collected polymer was evaporated to dryness and the yield related to the sludge suspended solids (SS) content.

Dewaterability tests

Sludge filtrability was determined using the capillary suction time (CST) test (Triton CST filtrability tester, model 200, Triton Electronics Ltd., Essex, UK) with standard filter papers (Part No. 815095) supplied by Triton Electronics. Each sludge was analysed 5 times and the results averaged, before being standardised to a SS concentration of 1 g l⁻¹ as detailed in Standard Methods (APHA, 2000).

Sludge drainability was determined using a gravity drainage test (IWPC, 1981). A 200 ml sludge sample was poured into a Hartley pattern buchner funnel and allowed to drain under gravity through a section of belt press filter cloth (Material: 100 % polyester; Wire diameter: 0.35 mm warp, 0.80 mm weft; Yarn count: 40/cm warp, 8.5/cm weft; Weave type: twill; Weight: 1200 g m²; Air permeability: 2790 l m² s⁻¹ 200 pa; Simon-Hartley Ltd. [now discontinued]). The volume of filtrate was measured and recorded at 30 s intervals over a 4 min period. Each sludge was analysed 3 times and the results averaged.

Particle size analysis

The particle size distribution was determined using laser diffraction. Analysis was carried out on a Malvern Mastersizer 2000[®] (Malvern Instruments Ltd., Malvern, UK) at the following settings: stirrer speed 350; pump speed 500; ultrasonic off. The results were recorded as the particle volume percent in 70 discrete size ranges between 0.45

and 2000 μm . All samples were diluted approximately 500-fold in tap water before being analysed 10 times and the results averaged to produce the particle size distribution.

Analytical methods

Total solids (TS), volatile solids (VS) and SS were determined in accordance with Standard Methods (APHA, 2000). Chemical oxygen demand and volatile acid (VA) concentration were determined using HACH Methods 8000 and 8196 respectively (Camlab Ltd., Cambridge, UK). Digester gas was collected over acidified water (0.1 M H_2SO_4), and the volume produced in a set time period measured. All gas measurements were normalised to standard temperature and pressure (stp).

The extracted ECP was re-suspended in distilled water at a concentration of 1 mg ml^{-1} , before being analysed for both protein and carbohydrate content. Protein analysis was carried out using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Carbohydrate analysis was carried out using the method of Dubois *et al.* (1956) with glucose as the standard. The organic fraction of the extracted ECP was determined gravimetrically after ashing the dried ECP in a furnace at 550 $^{\circ}\text{C}$ for 4 h.

RESULTS

The characteristics for each type of feed used are listed in Table 1. As part of the primary sludge was substituted with glucose or propionic acid, the TS decreased. All of the digesters performed satisfactorily for the 40 d of operation, with a mean operating temperature of 35.1 - 35.5 $^{\circ}\text{C}$ and a mean pH of 7.02 - 7.21 (Table 2). The VS loading rate of each digester varied, depending on the feed type used. Substituting part of the

primary sludge with glucose increased the VS content of the feed, whilst substitution with propionic acid reduced the VS content (Tables 1 and 2). This was most apparent when propionic acid was used (Table 1).

The control digesters fed 100 % primary sludge had a mean volatile solids reduction (VSR) of approximately 55 %. The VSR increased to 61 - 63 % when the feed sludge was partially substituted for pure glucose, and decreased to 39 - 41 % when the feed sludge was partially substituted for propionic acid (Table 2). The greatest quantity of gas produced per g VSR occurred when propionic acid was used as part of the influent feed, whereas the lowest level of gas production occurred in the control digesters fed 100 % primary sludge (Table 2).

The VA content of the digester feed varied between the different treatments. The primary sludge and primary sludge with glucose had similar VA concentrations at approximately 2,000 mg l⁻¹, but the VA concentration of the primary sludge with propionic acid increased as the proportion of propionic acid increased (Figure 1). The final concentration of VA in the primary sludge with propionic acid feed was approximately 20,000 mg l⁻¹. High levels of VA did not appear to affect the digester operation, with the waste sludge from each digester having VA concentrations of 12 - 220 mg l⁻¹.

Digestion reduced the dewaterability of the primary sludge, both in terms of filtrability and drainability. At the same time, the SS content of the sludge decreased from 36.8 g l⁻¹ to 13.5 - 16.7 g l⁻¹ (Table 3). The final SS content of the digested sludge was dependent on the feed type, the highest concentration (16.4 - 16.7 g l⁻¹) determined in digested primary sludge, and the lowest concentration (13.5 - 13.6 g l⁻¹) in digested primary sludge with propionic acid.

The filtrability of the digesters fed primary sludge or primary sludge with propionic acid was similar, 6.9-8.5 s, but nearly doubled to 14.0-16.3 s when glucose was used in the digester feed. The sludge drainability results in Table 3 indicated how easy water is released from the sludge under gravity, irrespective of the solids concentration. Prior to digestion, the volume of filtrate that drained from 200 ml of primary sludge was greater, even though the SS concentration was more than double that of the digested sludge. The most difficult sludge to dewater by draining under gravity was the control digester sludge. Supplementing a proportion of the feed with either glucose or propionic acid slightly improved the drainability of the sludge after digestion.

The organic content of the digester feed had an impact on the extracted ECP yield and composition (Table 4). Digestion of primary sludge and primary sludge with propionic acid led to a reduction in the ECP yield from 19.6 mg g⁻¹ SS to 16.3-19.2 mg g⁻¹ SS and 14.1-15.3 mg g⁻¹ SS respectively. In contrast, the ECP yield from the digested primary sludge with glucose increased to 24.4-25.3 mg g⁻¹ SS.

Digestion of primary sludge with glucose had the greatest impact on the ECP organic composition, increasing the organic fraction from 72.8 % to 79.3-79.4 % (Table 4). Where propionic acid was used in the digester feed the ECP organic fraction decreased from 72.8 % to 68.3-71.7 % after digestion. In all cases, the protein content of the extracted ECP increased after digestion from 19.9 % to 22.7-28.6 %, and the carbohydrate content decreased from 21.2 % to 18.6-21.1 %. The influence of the type of feed on the protein and carbohydrate content of the ECP was not conclusive. The composition of the remaining ECP was thought to consist of nucleic acids, humic substances and lipids, but these were not assayed for in this case.

The particle size distribution of digested primary sludge and digested primary sludge with propionic acid were similar, whereas digestion of primary sludge with glucose led

to an overall increase in the size of sludge particles (Figure 2). The mean particle size was 17.4-21.4 μm for digested primary sludge with glucose, and 13.2-15.7 μm for the other feed types. Digested primary sludge with glucose had a low sludge surface area, 0.28-0.35 $\text{m}^2 \text{g}^{-1}$, compared to the other feed types, 0.38-0.46 $\text{m}^2 \text{g}^{-1}$.

Increased sludge filtrability was related to higher levels of ECP and an increase in the mean particle size of the sludge, with the ECP composition having no effect. The sludge drainability was not influenced by the particle size distribution nor the ECP composition, whilst the impact of the ECP yield was less in comparison to the sludge filtrability.

The relationship between the sludge filtrability and the ECP yield was significant at the $P = 0.01$ level (Figure 3). Increasing concentrations of ECP in the sludge initially made the sludge slightly easier to filter, before further increases in ECP made the sludge progressively more difficult to filter. From Figure 3, the level of ECP that may be related to the optimum sludge filtrability was calculated as 17.2 mg g^{-1} SS.

Figure 2 illustrated changes in the particle size distribution. Examination of the mean $D(v,0.1)$, $D(v,0.5)$ and $D(v,0.9)$ values for each sludge, i.e. the 10 %, 50 % and 90 % cut-off points for the distribution, showed similar results for digested primary sludge and digested primary sludge with propionic acid, but very different results for digested primary sludge with glucose (Table 5). Where glucose was used as part of the digester feed the $D(v,0.1)$ and $D(v, 0.5)$ values have increased whilst the $D(v,0.9)$ value is approximately half that of the other digested sludge samples. This indicated that the high CST values obtained for the digested primary sludge with glucose were not due very small particles blinding the filter paper, but the ability of the sludge to hold on to the water present within the sludge.

To compare the effect of different ECP yields on the sludge drainability, the level of filtrate removed from the digested sludge was adjusted for the different sludge SS concentration as detailed in Standard Method 2710H for the time to filter test (Table 3). Increasing levels of ECP in the sludge appeared to make it harder for the water to drain from the sludge under gravity, but the relationship was not significant at the $P = 0.05$ level (Figure 4).

DISCUSSION

To date, the effect of microbial ECP production on digested sludge has not been extensively investigated. Alterations in the feed composition of anaerobically digested sludge had been shown to affect the level of ECP production and composition (Jia *et al.*, 1996) but this had not been related to the sludge dewaterability. The limited results available relating the ECP yield of digested sludge to the sludge dewaterability were inconclusive, with no significant relationship found for samples analysed from full-scale treatment works (Poxon and Darby, 1997; Houghton *et al.*, 2000a) and a strong positive correlation from laboratory-scale results (Houghton *et al.*, 2000b). Substituting a proportion of the digester feed primary sludge for glucose solution or propionic acid encouraged the activity of different microbial populations, acidogenic and acetogenic/methanogenic bacteria respectively, in the digesting sludge, allowing the impact on ECP production and dewaterability to be assessed under controlled conditions.

Increasing the feed sludge carbohydrate content with glucose, gave a large increase in the ECP yield from the digested sludge, (Table 4) as observed previously from full-scale (Poxon and Darby, 1997) and laboratory-scale (Jia *et al.*, 1996) samples. The provision of glucose, an easily assimilated substrate that is readily available to bacteria

in the initial stages of digestion, has stimulated ECP production in contrast to primary sludge and primary sludge with propionic acid feed types, indicating that acidogenic bacteria are responsible for the excess ECP production.

Jia *et al.*, (1996) found that the protein and carbohydrate content of the extracted ECP from digested glucose was higher (approximately 200 % and 2000 % respectively) than that from digested propionate. This was not observed in this study, where the extracted ECP mean protein content was approximately 21 % higher and the mean carbohydrate content was slightly lower than that for ECP extracted from the primary with propionic acid digested sludge. This could be due to a number of reasons. Jia *et al.* (1996) used a nutrient and trace metal solution with the glucose and propionate substrates rather than primary sludge. Degradation of primary sludge results in the presence of numerous compounds, some of which may interfere with determination of protein (Davis, 1988). Primary sludge also contains ECP that may have been degraded differently by each dominant microbial population when glucose or propionic acid were added to the feed, with differing fractions of the undigested ECP remaining in the digested sludge. The ECP extraction methods used were different in each case and this can affect the composition of the extracted ECP. Jia *et al.* carried out the experiment using 100 ml aliquots of digesting sludge rather than 5 l fermentation vessels, and the HRT of each experiment were different.

Anaerobic digestion resulted in an increase in the protein content and a slight decrease in the carbohydrate content of the extracted ECP, irrespective of the feed type (Table 4), indicating that during digestion carbohydrate is preferentially degraded compared to protein.

The sludge filtrability was strongly affected by the level of ECP present in the sludge (Tables 3 and 4, Figure 3). Digested primary sludge and primary sludge with propionic

acid had similar CST results, 6.9-8.5 s, and extractable ECP yields, 14.1-19.2 mg g⁻¹ SS. Adding glucose to the primary sludge increased the digested sludge ECP yield to > 24 mg g⁻¹ SS and doubled the CST to 14.0-16.3 s. The CST is also affected by the particle size distribution, with an increase in surface area increasing the frictional resistance to the withdrawal of water (Lawler *et al.*, 1986). The digested primary sludge with glucose had fewer fine particles (Table 5) and hence a lower surface area than the other digested feed types. This indicated that it was likely that the extra ECP present in the digested primary sludge with glucose was making it harder to remove water from the sludge (Wu *et al.*, 1982), rather than the increased frictional resistance of the particles (Lawler *et al.*, 1986). The gravity drainage test also indicated that the presence of extra ECP in the digested sludge made the sludge harder to drain (Figure 4) but the result was not significant at the P = 0.05 level.

The effect of the different feed types on the particle size of the digested sludge clearly showed the different level of activity of the separate microbial groups. Digested primary sludge with glucose had an increased particle size at the low end (D(v,0.1) and D(v,0.5)) of the distribution. This correlated with increased levels of ECP, which was likely to result in flocculation of the smallest particles present (Wingender *et al.*, 1999). In contrast, the mean D(v,0.9) was nearly half that of the digested primary sludge and primary sludge with propionic acid. The first stage in anaerobic digestion is hydrolysis, using hydrolytic enzymes to break down the large particles of organic matter into compounds that can then be assimilated by the bacteria and degraded further (Gujer and Zehnder, 1983). The availability of an easily assimilated substrate, glucose, has increased this initial stage of digestion, reducing the largest particles present in the sludge in comparison to the other treatments.

Previous work has indicated that the particle size distribution of digested sludge has the greatest impact on the sludge dewaterability (Karr and Keinath, 1978; Lawler *et al.*, 1986). This may be true when similar levels of ECP are present in the sludge, but the results presented here indicate that when the ECP content of a sludge is increased to a high concentration, it appears to have a direct impact on the sludge dewaterability that is not related to the size distribution of the particles.

CONCLUSIONS

The use of a high carbohydrate feed had a significant impact on the ECP content and dewaterability of digested sludge: the extractable ECP yield doubled; the measured CST doubled; the mean gravity drainage time increased; the particle size distribution altered with fewer fine particles and a reduction in the largest particle size.

Substitution of part of the primary sludge feed with propionic acid had no impact on the digested sludge ECP content and dewaterability compared to the control digesters fed 100 % primary sludge.

The relationship between the extracted ECP yield and sludge filtrability was significant at the $P = 0.01$ level. For the sludge produced, a calculated ECP yield of $17.2 \text{ mg g}^{-1} \text{ SS}$ appeared to be necessary for optimum sludge dewaterability.

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Table 1. Digester feed characteristics: total solids, volatile solids and chemical oxygen demand.

Feed type	TS (g L ⁻¹)		VS (g L ⁻¹)		COD (g L ⁻¹)	
	Mean	Range	Mean	Range	Mean	Range
Primary	46.3	39.5- 57.0	35.6	30.3- 46.6	67.6	44.6- 88.4
Primary with glucose	43.4	37.5- 51.4	39.0	33.1- 46.9	73.6	58.0- 88.9
Primary with propionic acid	28.8	21.1- 37.8	23.6	17.0- 31.0	70.5	58.1- 83.5

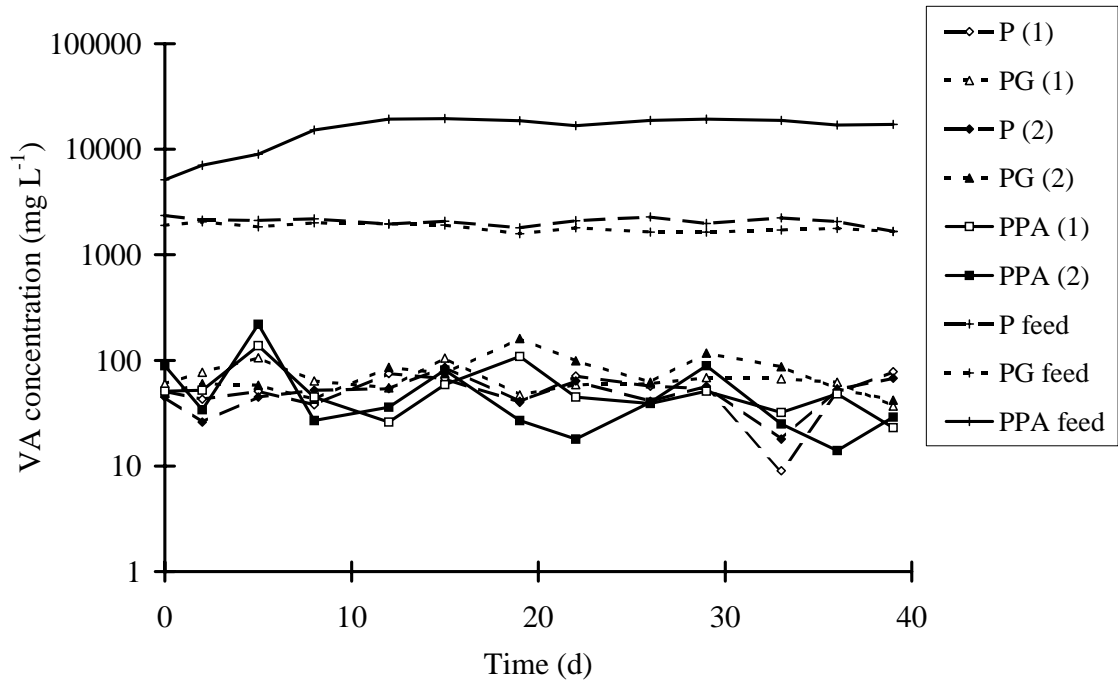


Figure 1. Volatile acid content of digester feed and waste sludge throughout the 40 days of operation. P = primary sludge; PG = primary sludge and glucose; PPA = primary sludge and propionic acid.

Table 3. Sludge dewatering characteristics.

Sludge sample	CST (s) (SS = 1 gL ⁻¹)	Sludge drainability (ml filtrate 4 min ⁻¹)	SS (gL ⁻¹)
Primary feed	2.1	108	36.8
Digested primary (1)	7.1	50	16.4
Digested primary (2)	8.5	61	16.7
Digested primary and glucose (1)	16.3	85	15.3
Digested primary and glucose (2)	14.0	59	15.1
Digested primary and propionic acid (1)	8.3	73	13.5
Digested primary and propionic acid (2)	6.9	59	13.6

Table 4. Sludge extracellular polymer yield and organic composition.

nd = not determined; P = primary sludge; PG = primary sludge with glucose; PPA = primary sludge with propionic acid.

Sludge sample	ECP yield (mg g ⁻¹ SS)	Organic fraction (%)	Analysis of organic fraction		
			Protein (%)	Carbohydrate (%)	nd (%)
Primary	19.6	72.8	19.9	21.2	58.9
Digested P (1)	19.2	71.6	28.6	21.1	50.3
Digested P (2)	16.3	75.0	24.6	18.6	56.8
Digested PG (1)	25.3	79.4	27.3	19.8	52.9
Digested PG (2)	24.4	79.3	27.9	20.8	51.3
Digested PPA (1)	14.1	71.7	22.7	18.9	58.4
Digested PPA (2)	15.3	68.3	23.0	20.1	56.9

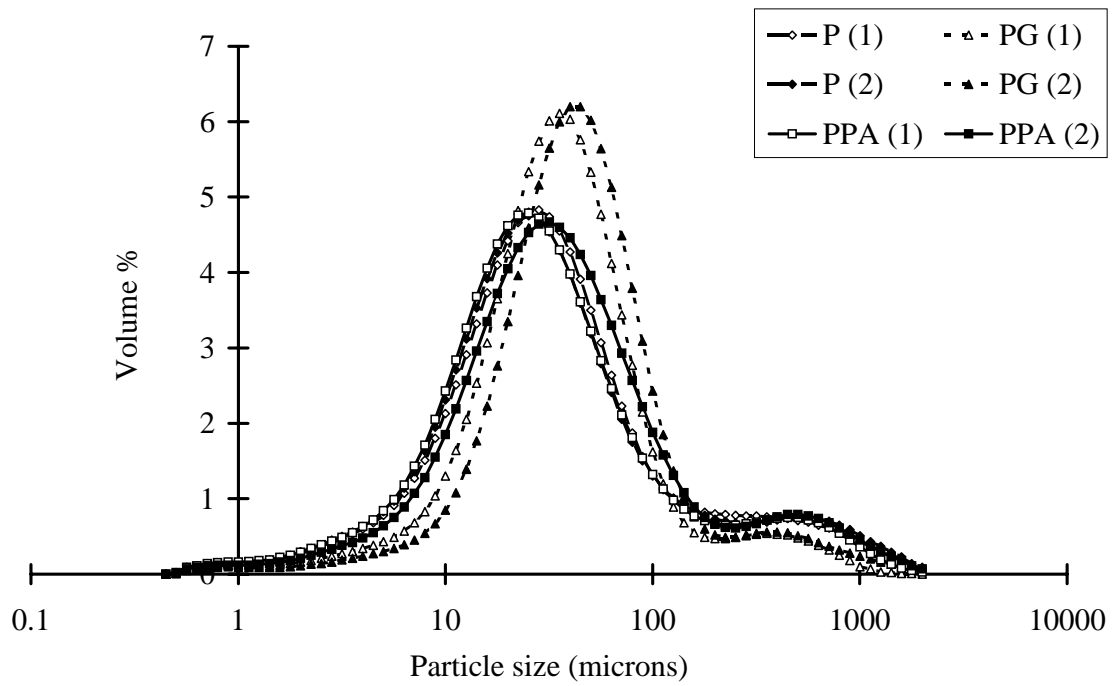


Figure 2. Particle size distribution of digested sludge samples.

P = primary sludge; PG = primary sludge and glucose; PPA = primary sludge and propionic acid.

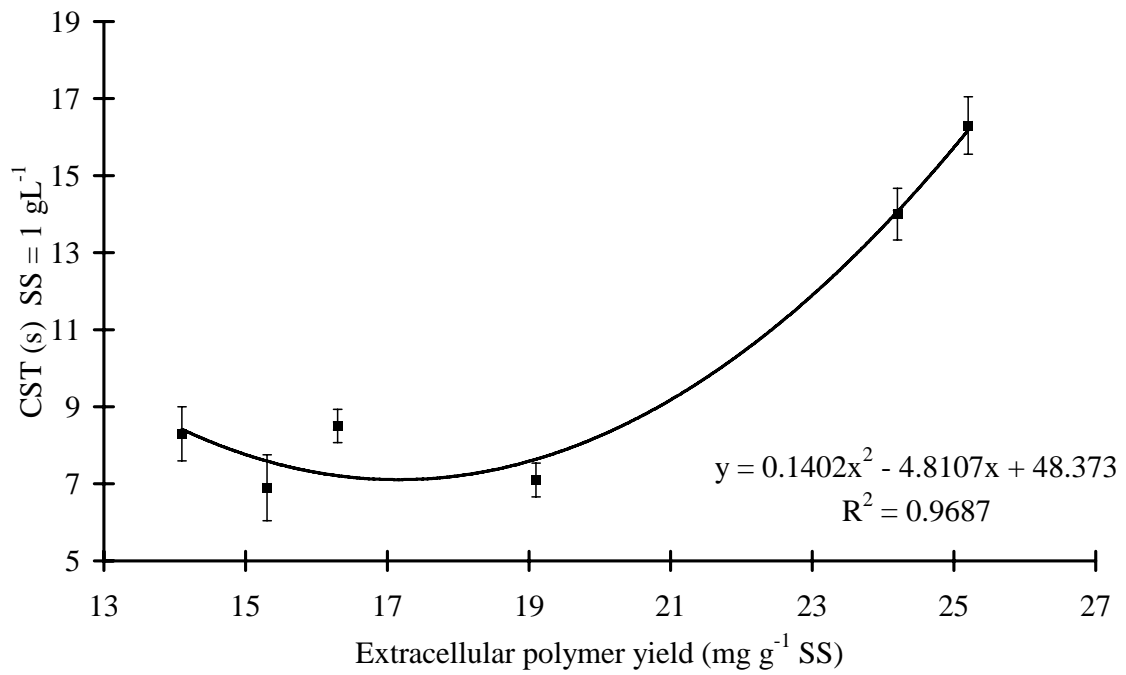


Figure 3. Relationship between extracted ECP yield and digested sludge filtrability.

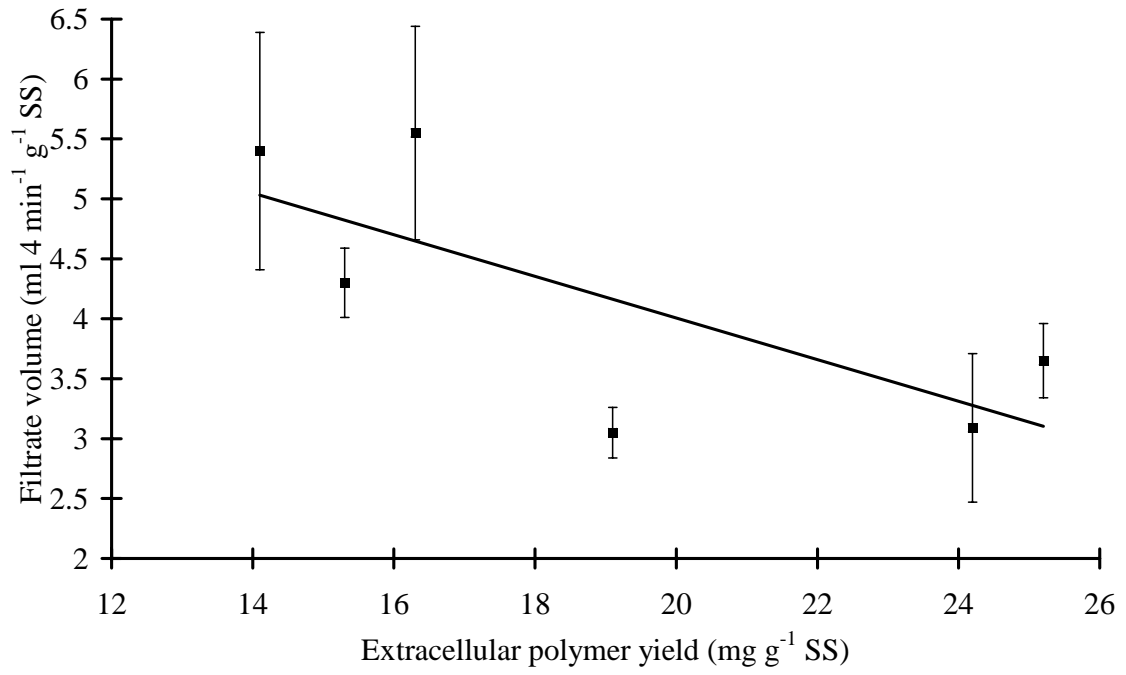


Figure 4. Effect of extracellular polymer yield on the drainability of digested sludge.

Table 5. Digested sludge mean particle size: 10, 50 and 90 percentile values.

Feed type	Mean particle size (μm)		
	D(v,0.1)	D(v,0.5)	D(v,0.9)
Digested primary (1)	7.49	28.22	226.4
Digested primary (2)	7.09	26.83	240.2
Digested primary and glucose (1)	11.02	33.08	101.6
Digested primary and glucose (2)	14.02	39.86	125.3
Digested primary and propionic acid (1)	7.03	25.98	191.4
Digested primary and propionic acid (2)	8.65	32.24	224.1

Table 2. Digester operation and performance: temperature, pH, loading rate, volatile solids reduction and gas production.

Digester feed type	Temperature (°C)		pH		Loading rate (g VS L ⁻¹ d ⁻¹)		VSR (%)		Gas production (ml g ⁻¹ VSR d ⁻¹)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Primary (1)	35.1	34.1-36.6	7.21	7.05-7.33	1.78	1.52-2.33	54.6	41.9-65.7	390	260-610
Primary (2)	35.5	34.6-36.6	7.19	7.04-7.37	1.78	1.52-2.33	55.9	43.2-65.9	330	110-610
Primary with glucose (1)	35.5	34.3-36.8	7.02	6.63-7.33	1.95	1.66-2.23	60.8	52.1-69.2	580	420-890
Primary with glucose (2)	35.5	34.6-36.7	7.04	6.72-7.33	1.95	1.66-2.33	62.6	52.6-71.0	570	390-960
Primary with propionic acid (1)	35.3	34.1-36.6	7.17	6.86-7.46	1.19	0.85-1.51	41.2	20.4-52.3	990	470-2050
Primary with propionic acid (2)	35.2	34.2-36.7	7.17	6.95-7.46	1.19	0.85-1.51	38.9	17.5-52.3	1160	480-2700