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A comprehensive insight into floc characteristics and their impact on compressibility and settleability of activated sludge

Bo Jin [a,*], Britt-Marie Wilén [a,b], Paul Lant [a]

[a] Department of Chemical Engineering, The University of Queensland, St. Lucia, Qld 4072, Australia
[b] Water Environment Transport, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

Abstract

This paper presents a comprehensive study of sludge floc characteristics and their impact on compressibility and settleability of activated sludge in full scale wastewater treatment processes. The sludge flocs were characterised by morphological (floc size distribution, fractal dimension, filament index), physical (flocculating ability, viscosity, hydrophobicity and surface charge) and chemical (polymeric constituents and metal content) parameters. Compressibility and settleability were defined in terms of the sludge volume index (SVI) and zone settling velocity (ZSV). The floc morphological and physical properties had important influence on the sludge compressibility and settleability. Sludges containing large flocs and high quantities of filaments, corresponding to lower values of fractal dimension (D_f), demonstrated poor compressibility and settleability. Sludge flocs with high flocculating ability had lower SVI and higher ZSV, whereas high values of hydrophobicity, negative surface charge and viscosity of the sludge flocs correlated to high SVI and low ZSV. The quantity of the polymeric compounds protein, humic substances and carbohydrate in the sludge and the extracted extracellular polymeric substances (EPS) had significant positive correlations with SVI. The ZSV was quantitatively independent of the polymeric constituents. High concentrations of the extracted EPS were related to poor compressibility and settleability. The cationic ions Ca, Mg, Al and Fe in the sludge improved significantly the sludge compressibility and settleability.

Keywords: Activated sludge flocs; Settleability; Compressibility; SVI; ZSV; Floc size; Fractal dimension; Filament index; Viscosity; Flocculating ability; Hydrophobicity; Surface charge; Polymeric constituents; EPS

1. Introduction

Biosolid–liquid separation by gravity settling is one of the most critical operations in the activated sludge process. Settling and compaction ability of activated sludge is crucial to overall performance and efficiency of the treatment process as well as the quality of the receiving water. Previous investigations indicate that many activated sludge systems have experienced various biomass separation problems in the settling tanks [1–3]. There are two main types of settling problems: (i) bulking sludge due to the proliferation of filamentous bacteria and (ii) poor flocculation properties, e.g. formation of small and light flocs. It is agreed that both aspects are equally important for the separation properties but the latter one has been much less investigated. In most cases, large, dense and strong flocs are desirable for good settling and compaction of activated sludge.

The flocculation of activated sludge is an active process and depends on physical, chemical and biological factors. Activated sludge flocs are aggregates of suspended solids containing different groups of microorganisms and organic and inorganic particles embedded in a polymeric network of extracellular polymeric substances (EPS) [4–7]. Due to the complex nature of the flocs, they display a wide variation in physical, chemical and biological properties. It has been suggested that the binding of the different entities is due to various types of intermolecular interactions such as DLVO-type interactions [8]; bridging of EPS by means of divalent and trivalent cations [1,9] and hydrophobic interactions [10]. The floc properties, such as size distribution and morphology, can differ substantially as a result of differences in the environment in the treatment plant. A number of floc characteristics could be expected to exert some direct and/or indirect influence on the activated sludge properties. The complicated interrelationships with respect to physical, chemical and biological factors affecting the floc characteristics and activated sludge properties are summarised in Fig. 1.

It is recognised that the amount of EPS, surface properties (colloidal properties), floc size distribution, density, and filament length are the major factors associated with activated

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[*] Corresponding author. Tel.: +61-7-3365-4479; fax: +61-7-3365-4199.
E-mail address: bojin@cheque.uq.edu.au (B. Jin).

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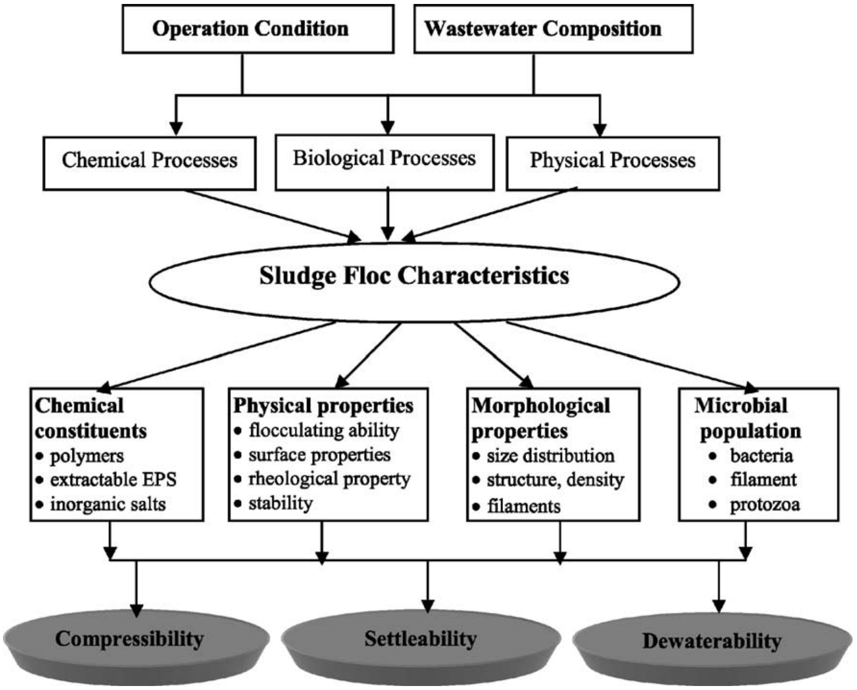

Fig. 1. Network of physical, chemical and biological factors affecting sludge floc characteristics and activated sludge properties.

sludge properties [10–15]. However, only a few isolated parameters have been examined in any of the previous studies. The relative importance of each property and its impact on the bioflocculation of activated sludge have, therefore, not been established. In fact, the fundamental mechanisms of bioflocculation and their impact on the sludge characteristics have neither been thoroughly investigated nor successfully quantified. Therefore, it is not well understood how the physical and biochemical characteristics of sludge flocs affect the settling and dewatering properties of activated sludge.

The goal of this study was to gain a comprehensive insight into sludge floc characteristics and to identify the key factors which determine the solid–liquid separation properties of activated sludge. Extensive laboratory examinations of the physical and chemical properties of activated sludge samples from sewage and industrial wastewater treatment plants (WWTPs) were performed. Of particular interest was the characterisation of the floc morphological (floc size distribution, fractal dimension, filament index), physical (flocculating ability, viscosity, hydrophobicity and surface charge) and chemical (polymeric constituents and metal contents) properties, and to identify their impact on compressibility, settleability and dewaterability of the activated sludge. The results of this study are presented in two papers dealing with: (I) compressibility and settleability; and (II) dewaterability [16]. This paper presents part of an extensive study into the impact of various floc characteristics on the compressibility and settleability of activated sludge.

2. Materials and methods

2.1. Integrated investigation and approach for activated sludge flocs

The investigations were conducted by collecting activated sludge samples from the target WWTPs and a series of tests were carried out in the laboratory to obtain an extensive amount of information on the physical, chemical and biological properties of the sludge flocs. The major factors affecting the activated sludge properties can be classified accordingly:

(1) influent and process conditions;
(2) chemical constituents of the sludge flocs;
(3) microbial community and activity;
(4) flocculating ability and floc surface properties; and
(5) floc structure.

An overview of the experimental approach is presented in Fig. 2. The results from the studies of the impacts of structural and microbial characteristics on the floc stability, and the influence of key chemical constituents in activated sludge on surface and flocculating properties are presented elsewhere [6,7].

2.2. Activated sludge samples

Activated sludge samples were taken from seven different full-scale activated sludge WWTPs in Brisbane, Australia, including five sewage treatment plants (STPs), treating domestic wastewater, and two industrial activated sludge

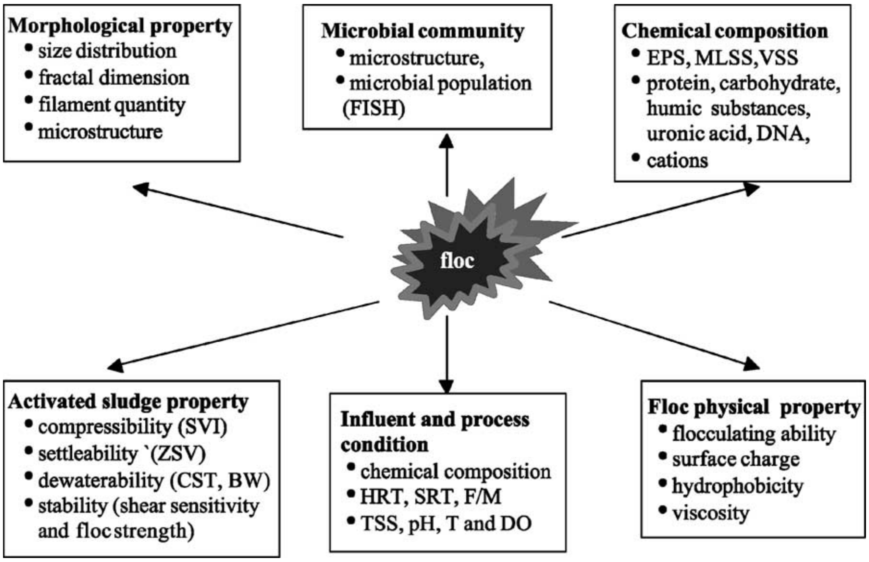

Fig. 2. Integrated investigation and approach to floc characteristics and sludge properties in activated sludge process.

treatment processes for oil refinery and leachate effluent (Table 1). The sludge samples from each WWTP were collected from the aeration tanks and maintained in filled plastic containers placed in ice cooler during the transportation from WWTP site to laboratory. Sample tests started immediately and were completed within 20 h, while being kept in a refrigerator at 4 °C. Sludges from each WWTP were generally examined twice with up to 4 months in between.

2.3. Extraction of EPS

The EPS were extracted from the activated sludge by mixing with a cation exchange resin (Dowex 50 × 80, Na^+ form, 20–50 mesh, Aldrich–Fluka 44445). This extraction procedure is based on the method as described by Frølund et al. [4]. This method has been widely accepted and used for EPS extraction from activated sludge since this procedure results in high extraction efficiency and little or no cell lysis or exopo1ymer disruption [4,17,18]. Two hundred grams of Dowex, previously washed in a buffer solution, and 0.5 l of thickened sludge (8 g/l) were transferred to an extraction vessel with four baffles and stirred at 900 rpm for 4 h at 4 °C. The extracted EPS was harvested by centrifugation at 12,000 × g for 15 min and filtration through a 0.45 μm cellulose acetate membrane. Total amount of EPS was expressed by sum of carbohydrate, protein, and humic substances.

2.4. Flocculating ability

The flocculating ability of activated sludge was determined as the reflocculation ability of sludge flocs after disruption. The method described by Jorand et al. [19] was modified and used in this study. 80 ml of sludge sample with a concentration of mixed liquid suspended solids (MLSS) approximately 4 g/l was transferred in a beaker placed on ice bath and sonicated at 50 W for 15 s. This was sufficient to disrupt the flocs, but did not cause cell rupture [5]. A 10 ml aliquot of the suspension was centrifuged at 1200 rpm for 2 min and the absorbance of the supernatant was measured at 650 nm (A). The rest of the sonicated suspension was stirred on a magnetic stirrer (set at a specific speed to keep the sludge flocs suspended) at ambient temperature

Table 1
Process descriptions of the wastewater treatment plants

Treatment plant	Source of wastewater	Biological process	Chemical dosage	SRT[a] (days)
Wacol (A)	Domestic 50%; industrial 50%	C, N, P		35
Oxley Creek (B)	Mainly domestic	C		4
Gibson Island (C)	Mainly domestic	C, N		18
Thorneside (D)	Mainly domestic	C, N, P	Lime and alum	12
Capalaba (E)	Mainly domestic	C, N, P	Alum	15
Caltex (F)	Oil refinery effluent	C		30
Tip (G)	Leachate	C, N (sequencing batch reactor)	$Mg(OH)_2$	20

The biological processes are classified as carbon removal (C); nitrogen removal (N); and phosphorus removal (P).

[a] Solids retention time (SRT) or sludge age.

for 15 min after which a 10 ml aliquot was analysed in the same way as before (B). The standard deviations for triplicate samples were 0.2–5%. The flocculating ability of the flocs was calculated as Eq. (1):

$$\text{flocculating ability (\%)} = \frac{1-B}{A} \times 100 \qquad (1)$$

2.5. *Relative hydrophobicity*

The relative hydrophobicity of the sludge flocs was measured as adherence to hydrocarbons, as detailed by Chang and Lee [20]. 30 ml of the sludge samples were washed and suspended in Tris buffer (0.05 mM at pH =7.1). The activated sludge suspension (thickened to approximately MLSS 4 g/l) was homogenised by sonication (50 W for 2 min) at 4 °C to disrupt the flocs to single cells and small micro-colonies. The suspension was agitated uniformly for 5 min with 15 ml hexadecane (Sigma) in a separatory funnel. After 30 min when the two phases had separated completely, the aqueous phase was transferred into another glassware. The standard deviation for the three duplicate samples tested was 1–6%. The relative hydrophobicity was expressed as the ratio of MLSS concentration in the aqueous phase after emulsification (MLSS_e) to the concentration of MLSS in the aqueous phase before emulsification (MLSS_i):

$$\text{relative hydrophobicity (\%)} = \left[1 - \frac{\text{MLSS}_\text{e}}{\text{MLSS}_\text{i}}\right] \times 100 \qquad (2)$$

2.6. *Surface charge*

The measurement of surface charge of the sludge flocs was performed by colloidal titration [21]. A known volume of sludge sample diluted in distilled water was reacted with an excess amount of polybrene (Polyscience Inc.) (0.001N), followed by back-titration with polyvinyl sulphate (Aldrich–Fluka) (0.001N) to a colorimetric end-point indicated by Toluidin Blue. The surface charge was expressed as milliequivalents per gram of mixed liquor suspended solids of positive or negative colloidal charge, i.e. meq./g MLSS. The standard deviations for triplicate samples were 3–10%.

2.7. *Compressibility and settleability*

The compressibility and the settleability of the activated sludges were evaluated by the sludge volume index (SVI), and the zone settling velocity (ZSV), respectively. SVI is the volume of 1 g of the total suspended solids after 30 min of settling. A higher SVI is related to a poorer compressibility. The ZSV is a key parameter to evaluate the sludge settling properties, since it determines how much the secondary settlers can be loaded. ZSV and SVI were measured according to Standard Methods [22] in a 2 l settling cylinder. When the settled sludge volume exceeded 25% of the total volume (2 l), the sludge was diluted accordingly to minimise the wall effects during settling.

2.8. *Floc size distribution*

The floc size distributions were determined by a Malvern Mastersizer/E instrument with a 300 mm lens which enables the measurement of particles in the range 0.9–546 μm. This instrument measures the size of particles by means of light scattering. The samples were diluted in filtrated effluent (0.45 μm millipore filter) to avoid multiple scattering. The activated sludge suspension was then continuously recycled through the sample cell of the Malvern with a peristaltic pump to be exposed to a 2 mW He:Ne laser (wavelength 633 nm). Each sample was measured three times with a standard deviation 0.1–4.5%. The scattered light is detected by means of a detector that converts the signal to a size distribution based on volume. The average size of the flocs was given as the mean based on the volume equivalent diameter (D) [4,3].

2.9. *Fractal dimension*

The structure of the flocs was quantified in terms of fractal dimensions (D_f), D_f corresponds to the space filling capacity of an object and is thus a measure of the aggregate structure. The D_f dimensions were calculated from the raw light scattering data from the Malvern Mastersizer/E instrument according to the method by Spicer et al. [23]. This technique is based on a power law relationship between the total scattering intensity of the light from the aggregates and the magnitude of the scattering vector. The scattering intensity at each detector was calculated from the raw scattering data by means of information from the Malvern Mastersizer/E. By plotting the log of the light scattering intensity as a function of the log of the light scattering vector, the linear slope is the D_f. D_f varies between 1 and 3. Guan et al. [24] used this method to calculate the fractal dimensions for activated sludge. The high value of the D_f is related to compact and dense flocs.

2.10. *Filament index*

The sludge flocs were examined by light microscopy and images were captured on a Nikon Microphot FXA microscope via a charge-coupled device connected to a PC. Filamentous organism content was quantified as filament index using the method by Jenkins et al. [25]. The number of filamentous organisms was rated on a scale of 1–5, where 1 corresponds to no filamentous organism presents and 5 corresponds to excessive growth of filamentous organisms, according to Eikelboom and van Bijsen [11].

2.11. *Sludge viscosity*

The apparent viscosity was determined using a rotational viscosity meter (Model LVDVII, Brookfield, England). It was measured at the shear rate 100 s^{-1} for 5 min to keep the sludge in suspension and is expressed as mPa s. The measurements were carried out at similar suspended solids concentrations, approximately 3 g/l, at 20 °C. The apparent viscosity of the sludge is a reflection of internal and external interactions and forces occurring within the sludge flocs and fluids, and describes the deformation of the flocs under the influence of stresses [26–28].

2.12. Chemical analysis

The sludge and the extracted EPS were analysed for protein, carbohydrate and humic substances as detailed by Frølund et al. [4]. All analyses were carried out in triplicate. Mixed liquid suspended solids and volatile suspended solids (VSS) were measured according to Standard Methods [22]. Metals in the sludge were analysed by flame-AAS in centrifuged samples adjusted to pH 1 with supra pure HNO_3. The standard deviation for the determinations was 1–10% for protein and carbohydrate, 1–20% for humic substances and 1–5% for cationic ions.

2.13. Statistical analysis

Statistical analyses were performed to identify the major cause and effect relationships. To simplify the analysis, univariate linear correlations were used. All statistical analyses were carried out using the software *Statistica* (Statsoft, Tulsa, OK, USA). Since a normal distribution was not obtained for many of the properties examined, a distribution-free statistical method was used. The Pearson's product momentum correlation coefficient (r_p) was used to estimate linear estimations. The Pearson's coefficient r_p is always between −1 and +1, where −1 means a perfect negative correlation and +1 a perfect positive correlation while 0 means absence of relationship. Non-linear relationships were conducted by regression analysis. Correlations were considered statistically significance at a 95% confidence interval ($P < 0.05$).

3. Results and discussions

3.1. Sludge floc characteristics

The floc characteristics and physical properties of the activated sludges are summarised in Table 2, and chemical composition of the sludge and the extracted EPS is detailed in Table 3. It can be observed that the sludge samples were intentionally chosen to cover a broad range of floc characteristics.

The mean size of the sludge flocs varied between 40 and 320 μm and most samples had an average floc size smaller than 150 μm. The D_f was measured between 1.9 and 2.5. The quantity of filamentous organisms determined as filament index (FI) had a wide range from 1 to 5. Most of the sludges had an FI lower than 3, corresponding to low to moderate numbers of filamentous bacteria. The sludges from the STPs contained different levels of filamentous microorganisms, whereas no filamentous species was found in the sludge samples from the two industrial treatment plants.

The sludge flocs performed different flocculating abilities varying between 30 and 70%, but had relatively small variations in relative hydrophobicity between 50 and 70%. The sludges had negatively charged surfaces varying from −0.08 to −0.55 meq./g MLSS. Table 2 shows that more than 60% of the biosolids were in the volatile fraction. Apart from one of the STP sludge sample (C), which contained excessive quantities of filamentous microorganisms, the viscosity of the sludge was between 4.5 and 5.5 mPa s.

Protein, humic substances and carbohydrate were the major polymeric materials in both the sludge and the extracted EPS. Sum of the amount of protein, humic substances and carbohydrate is referred as total biopolymers in this study. Protein was the dominating biopolymer and represented more than 45% of the total polymeric fractions, followed by humic substances, and carbohydrate constituting approximately 15–30 and 13% of the biopolymers in the sludge and the extracted EPS, respectively. All sludge sam-

Table 2
Summary of floc characteristics of the sludge flocs and physical properties of the activated sludge

Parameter	Unit	Activated sludge sample						
		A	B	C	D	E	F	G
Floc size	μm	122 ± 2	311 ± 2	176 ± 3	122 ± 5	63 ± 3	55 ± 2	124 ± 3
D_f	–	2.16 ± 0.23	1.96 ± 0.06	2.12 ± 0.02	2.15 ± 0.01	2.30 ± 0.04	2.44 ± 0.04	2.09 ± 0
Filament index	–	2	4–5	5	3–4	2–4	1	1
Flocculating ability	%	60 ± 1	55 ± 3	55 ± 1	56 ± 2	37 ± 4	69 ± 1	32 ± 5
Hydrophobicity	%	65 ± 2	68 ± 3	70 ± 2	60 ± 2	60 ± 6	64 ± 6	48 ± 1
Negative surface charge	meq./g MLSS	0.26 ± 0.07	0.54 ± 0.02	0.34 ± 0.06	0.32 ± 0.12	0.30 ± 0.01	0.19 ± 0.03	0.13 ± 0.05
Viscosity	mPa s	4.75 ± 0.13	4.59 ± 0.43	10.5 ± 0.46	5.19 ± 0.38	4.53 ± 0.13	4.96 ± 0.02	3.97 ± 0.12
SVI	ml/g	97 ± 18	235 ± 11	255 ± 5	148 ± 5	109 ± 11	74 ± 8	45 ± 5
ZSV	m/h	2.89 ± 1.26	1.49 ± 1.10	0.52 ± 0.22	3.04 ± 0.40	4.02 ± 0.60	2.51 ± 0.26	5.94 ± 0.66
Volatile fraction (VSS/MLSS)	%	80 ± 2	73 ± 5	80 ± 5	79 ± 3	71 ± 2	84 ± 4	59 ± 1

Table 3
Summary of chemical constituents (mg/g MLSS) in the sludge and the extracted EPS of the activated sludge

Parameter	Activated sludge sample						
	A	B	C	D	E	F	G
Polymer							
Protein							
Sludge	217 ± 3	353 ± 35	254 ± 7	302 ± 11	224 ± 11	336 ± 7	191 ± 18
EPS	45 ± 3.6	56 ± 4.5	41 ± 11	55 ± 2.2	45 ± 6.5	30 ± 4.0	28
Humic substances							
Sludge	195 ± 45	191 ± 54	127 ± 3	131 ± 1	111 ± 18	73 ± 3	183 ± 17
EPS	28 ± 9.0	48 ± 6.8	23 ± 2.8	43 ± 12	33 ± 1.0	17 ± 0.2	51
Carbohydrate							
Sludge	83 ± 8.3	64 ± 6.3	89 ± 2.1	93 ± 3.5	61 ± 1.2	64 ± 1.2	55 ± 0.2
EPS	7.8 ± 0.6	11 ± 0.5	7.0 ± 1.9	9.8 ± 0.04	9.2 ± 1.8	5.7 ± 1.1	40
Total extracted EPS	81 ± 14	114 ± 12	71 ± 15	108 ± 14	87 ± 9	52 ± 5	119
Cations in sludge							
Al^{3+}	2.9 ± 0.9	5.8 ± 1.9	1.4 ± 0.1	16 ± 0.4	17 ± 2.3	3.2 ± 0.5	2.7 ± 0.1
Fe^{3+}	6.0 ± 1.6	11 ± 0.3	6.5 ± 0.1	3.9 ± 0.1	3.0 ± 0.4	16 ± 0.1	76 ± 1.7
Ca^{2+}	13 ± 1.1	13 ± 1.5	11 ± 0.2	14 ± 0.5	15 ± 1.3	6.2 ± 0.1	7.9 ± 0.5
Mg^{2+}	5.0 ± 0.5	3.8 ± 0.7	4.4 ± 0.7	3.5 ± 0.8	5.9 ± 0.4	2.6 ± 0.3	19 ± 2.7

ples contained similar amounts of Ca^{2+}, Na^+ and K^+, and the contents of Al^{3+} and Fe^{3+} varied from plant to plant, mainly depending on the use of flocculants containing aluminium and iron salts [6]. The sludge from the leachate treatment plant had very high concentrations of Mg^{2+} due to the addition of $Mg(OH)_2$ for pH adjustment in the WWTP.

3.2. Relationship between SVI and ZSV

The activated sludges had very different compaction and settling properties with SVIs varying from 40 to 260 ml/g and the ZSV ranging from 0.3 to 6.6 m/h. There was a clear non-linear correlation found between SVI and ZSV, as shown in Fig. 3. For SVIs higher than 150 ml/g, the sludge demonstrated extremely low settleability. At such high SVI the values of ZSV are strongly affected by the suspended solids concentration. This indicates that the settling and compressibility are governed by different sludge characteristics, and no simple and consistent relationship exists.

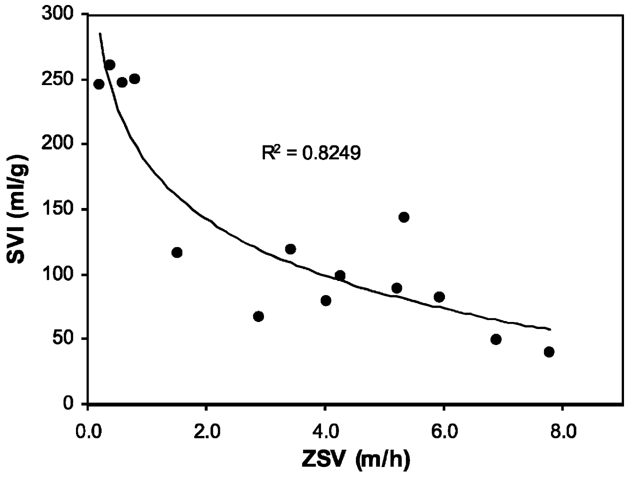

Fig. 3. Relationship between SVI and ZSV.

It is well known that the SVI as well as the ZSV are influenced by the geometry of the settling device, the concentration of MLSS, the sludge volume, the temperature as well as the floc structure [29,30]. This shows that the SVI as well as ZSV are rather non-specific measures of the physical characteristics of activated sludge. For a specific sludge, the ZSV is a function of the suspended solids concentration, generally described by the Vesilind relationship [31,32]. Attempts have been made to correlate the SVI to the ZSV with varying success [30,32–35]. Bye and Dold [36] found that it was questionable to correlate the SVI to the Vesilind correlation. Dick and Vesilind [29] reported that there was not a consistent relationship between ZSV and SVI. From the literature survey it is evident that there is a correlation between SVI and ZSV, i.e. sludges that settle poorly also compact poorly, especially when the sludge from one specific treatment plant is considered. When it comes to comparing sludges from different plants it becomes more complicated since the variations in settling properties are due to different factors (e.g. presence of filaments, floc size and composition), and the SVI and ZSV are probably not related to exactly the same sludge properties. A number of investigators have stated that SVI may not be a useful parameter for comparison of sludge settling characteristics from different plants [29,30,33]. It is also important to note that the settling characteristics in a cylinder are not the same as in a full scale settling tank. Nevertheless, SVI is one of the few ways of estimating the function of the secondary settlers and it is routinely measured at most treatment plants. SVI has been employed by a lot of previous researchers to characterise sludge settling properties, due to the simple and easy measurement involved [3,30,37,38]. The ZSV has been paid less attention and little information is available in the literature regarding its correlation to the sludge characteristics. This is mainly due to the difficulties involved in the determination of ZSV.

The purpose of this study was not to assess the precise relationship between these two sludge properties. Furthermore, when analysing the results, the floc morphology must be taken into consideration. In spite of the problems encountered when measuring SVI and ZSV, our results show that these measurements can be used to evaluate the settleability and compressibility, and the results clearly reaffirm that SVI and ZSV are lumped parameters, which should be used neither alone to infer sludge properties, nor to infer each other.

3.3. Effect of flocs properties on compressibility and settleability

To facilitate statistical analysis, the SVI and ZSV values were paired with each of the measurements of the floc properties. This analysis provided an initial estimate of the strength of the correlations. Correlation coefficients were determined for those parings frequently cited in the literature, as well as for those that visually suggested a strong relationship. The results of the statistical analysis calculated by Pearson's correlation, and r_p and P-values are summarised in Table 4.

3.3.1. Morphological characteristics

The results of the statistical analysis revealed that the *floc size*, D_f and FI were important parameters which influenced the compressibility and settleability (Table 4). With the exception of sludge samples from the G plant (highlighted in Fig. 4a and b), SVI correlated significantly ($P < 0.05$) with floc size, D_f and FI (Fig. 4). Sludges containing large flocs demonstrated a poor compressibility. Sludge flocs with high quantities of filaments were relatively large and had low values of D_f, indicating an open structure [7]. A high D_f value corresponded to relatively compact flocs and low SVI. The sludge from leachate treatment plant (G) had an almost granule-like structure, which contain relatively large and very compact flocs with no filaments, and demonstrated the highest settleability. The statistical results revealed that the D_f and FI had strong non-linear correlations with SVI Fig 4b and c). A better statistical correlation between SVI and D_f was found for SVI < 150 ml/g (Fig. 4b) This was particularly evident for the sludges with low FI.

Compared with SVI, the ZSV correlated relatively weakly with the morphological properties of the sludge flocs (Table 4). The results from the statistical analysis revealed that sludge containing large flocs and high quantity of filament demonstrated low settleability. Although no statistically significant correlation was found between ZSV and D_f, highly compact sludge flocs had an obvious tendency to settle fast (Table 4).

In previous studies, it has been found that the SVI is related to the median floc size for non-filament sludge [13,37].

Table 4
Summary of Pearson's correlation coefficient (r_p) and P-value between the floc characteristics and the compressibility and settleability

Parameter	Unit	SVI		ZSV	
		r_p	P-value	r_p	P-value
Floc size[a]	µm	0.8510	0.0033	−0.7365	0.0116
Fractal dimension[a]	–	−0.7689	0.0137	0.5206	0.4270
Filament index	–	0.9408	0.0010	−0.7204	0.0335
Flocculating ability[b]	%	−0.8012	0.0048	0.6663	0.0284
Relative Hydrophobicity	%	0.7630	0.04502	−0.6972	0.0147
Negative surface charge	meq./g MLSS	0.8529	0.0026	−0.7826	0.0106
Viscosity	mPa s	0.7589	0.0274	−0.4397	0.5213
Protein					
Sludge	mg/g MLSS	0.8369	0.0183	0.6834	0.0332
EPS		0.6344	0.0349	0.4278	0.1755
Humic substances					
Sludge	mg/g MLSS	0.7643	0.0341	0.5355	0.0896
EPS		0.7870	0.0205	–	–
Carbohydrate					
Sludge	mg/g MLSS	0.7538	0.0445	0.3941	0.1768
EPS		0.6138	0.0549	–	–
Total EPS	mg/g MLSS	0.7540	0.0391	–	–
Ca + Mg[c]	meq./g MLSS	−0.7255	0.0209	0.6670	0.0387
Fe + Al[c]	meq./g MLSS	−0.9156	0.0047	0.8602	0.0134

[a] Sludge G excluded.
[b] Sludge E excluded.
[c] Sludge F excluded.

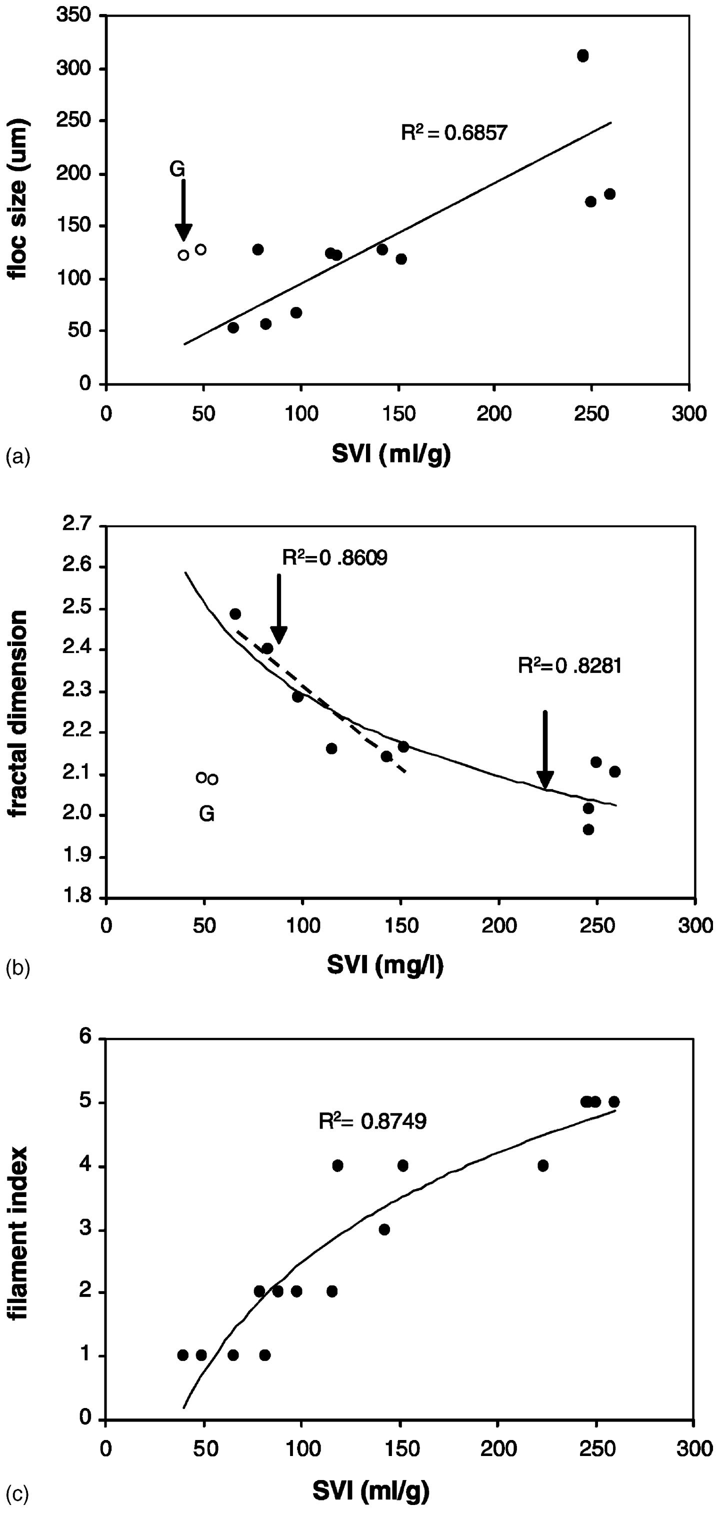

Fig. 4. SVI vs. (a) floc size, (b) fractal dimension and (c) filament index.

Andreadakis [13] found that large flocs had a lower density and larger surface area than small ones which might explain why they compact less well. The present study supported these findings, since sludges with SVI $\leq$ 150 ml/g containing low to moderate numbers of filaments had better compaction ability, and increasing floc size and decreasing D_f resulted in an increase in SVI. For the sludges with higher indices, the presence of filaments probably influenced the compressibility rather than the floc size. The sludge flocs from the leachate treatment plant (G) had a rather different morphology compared to the other sludges [7]. Due to their dense and very round morphology, these flocs could compact efficiently even though they were large, giving a low SVI.

It is well known that the SVI is affected by the amount of filamentous bacteria [33,37]. These variations in SVI with filament quantity can be explained by the effect of filamentous microorganisms on aggregation of activated sludge flocs and on their compressibility. Apparently, as filament lengths increase, flocs tend to become looser, thereby increasing their apparent size. Sezgin [33] reported that filament lengths below 10^7 μm/mg MLSS had no effect on SVI if the MLSS concentration was in the range 0.7–4.8 g/l, whereas when the filament length was over 10^7 μm/mg MLSS, SVI increased sharply with increasing filament length. It is very hard to measure the quantity of filaments accurately. Not only length, but probably also filament morphology and surface properties are important for the settling and compression behaviour of the sludge. It can be hypothesised that, depending upon the quantity of filamentous microorganisms protruding from activated sludge flocs, different types of aggregates are formed during settling and compaction. At very low extended filament index, the flocs make a particle to particle contact when they approach each other, resulting in the forming of compact, small size and high density “floc–floc” aggregates. On the other hand, at high filament index, “filament-to-filament” and “floc-to-filament” aggregations occur and large agglomerates of flocs are formed leaving large voids in between them. These flocs or aggregations are rather loose and have low density which makes them settle and compact poorly. A microscopic investigation of the sludges showed that the flocs with high SVI were more irregularly shaped. Furthermore, they were less stable towards shear, indicating a very loose structure [7]. Not only the abundance of filaments, but also the density of the core of the flocs affect the settling and compaction. It was observed that two sludges (D and E) with relatively high quantity of filaments settled well due to its high density (judged by microscopy), whereas another sludge (F) with dense flocs containing no filaments settled relatively slowly due to their small size. The relatively poor correlation between the floc size and ZSV indicates that floc morphology and density are more important for the settling than the actual size. Since the ZSV is the velocity of the sludge flocs when they are under the influence of other flocs in the liquid, both structure and density can be expected to affect its value.

To define the structure of complex aggregates such as activated sludge is complicated since they have a multilevel structure consisting of primary particles, aggregates of primary particles, microflocs and porous flocs [39]. It is therefore not probable that the structure can be defined by a single fractal dimension. The structure of flocs can be considered on two size scales: microscale and macroscale. The small-angle laser light scattering technique used in this study probably reflects the structure on a microscale (surface smoothness), whereas other means of methods have to be used to characterise the surface structure of the entire floc [39]. Consequently, activated sludge flocs can be regarded as multifractal objects. The floc size reflects the globular property of the flocs as opposed to the D_f which reflects the surface smoothness. It can also not be excluded that the values of D_f obtained in this study are affected by multiple scattering due to the large floc size and in some cases relatively dense flocs [24]. Especially for sludge G, this could have been the case, since these flocs had a very different morphology compared to the other sludges [7]. Nevertheless, there was an significant correlation between D_f and SVI, especially for sludges with no or few filaments. More research is needed to elucidate the fractal dimensions required to describe the flocs adequately.

3.3.2. Physical properties

The *flocculating ability* for most sludges was significantly correlated with the SVI and ZSV (Figs. 5 and 6). Sludges with high flocculating ability demonstrated high compressibility and settleability. However, the results measured in the sludges from WWTPs E and G did not follow the same trend. As indicated in Figs. 5a and 6a, these sludges had low SVI and flocculating ability (<40%), but settled fast.

Little is known about what governs the reflocculation of deflocculated sludge flocs. Biggs and Lant [5] found that activated sludge flocs that had been disrupted by means of sonication could be reflocculated by stirring in a similar way as inorganic flocs. The method for determining flocculation ability used in this study is essentially measuring the ability to re-flocculate after the flocs have been fractured. During the deflocculation, different floc constituents are released into the bulk water. As the stirring is initiated, the fragments start to move around, and some flocs and floc fragments collide with each other. Some of these collisions cause an attachment whereas others do not. Depending on differences in physico-chemical properties, different sludge flocs have different abilities to flocculate. What causes the difference in flocculation ability is not known. EPS have been found to have a stabilising effect on the flocs [15] but it is not clear what affects the flocculation properties. The presence of carbohydrate and protein in the biopolymers seem to improve flocculation whereas humic substances have a negative effect on flocculating ability [6]. The results in this study infer that the flocculating ability reflects the internal molecular binding forces, and highly flocculated sludge corresponds to highly compacted sludge, resulting in low SVI and high ZSV. Another possibility is that floc fragments containing high quantity of filaments are released to the bulk water after deflocculation and they cannot flocculate readily again. Aerobic granules, such as sludge G, have a very different structure compared to flocs and they do not aggregate readily with each other. The E-sludge had poor flocculating ability, but demonstrated high compressibility and settleability. This might be due to the high concentration of Al and Ca in the sludge, resulting in highly compacted flocs ($D_f = 2.3$). Since the interactions between the floc

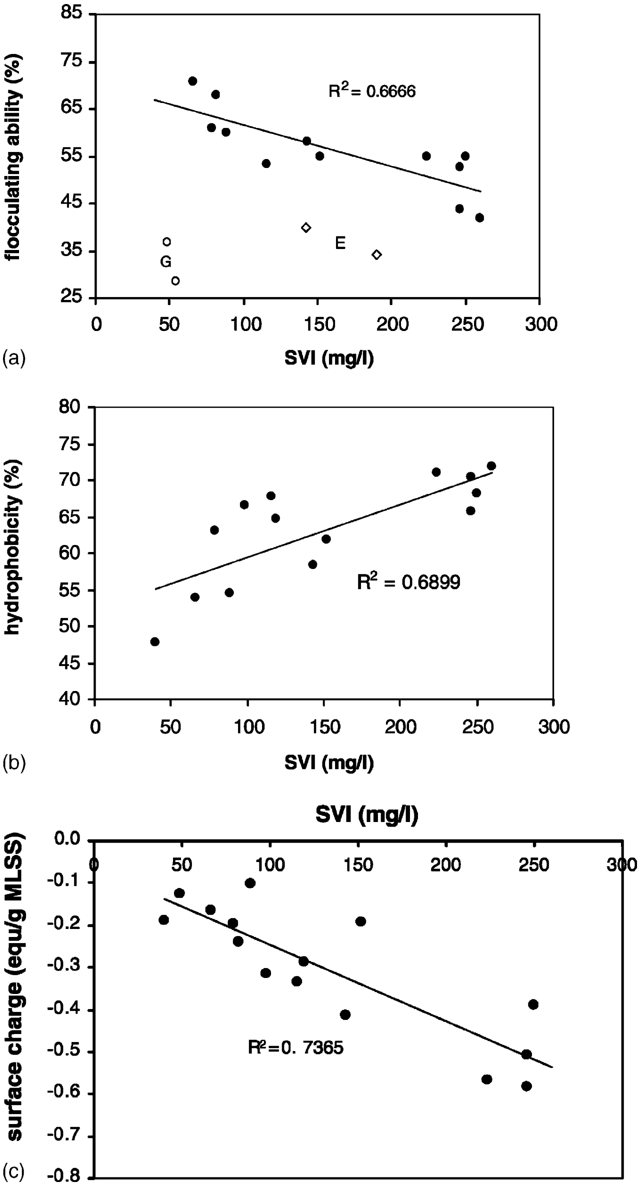

Fig. 5. SVI vs. (a) flocculating ability, (b) hydrophobicity and (c) surface charge.

constituents are determined by both physical and chemical mechanisms in a complicated way, it is difficult from this type of measurements to elucidate what determines these interactions in detail. It can be hypothesised that the physical as well as chemical properties of the polymer chains are important for the binding of the different floc fractions, and floc structure or morphology may be the most important aspects effecting the sludge compressibility and settleability.

The surface properties *hydrophobicity* and *surface charge* corresponded significantly to SVI and ZSV (Table 4). Highly hydrophobic or negatively charged floc surfaces were associated with a relatively poor compressibility and settleability (Figs. 5 and 6). The correlation between negative surface charge and SVI was stronger if only sludges with SVI $<$ 150 ml/g (FI ≤ 2) were considered (Fig. 5c). Although the increase in SVI is probably mainly caused by increasing amounts of filaments, the contribution of hydrophobic interactions to SVI may be significant, especially for sludges with low content of filaments.

Sludge floc surface charge has been related in various ways to both flocculation and bulking. The presence of a net negative surface charge on floc surfaces may create repulsive electrostatic interactions which prevents close contact. A high value of negative surface charge indicates that there may be more free negative charges and less electrostatic binding of cations on the surfaces of the sludge flocs. The positive correlation between negative surface charge and SVI could therefore be due to an increased electrostatic repulsion between floc components as described by the DLVO theory [14]. According to the DLVO theory, the degree of interaction between colloidal particles depends on the surface potential and on the thickness of the electrical double layers surrounding the particles. The double layer thickness is inversely proportional to the square root of the ionic strength. This means that, if the electrolyte concentration is high or if there are polyvalent counterions present, the electrostatic repulsion is reduced and the bacteria and other floc constituents can more easily adhere to each other. Barber and Veenstra [37] reported that if the surface charge itself is important as a repelling force, it could only be significant in cases of truly non-filamentous sludges. Otherwise, the filaments themselves would act as a physical bridge between flocs. In this study, however, there was a significant correlation ($P < 0.05$) between SVI and negative surface charge also for sludges containing high numbers of filaments. However, the correlations for the sludges with low content of filaments were better. As pointed out by Mikkelsen and Keiding [15], the measurement of surface charge is associated with some problems: since the method is based on adsorption of cationic polymers to the sludge surface, it is likely that surface charge measured can be affected by the surface area of the floc surface. This could be the reason to the relatively large scattering in the data for sludge flocs with high numbers of filaments since it is likely that these flocs have a relatively larger surface area than the ones with low numbers of filaments. When sludge flocs settle during the hindered settling, they interact with each other. It can be hypothesised that if the negative surface charge is high they repel each other more and thereby keeping the flocs in a more expanded state. This might make the whole blanket of flocs settle slower than otherwise would be the case.

The relative hydrophobicity of the sludge expressed in this study indicates the presence of both hydrophobic and hydrophilic groups in the sludge polymers, and is an average of the hydrophobicity of polymeric compounds and the bacterial cells [6,40]. There are no really suitable methods for measuring the hydrophobicity for heterogeneous aggregates such as sludge flocs and most available techniques are mainly applicable for homogenous suspensions of pure cultures. It was therefore not surprising to find a relatively modest correlation between the hydrophobicity and settling

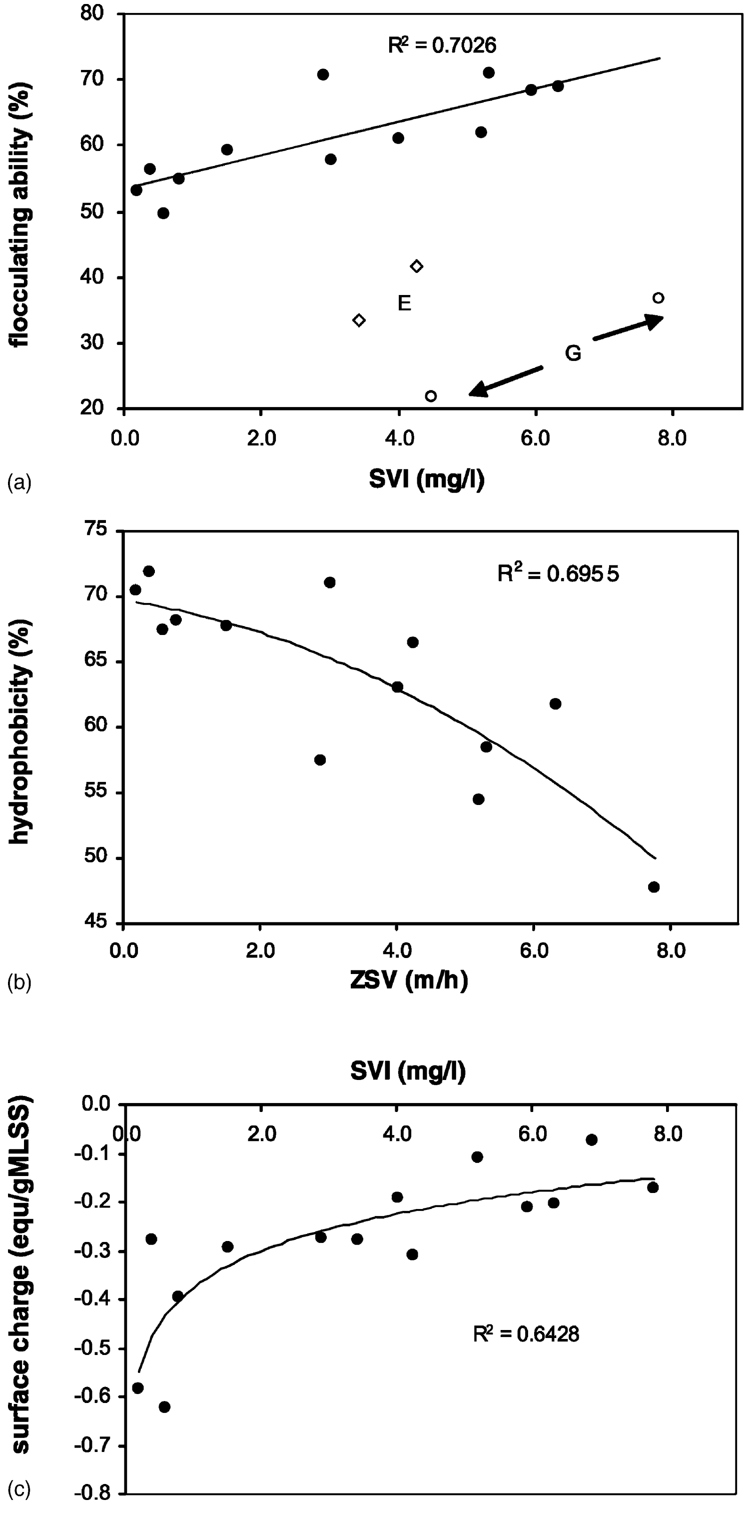

Fig. 6. ZSV vs. (a) flocculating ability, (b) hydrophobicity and (c) surface charge.

and compressibility. Generally, hydrophilic molecules are polar or charged while hydrophobic molecules are non-polar. Since non-polar molecules less readily mix in water compared to polar molecules, they should contribute to the binding together of the sludge flocs. It is also known that hydrophobic interactions are important for the attachment of bacteria to surfaces, but it is not well known how it affects the interactions between larger aggregates such as activated sludge flocs. Nielsen et al. [41] investigated the hydrophobicity of different types of filamentous bacteria in situ in activated sludge by adhesion of fluorescent microspheres with defined surface properties. They found that different filaments have very different degrees of hydrophobicity which might affect the flocculation and settling properties. Due to the complex composition and structure of the sludge surfaces, molecular determination for hydrophobicity and surface charge of the sludge has not yet been fully established.

Sludge viscosity was measured in the range 4–5 mPa s for sludges with SVI lower than 150 ml/g. For these sludges there was a linear correlation between viscosity and SVI (Table 4). Sludges with very high SVI (>200 ml/g) appeared to deviate from this correlation: sludge C with high FI had a high viscosity in the range 9–12 mPa s. With an increased in SVI, the flocs were larger and more irregularly shaped with filaments or other irregularities protruding from the floc surfaces, resulting in resistance to shear due to physical interaction forces between the flocs or aggregates of flocs. One sludge B with excessive numbers of filaments had relatively low viscosity. These flocs had a very dense interior with a very large average diameter. The other sludge C which had extremely poor settling and compaction properties contained flocs virtually lacking a floc core [7]. It is clear that the quality of filaments as well as the structure of the core of the flocs influence the viscosity. Although the relationship between the ZSV and sludge viscosity was insignificant, highly viscous sludges performed obviously low ZSV. These results indicate that the viscosity could be used as an indication of the compressibility of activated sludge. There is a lack of information in the literature regarding the relationship between the rheological properties of activated sludge and the compressibility and settleability.

3.3.3. Chemical constituents

Since the extracted amount of EPS might not necessarily reflect the *true* concentration of EPS in the sludge due to the probability of incomplete extraction, the settleability and compressibility were also correlated to the total concentration of the polymers in the sludge. For sludges with SVI $<$ 150 ml/g, the concentration of the three main polymers protein, humic substances and carbohydrate in both the sludge and the extracted EPS was significantly correlated ($P < 0.05$) with the SVI (Table 4). High concentration of these polymers measured in the sludge and extracted EPS were related to high SVI. Protein, as the dominating polymers, had stronger correlations with SVI than other polymers. However, the industrial sludges (F and G) had a somewhat different composition compared to the sludge from STPs and did not follow the general trend in relation to the SVI. The oil refinery sludge (F) contained relatively more protein in the total sludge even though the SVI was relatively low. The extracted EPS in sludge F, however, contained low concentrations of protein. This particular sludge contained very small, dense and round flocs that had good compaction properties. The EPS of the leachate sludge (G) contained high amounts of humic substances and carbohydrates but had low values of SVI. As mentioned earlier, this sludge had very good settling and compaction properties due to its dense and round aggregates. These results may indicate that floc structure and excessive numbers of filaments affect the SVI more than the composition of the biopolymers, since the sludge with SVI higher than 150 ml/g consisted of flocs with high FI (4 and 5). Nevertheless, it is interesting to note that high amount of total extracted EPS as well as the individual polymers corresponded to high SVI, indicating poor compressibility.

Poor correlations between ZSV and individual chemical constituents were established in the statistical analysis (Table 4), indicating that the sludge settleability was quantitatively independent of the content of the individual polymeric fractions. Only the content of protein in the sludge had a negative statistically significant correlation with ZSV. The results from this study may reveal that the precise role of polymer with respect to the sludge properties is complicated. It is likely that the polymers may take an important role in the floc formation, hydrophobic interaction and binding of the various floc constituents and may therefore also be associated with the morphological and physical characteristics of the polymeric network, i.e. flocs. More research is needed to assess the role of the EPS in stabilising the polymeric network and its effect on the floc structure.

The EPS have been paid much attention in the literature. It is generally believed that EPS contribute to better bioflocculation, resulting in better settling and dewatering properties [1,4,10,19,42–44]. However, the precise role of the EPS in controlling flocculating ability, compressibility and settleability of the sludge is very difficult to resolve, considering the complexity of the sludge matrix and the variability associated with different extraction methods employed in different studies [7,10,42–45]. A few workers have reported that the concentration of protein in the EPS is positively correlated to the SVI, whereas no correlations were found between concentration of carbohydrate in the EPS and the compressibility of sludge [21,41,43]. Large amounts of loosely bound and easily extracted polymers lead to a weakly flocculated sludge with a large volume fraction [6]. A large fraction of the sludge consists of EPS which are negatively charged [15,40]. According to the DLVO theory, the electrostatic repulsion between surfaces increases as the surface charge increases. Increased concentrations of EPS in the sludge would therefore increase the negative charge of the sludge surfaces and therefore also the repulsive forces between the polymer chains of the EPS. Since EPS are gel-like biopolymers and highly charged, they take up water to reduce the osmotic pressure difference between the biological aggregate and the surrounding liquid [15,42]. It is therefore not surprising that an increase in total amount of the extracted EPS corresponded to an increase in SVI. Our results showed that the EPS might also be important for the compressibility of the sludge and that the charged polymers swell and thereby increase the biopolymer volume. The EPS molecules probably create the relatively stable network holding the different floc fragments together. This means that the flocs should be regarded more as gels than rigid particles. The increase in SVI could therefore be partly due to the DLVO theory and partly due to steric interactions. The steric forces that the EPS may create when the EPS molecules extend out from cell surfaces can therefore physically prevent the cells and polymers from forming close contact.

As expected, increased concentrations of the multivalent cations Ca, Mg, Fe and Al in the sludge flocs contributed to improve compressibility and settleability (Table 4). Statistical relationships between SVI and ZSV and concentrations of the multivalent cations were significant. However, the oil refinery sludge (F) contained low concentrations of divalent and trivalent ions but had good settling and compaction properties. Since the biopolymers have high negative charge they have a high affinity for cations. Cations bound on the floc surfaces partially neutralise the negative charged groups and affect the surface charge [6]. Thus, the improved compressibility and settleability could be due to bridge binding interactions occurring between negatively charged polymeric groups and cationic ions and/or surface charge reduction due to binding to negatively charged sites on the floc surface.

4. Conclusions

This work has provided a comprehensive insight into the morphological, physical and chemical characteristics of activated sludge flocs. The impacts of the floc characteristics on the compressibility and settleability were assessed by a series of statistical data analyses and expressed with significant relationships. The results revealed that the morphological and physical properties of the sludge flocs had relatively more significant influence on the compressibility and settleability than the chemical properties. The main observations were:

1. The flocs size, fractal dimension and filament index were the major parameters associated with SVI and ZSV. Activated sludges containing relatively small and compact flocs with low numbers of filaments had better compressibility and settleability. The sludge properties SVI and ZSV were strongly influenced by the fractal dimension and filament index of sludge flocs.
2. Flocculating ability and surface properties of the sludge flocs played important roles in sludge compressing and settling abilities. Sludge with higher flocculating ability demonstrated better compressibility. High values of hydrophobicity and negative surface charge corresponded to high SVI and low ZSV. Highly viscous sludges demonstrated high SVI and low ZSV.
3. The quantity of the polymeric compounds protein, humic substances and carbohydrate in the sludge and the extracted EPS had significant positive correlations with SVI, whereas ZSV was independent of the polymeric constituents of the sludge. Although the contribution of the polymeric constituents to the sludge properties is complicated, it is evident that sludge containing high concentration of the extracted EPS had poor compressibility and settleability. High contents of Ca, Mg, Al and Fe in the sludge improved significantly both compressibility and settleability.

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