

Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering

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Abstract

The management of wastewater sludge, now often referred to as biosolids, accounts for a major portion of the cost of the wastewater treatment process and represents significant technical challenges. In many wastewater treatment facilities, the bottleneck of the sludge handling system is the dewatering operation. Advanced sludge treatment (AST) processes have been developed in order to improve sludge dewatering and to facilitate handling and ultimate disposal. The authors have extensively reported lab-scale, semi-pilot and pilot investigations on either thermal and thermochemical processes, or chemical oxidation using hydrogen peroxide. To understand the action of these advanced sludge technologies, the essential role played by extracellular polymeric substances (EPS) needs to be understood. EPS form a highly hydrated biofilm matrix, in which the micro-organisms are embedded. Hence they are of considerable importance in the removal of pollutants from wastewater, in bioflocculation, in settling and in dewatering of activated sludge. The present paper reviews the characteristics of EPS and the influence of thermochemical and oxidation mechanisms on degradation and flocculation of EPS. Experimental investigations on waste activated sludge are conducted by the authors to evaluate the various literature findings. From the experiments, it is concluded that AST methods enhance cake dewaterability in two ways: (i) they degrade EPS proteins and polysaccharides reducing the EPS water retention properties; and (ii) they promote flocculation which reduces the amount of fine flocs.

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1. Introduction

Although biological processes are an effective way of treating wastewater and ensuring minimum residual impact on the aquatic environment, they have the serious drawback of producing huge amounts of excess sludge: the more effective the process is in removing contaminants from the wastewater, the more sludge is produced. Historically, it was common to see schematics that showed the water treatment scheme in detail with all of the appropriate unit processes and an arrow at the end that simply said “sludge to disposal”. The assumption that the sludge would somehow be dealt with was supported by the fact that only a

minor fraction of the design and construction budget related to sludge management. Unfortunately this approach does not represent the reality and today it is recognised that the management of sludge, or biosolids, represents major investment and operating costs and forms an important technical challenge. The associated capital and operating costs may be as high as 25–50% of the total cost of the wastewater treatment process (Karr and Keinath [1]; Baeyens et al. [2]).

The bottleneck of the sludge handling system is the dewatering operation. Advanced sludge treatment (AST) processes have been developed and investigated in order to improve sludge dewatering, and facilitate the ultimate disposal. Different processes using thermal hydrolysis (neutral, acid, alkaline) or chemical oxidation (H₂O₂, O₃, O₂, ...) have been proposed in literature. The authors (Neyens and Baeyens [3,4]; Neyens et al. [5–9]) have recently summarized the literature findings and added extensive experimental results concerning the optimum treatment conditions of thermal and thermochemical processes, as

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Nomenclature

| | |
|----------|--|
| AST | advanced sludge treatment |
| COD | chemical oxygen demand |
| CST | capillary suction time |
| d | daily |
| DS | dry solids |
| EPS | extracellular polymeric substances |
| FTIR-ATR | Fourier transform infrared attenuated total reflection |
| IE | equivalent-inhabitant |
| ODS | organic dry solids |
| ORD | oxidative reductive oxidation |
| <i>P</i> | pressure |
| <i>T</i> | temperature |
| WWTP | wastewater treatment plant |

well as peroxidation (H_2O_2) for thickened sludge (5–6% DS) from a municipal sewage treatment plant.

Effective dewatering requires the attack of the micro-organisms. The vast majority of these micro-organisms live in aggregates such as films, flocs and sludges. A common feature is that the micro-organisms are embedded in a matrix of extracellular polymeric substances (EPS). To understand the action of the AST technologies in the sludge dewatering process, the essential role played by the EPS must be assessed and understood.

The present paper reviews the characteristics of EPS and assesses the mechanisms in, and results of, AST processes.

2. Extracellular polymeric substances: generalities

The production of EPS is a general property of micro-organisms in natural environments (Wingender et al. [10]). The abbreviation EPS is used as a more general and comprehensive term for different classes of organic macromolecules such as polysaccharides, proteins, nucleic acids, lipids, and other polymeric compounds which have been found to occur in the intracellular space of microbial aggregates, more specifically at or outside the cell surface (Flemming and Wingender [11]).

Films, flocs and sludges are accumulations of micro-organisms, EPS, multivalent cations, biogenic and inorganic particles as well as colloidal and dissolved compounds. EPS are mainly responsible for the structural and functional integrity of the aggregates and are considered as the key to the physicochemical and biological properties (Flemming and Wingender [11]). EPS form a three-dimensional, gel-like, highly hydrated and often charged biofilm matrix, in which the micro-organisms are embedded and more or less immobilized (Wingender et al. [10]). The total mass of EPS (EPS and water held within the EPS-structure) has been found to represent up to 80% of the mass of activated sludge (Frolund et al. [12]).

Polysaccharides have often been assumed to be the most abundant components of EPS in early biofilm research (approximately 60% of the dry solids content). However, proteins, nucleic acids and amphiphilic compounds including (phospho)-lipids have also been shown to appear in significant amounts (up to 40% of the DS content) (Wingender et al. [10]).

EPS are involved in both detrimental and beneficial characteristics of microbial aggregates (Flemming and Wingender [13]). In biofouling they are responsible for the increase of friction resistance, change of surface properties such as hydrophobicity, roughness, colour, etc. In biocorrosion of metals they are involved by their ability to bind metal ions. In bioweathering, they contribute by their complexing properties to the dissolution of minerals. The EPS present adsorption sites for pollutants such as heavy metal ions and organic molecules hence contributing to the water purification, but possibly increasing the hazardous properties of the sludge produced. Other biotechnological uses of EPS exploit their contribution to viscosity, e.g. in food, paints and oil-drilling ‘muds’, whereas their hydrating properties find applications in cosmetics and pharmaceuticals (Flemming and Wingender [13]). EPS may furthermore have potential uses as biosurfactants, e.g. in tertiary oil production, and as biological glue (Flemming and Wingender [13]).

3. EPS in activated sludge

EPS are present in varying quantities in sewage sludge, occurring both as a highly hydrated capsule surrounding the bacterial cell wall and loose in solution as slimy polymers.

Extracellular polymers are thought to be of considerable importance in the removal of pollutants from wastewater, in bioflocculation and settling (Eriksson and Alm [14]; Bruus et al. [15]; Urbain et al. [16]), and in the dewatering of activated sludge (Urbain et al. [16]; Pere et al. [17]; Forster and Lewin [18]; Li and Ganczarczyk [19]; Sanin and Vesilind [20]). Li and Ganczarczyk [19] found that EPS form the third biggest component in an activated sludge floc after the cells and water.

3.1. Characterization of the organic matter in EPS

In activated sludge, between 70 and 80% of the extracellular organic carbon could be attributed to proteins and saccharides (Dignac et al. [21]). Some researchers have suggested that polysaccharides play the major role in flocculation: Bruus et al. [15] indicated that divalent cations bridge negatively charged groups on alginate-like polysaccharides within bioflocs. Forster and Dallas-Newton [22] also suggested cations may bridge between negatively charged carboxyl groups on uronic acids. Most research on the characterization of EPS from activated sludge has focused on exocellular polysaccharides (Forster [23]; Horan and Eccles [24]; Jorand et al. [25]). However, many studies

have reported that the exocellular protein concentration in activated sludge systems exceeded the exocellular polysaccharide concentration (Tenney and Verhoff [26]; Brown and Lester [27]; Barber and Veenstra [28]; Eriksson and Alm [14]; Urbain et al. [16]; Jorand et al. [25]; Frolund et al. [12]; Higgins and Novak [29]). The predominance of proteins in activated sludge EPS could be due to the presence of large quantities of exoenzymes in the floc (Frolund et al. [30]). The extracellular proteins could also originate from wastewater compounds or from cell lysis compounds.

On the basis of their relatively high content of negatively charged amino acids, proteins are supposed to be more involved than sugars in electrostatic bonds with multivalent cations, underlining their key role in the floc structure (Frolund et al. [30] and Sutherland [31]). The difference in protein content between activated sludge and pure cultures is possibly the result of the complexity of the substrate present in wastewater which necessitates the presence of exoenzymes around bacteria in activated sludge, while the synthetic substrate added to pure cultures is much more simple and easy to degrade.

The remaining 20–30% uncharacterised organic carbon of EPS are possibly composed of humic compounds, uronic acids, nucleic acids and lipids.

3.2. EPS and sludge dewatering

3.2.1. Generalities

The fact that EPS are highly hydrated and are able to bind a large volume of water was discovered by Forster [32]. EPS therefore play an important ecological role in protecting biofilm organisms from desiccation (Flemming and Wingender [13]): it maintains an environment in which microbial life is possible.

The technical impact of this water retention affects the dewaterability of activated (biological) sludge: EPS water needs to be removed. This is a significant economical drawback of the water retention by EPS, considering the energy required for dewatering and the millions of tons of sewage sludge which have to be dewatered every year.

The influence of EPS on dewatering has been investigated in various studies. Kang et al. [33] found that extracellular polymer substance extracted from one activated sludge and then added to another activated sludge has a detrimental effect on the dewatering process of the latter sludge. According to Houghton et al. [34] however, there appears to be a level of EPS at which the sludge should be easiest to dewater. Increasing relatively low levels of EPS can aid sludge dewaterability by improving the level of sludge flocculation (Sanin and Vesilind [20]). This reduces the number of small particles present in the sludge, a factor that has been shown previously to make a sludge easier to dewater (Karr and Keinath [1]). Once optimum sludge flocculation has been attained, further increases in EPS become detrimental to sludge dewaterability. It is envisaged that this is due to the highly hydrated nature of the EPS retaining wa-

ter within the sludge matrix, which counteracts the benefit of flocculation. The water in the EPS will shield many of the potential binding sites which could otherwise bridge the macromolecules by electrostatic interactions, hydrogen bonds and London dispersion forces (Flemming et al. [35]).

3.2.2. Water binding mechanisms

The water retained in the EPS-structure is bound mainly by the polysaccharides and proteins of the EPS in the activated sludge. The molecular mechanisms of water binding are of crucial importance for a rational basis of the improvement of dewatering techniques. Schmitt et al. [36] investigated water binding by measuring the exchange of D₂O against H₂O in an FTIR-ATR study. Their results showed that treatment with chlorine clearly facilitated the exchange, indicating that chlorine may have destroyed water binding structures, probably in the EPS.

According to Flemming [37], two types of binding mechanisms between water molecules and the EPS-structure must be considered, i.e. electrostatic interactions and hydrogen bonds:

- (i) *Electrostatic interactions* are active between the permanent dipole of the water molecules and ions or permanent/induced dipoles of the functional groups of the EPS-structure. Considering the strength of the potential interactions, carboxylate groups and hydroxyl groups are expected to play the most important roles among the functional groups present in EPS.
- (ii) *Hydrogen bonds* are mainly active between the EPS hydroxyl groups (particularly frequent in polysaccharides) and water molecules. They also support the tertiary structure of proteins.

Both types of binding forces contribute to the overall binding energy, although possibly to different extents. The individual binding force of any type of weak interaction is very small compared to a covalent C–C bond (Flemming [37]). However, the large number of functional groups of a macromolecule may result in an overall binding energy which is well in the range of several covalent C–C bonds. These interactions are affected by the water content and increase with increasing polymer concentration which is another argument to degrade the EPS to improve dewaterability.

4. Advanced sludge treatment and EPS

The authors have previously summarized the literature findings concerning the optimum treatment conditions of thermal and thermochemical pre-treatment processes (Neyens and Baeyens [3]) and chemical oxidation using hydrogen peroxide (Neyens and Baeyens [4]). These reviews were complemented by extensive lab-scale (Neyens et al. [5–7]), semi-pilot and pilot-scale investigations (Neyens et al. [8,9]) for treating thickened sludge (5–6% DS) from a municipal sewage treatment plant. From these experimental

results and reviews, we suspect that the AST methods improve the dewaterability of the sludge by affecting the extracellular polymeric substances in two ways: (i) they have the potential to degrade the EPS and; (ii) they affect the multifunctional groups of the EPS and promote their participation in several interactions. Both effects are studied below.

4.1. Degradation of EPS

Considering the high water binding properties of EPS (Forster [32]; Roberson and Firestone [38]; Wingender [10]; Flemming and Wingender [13]), together with the finding that the mass of total EPS represents up to 80% of the mass of activated sludge (Frolund et al. [12]), it is concluded that the dewatering efficiency of activated (biological) sludge can be increased by degrading the EPS. The susceptibility towards degradation depends both upon the agent which leads to the breaking of polymer chains, and upon the chemical nature of the polymer (Wingender et al. [10]). We therefore review the literature on EPS degradation and present some experimental results concerning the influence of the proposed AST methods on degradation of EPS.

4.1.1. Literature review of degradation of EPS by AST methods

4.1.1.1. Heat treatment. Thermal conditioning significantly improves the dewatering properties of waste activated and primary sludge (Hatfield [39]; Brooks [40]; Everett [41]; Marshall [42]). Semi-pilot-scale experiments by the authors (Neyens et al. [9]) demonstrated that thermal hydrolysis most effectively reduces the residual sludge amounts and enhances the dewaterability when performed at temperatures of 120 °C for a 60 min reaction time. The rate of filtration, as measured from CST-values, decreases compared to the initial untreated sludge sample. The amount of DS to be dewatered is reduced to 58% of the initial untreated amount and the DS-solid content of the dewatered cake increases from 30% DS (initial untreated) to approximately 43% DS (Table 1).

Watson et al. [43] found that protein, the main component of EPS in activated sludge, is degraded by heating. Extremes

of temperature cause them to lose their natural shapes (denaturation) and sometimes to precipitate irreversibly out of solution in an inactive form.

Results of Hatfield [39], Everett [41] and Neyens et al. [5] revealed that heat treatment alters the structure of the sludge, reduces the specific resistance and transforms some of the suspended organic solids into soluble compounds. Everett [41] confirmed the increase in COD of the water phase as a result of thermal hydrolysis of organic macromolecules: amino acids, volatile acids, and simple sugars are produced.

Brooks [40,44] treated sludge at 180 °C and found that hydrolysis of the exocellular and intracellular organic fraction of the sludge, i.e. proteins, carbohydrates and lipids, leads to destruction of the colloidal properties of these macromolecules with a resulting release of the water originally bound to the particles.

Edelhoc and Osborne [45] also concluded that the heat treatment reduces or denatures the substances with high affinity towards water such as proteins, carbohydrates and nucleic acids.

4.1.1.2. Acid/alkaline thermochemical treatment. Acids and alkalis act as catalysts in the thermal hydrolysis of organic macromolecules. Everett [41] already noted that their addition to the sludge prior to thermal conditioning resulted in an additional improvement of the rates of mechanical dewatering and increased percentages of DS in the filter cake.

The results of the sensitivity analysis by the authors (Neyens et al. [9]) concerning optimum treatment conditions are summarized in Table 1. Both acid thermal and alkaline thermal hydrolysis increase the rate of filtration (as measured from CST-values), the amount of DS to be dewatered is reduced to 45% of the initial untreated amount for acid hydrolysis and to 62% for alkaline hydrolysis; the DS-solid content of the dewatered cake increases from 25% DS (initial untreated) to approximately 45% DS for acid hydrolysis and from 28 to 48% DS for alkaline hydrolysis.

Extremes of pH (acidity or alkalinity) cause EPS proteins to lose their natural shapes (Watson et al. [43]).

Polysaccharides, another component of EPS, are generally unstable in strong acids, which leads to acid hydrolysis

Table 1
Comparison between acid and alkaline thermal hydrolysis: semi-pilot-scale experiments

| | Thermal hydrolysis | Acid thermal hydrolysis | Alkaline thermal hydrolysis |
|--|--------------------|--------------------------------|-----------------------------|
| Reagent | | H ₂ SO ₄ | |
| Optimum conditions | | | |
| <i>T</i> (°C) | 120 | 120 | 100 |
| pH | 7 | 3 | 10 |
| Time of reaction (min) | 60 | 60 | 60 |
| Rate of mechanical dewatering after neutralisation, as measured by CST | Decreased | Increased | Increased |
| Amount of DS to be dewatered as a fraction of the initial untreated amount (%) | 58 | 45 | 62 |
| % DS in filter cake | 43 | 45 | 48 |
| % DS initial untreated | 30 | 25 | 28 |

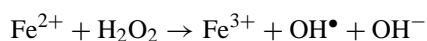
of the glycosidic linkages (Wingender et al. [10]). They are however quite stable towards degradation in alkali, especially at high temperature (Wingender et al. [10]).

Strong alkali may solubilize gels not only because of chemical degradation, but also because of the ionization of the hydroxyl groups ($-\text{OH} \rightarrow -\text{O}^-$) which leads to extensive swelling and subsequent solubilization (Wingender et al. [10]).

At extremes of pH and after the solubilization of gels (EPS), the cell loses its viability, it cannot maintain an appropriate turgor pressure and disrupts. Acids/alkali added to the cell suspension react with the cell walls in several ways, including the saponification of lipids in the cell walls, which leads to solubilization of membrane (Erducler and Vesilind [46]). Disruption of sludge cells leads to leakage of intracellular material and water out of the cell which further improves dewaterability.

4.1.1.3. Advanced oxidation. The oxidation processes utilizing activation of H_2O_2 by Fe(II)-salts are referred to as Fenton's reactions. The Fenton reaction causes the dissociation of the oxidant and the formation of highly reactive hydroxyl radicals that attack and destroy organic pollutants.

Fenton's reagent is a mixture of H_2O_2 and ferrous iron, which catalyses the formation of hydroxyl radicals according to the reaction (Kitis et al. [47]; Yoon et al. [48], Lu et al. [49]):



Advanced sewage sludge treatment by peroxidation was studied by the authors. Both lab-scale (Neyens et al. [6]) and pilot-scale experiments (Neyens et al. [8]) were conducted to investigate the efficiency of the process in function of the operating conditions (temperature, pH, Fe^{2+}). The same properties as in the case of hydrolysis were measured. The optimum results of these experiments are summarized in Table 2.

Table 2
Comparison between lab-scale and pilot-scale peroxidation

| | Pilot-scale peroxidation (1000l) |
|--|----------------------------------|
| Optimum conditions | |
| <i>T</i> (°C) | Ambient |
| <i>P</i> (atm) | Ambient |
| pH | 3 |
| H_2O_2 (g/kg DS) | 25 |
| Fe^{2+} (g/kg DS) | 1.67 |
| Time of reaction (min) | 60–90 |
| Reduction of DS and ODS during peroxidation | 30% |
| Rate of mechanical dewatering, as measured by CST | Increased |
| Amount of DS to be dewatered as a fraction of the initial untreated amount | 40% |
| % DS in filter cake | 45% (27% DS initial untreated) |

Extracellular polymers are susceptible to oxidative-reductive depolymerization (ORD) which results in cleavage of linkages in the polymeric backbone (Herp [50]; Christensen et al. [51]; Wingender et al. [10]). The degradation of alginate by ORD has been studied in detail (Smidsrod et al. [52–54]). ORD involves a series of free radical reactions which ultimately lead to chain scission. Molecular oxygen and transition metal ions (e.g. $\text{Fe}^{2+}/\text{Fe}^{3+}$) are efficient catalysts. The Fenton reagent effectively degrades polymers (Christensen et al. [55]). ORD has the advantage that relatively high depolymerization rates may be obtained at ambient temperature due to the low activation energies, whereas acid or alkaline hydrolysis require high temperatures. Acids or alkalis can be employed, but the degradative effects are non-specific and may severely affect the cells. Most cells have an intracellular defense system against toxic radicals. This means that peroxides may operate within a biofilm, but that they degrade only extracellular material such as the matrix (Christensen et al. [55]).

4.1.2. Experimental investigations

In the following, the Advanced Sludge Treatment methods are studied from the point of view of degrading the EPS. Sludge samples were obtained from the underflow of the thickener (approximately 6% DS) at the municipal sewage treatment plant of Kessel-Lo (Belgium).

To get a clear indication on how the proposed AST methods influence the EPS-content of the activated sludge, the presence of the main components of EPS, i.e. proteins and polysaccharides, in the untreated and treated sludges is measured. Proteins and polysaccharides are also the most water retaining components, hence influencing the dewaterability characteristics of the sludge. Since the mass of total EPS (EPS and water held within its structure) represents up to 80% of the mass of the activated sludge (Frolund et al. [12]), the measuring of *sludge* protein and polysaccharide content instead of EPS protein and polysaccharide content should give a reasonable indication of the degradation of EPS-components by the AST methods.

The content of protein compounds was determined by the modified Lowry method described by Frolund et al. [30] with bicinchoninic acid (BCA) as a standard. The polysaccharide content was determined by the anthrone method modified by Raunkjer et al. [56] and glucose was used as a standard.

The experimental results for the untreated sludge and the sludges treated with the various AST methods are indicated in Table 3. The protein and polysaccharide content are given as percentages of the dry solids content. All samples are treated at the optimum conditions (given in Table 1) derived from experiments reported in Neyens [9].

The results of Table 3 illustrate that proteins are more prevalent than polysaccharides and probably are the most abundant component of the sludge (EPS). Over one-third of the dry solids and over one half of the organic dry solids of the untreated sludge consists of proteins (Table 3). Over

Table 3
Protein and polysaccharide content of untreated and AST-treated sludges originating from WWTP Leuven (ODS/DS = 0.68)

| | Blanco | Thermal hydrolysis | Acid thermal hydrolysis | Peroxidation |
|---------------------------|--------|--------------------|-------------------------|--------------|
| Proteins (% of DS) | 36.2 | 30.1 | 25.7 | 24.3 |
| Polysaccharides (% of DS) | 22.4 | 20.7 | 19.3 | 18.5 |

85% of the organic carbon in the untreated sludge could be attributed to proteins and polysaccharides (Table 3).

It is clear from the results that the thermochemical pre-treatment methods cause a destruction of proteins and polysaccharides. It has to be remarked that the degradative effect caused by heating the sludge is strongly improved by the addition of acid. In case of thermal hydrolysis 13% of total mass of polysaccharides and proteins is destroyed, in the case of acid thermal hydrolysis 23%. However, peroxidation under ambient conditions is more efficient in degrading proteins and polysaccharides (27% destroyed). Each of the three tested AST methods caused a larger destruction for proteins than of polysaccharides.

Experimental investigations done by the authors (Neyens et al., [57]) moreover revealed that the degradation of EPS by the AST methods caused the release of a significant amount of heavy metals into the water phase. Therefore, the water phase (filtrate) will be subject to metal precipitation and separation prior to being recycled to the WWTP influent.

4.2. The role of EPS in flocculation

The stability of sludge flocs or floc strength is important in determining the efficiency of biosolids–liquid separation in the activated sludge process. The presence of a large number of fine flocs significantly reduces not only the effluent quality but also the dewatering properties of excess sludge. These fine flocs can result in the clogging of the pore structure in sludge cakes and may also lead to a higher level of bound water content of the sludge. Consequently, the minimization of fine flocs in the activated sludge process is essential for better effluent quality and for improved dewatering properties of sludge.

A number of mechanisms, such as charge neutralization, hydrophobic interactions, hydrogen bonds, ionic interactions, and physical enmeshment have been recognized as important in controlling the formation of the sludge floc and its stability. Since EPS (present at or near sludge surfaces) are composed of diverse high-molecular-weight biopolymers (e.g. polysaccharides, proteins, DNA, and lipids) that contain many functional groups capable of participating in interactions, it is likely that the stability of sludge flocs is related to the interparticle interactions at or near sludge surfaces. The effects of AST methods on interparticle interactions are now reviewed.

4.2.1. The effects of pH on surface charge and stability of sludge flocs (electrostatic interactions)

4.2.1.1. *Low pH.* Everett [41], Karr and Keinath [1] and Neyens et al. [5] showed that the dewaterability of activated sludge improved as the pH decreased. The presence of acids seemed to alter the particle size distribution as the concentration of supracolloidal solids was noted to have decreased with pH (Karr and Keinath [1]). It has furthermore been shown that the amount of supracolloidal particles in the size range 1–100 μm seems to be an important factor for the filterability of activated sludge (Karr and Keinath [1]; Barber and Veenstra [28]). The reason for this is generally believed to be blinding of the sludge cake or filter medium due to clogging with fine particles. Given this background, it can be assumed that the amount of fine particles in a sludge will be of importance for both clarification and filterability.

Liao et al. [58] illustrate that the sludge flocs were generally more stable (smaller dissociation constant) in the pH range of 2.5–9.5 than those in the pH range of less than 2. A minimum dissociation constant occurred in the pH range of 2.6–3.6, which is the pH range of the isoelectric point. The presence of a net charge on sludge surfaces creates electrostatic repulsion that prevents close contact of sludge micro-organisms (Liao et al. [58]). The variation in dissociation constants in terms of pH indicates that repulsive electrostatic interactions were involved in disrupting the stability of sludge flocs. The variation in the dissociation constants with pH may be explained by the presence, on sludge surfaces, of different functional groups, such as carboxyl, amino, and phosphate groups with different ionization constants ($\text{p}K_a$). The major source of surface charge was the ionisation of carboxyl and amino groups from proteins, the main component of EPS (Liao et al. [58]).

The repulsive electrostatic interactions were minimized in the pH range of 2.6–3.6, near the isoelectric point, so that fine flocs could approach each other closely, and the dissociation constants of sludge flocs were also at a minimum as mentioned earlier. The sharp increase in the dissociation constants at a pH less than the isoelectric point could be related to the adsorption of H^+ by amino groups (R-NH_2) to form R-NH_3^+ under low pH conditions. The relatively stable dissociation constants, in the pH range of 4.5–9.5, could be attributed to the combined influence of the amino and carboxyl groups on sludge surfaces ($\text{p}K_a$ values of 1.71–3.0 and 8.2–11, respectively (Smith and Martell [59]; Nelson and Cox [60]). The naturally occurring amino groups might form a stable five member ring chelated with divalent cations, such as Ca^{2+} and Mg^{2+} , and therefore neutralize the amino groups (Huang et al. [61]). At least 99% of the carboxyl groups of amino acids would ionise into COO^- groups when $\text{pH} = \text{p}K_a + 2$ i.e. pH 4–5), so that the magnitude of the surface charge was relatively stable, which further explains the stability of the dissociation constants in the pH range of 4.5–9.5.

4.2.1.2. High pH. Neyens et al. [7] showed that the treatment of activated sludge at high pH improved the dewaterability. This improved dewaterability is also illustrated in Table 1.

Katsiris and Kouzeli-Katsiri [62] stated that when the pH of sludge samples increased, the bacterial surfaces become increasingly negatively charged. This creates high electrostatic repulsion which causes desorption of some part of extracellular polymers. These dissolved extracellular polymers and intracellular material released by cell disruption are all high molecular weight polymeric materials that possibly act as polyelectrolytes and thereby promote flocculation (Valom and McLoughlin [63]) and dewaterability.

Since cells are disrupted both at low and high pH, the water-floc separation properties of sludge are improved by the release of interstitial water trapped inside the sludge cells and by the promotion of the flocculation, reducing the amount of small flocs.

4.2.2. The effects of cations on flocculation (ionic interactions)

Several researchers have suggested that cations in general, and bi-valent ones specifically, aid flocculation and dewatering by bridging negative sites on exocellular biopolymers which promotes an increase in floc size, floc density, floc resistance to shear and dewaterability (Tezuka [64]; Bruus et al. [15]; Eriksson and Alm [14]; Andreadakis [65]). Higgins and Novak [66] showed that the settling and dewatering properties of the activated sludge systems were dependent on both the concentrations and ratios of cations added with high concentration ratios of divalent/monovalent cations significantly improving settling and dewatering. This improvement correlates with an increase in the bound exocellular biopolymer content involved in the aggregation process. Divalent cations act to bind protein and not polysaccharide within the floc in these systems (Higgins and Novak [66]). As a result, the improvements in settling and dewatering are well correlated to the bound protein content in addition to the increase in divalent cation concentration. These results suggest that protein is an important biopolymer component in the flocculation process. It is likely that carboxyl groups from amino acids in proteins and phosphate groups from DNA were the dominant functional groups involved in salt bridging (Liao et al. [58]).

4.2.3. Experimental investigations

Most information on floc size diameter in the literature concerns activated sludge. Andreadakis [65]; Higgins and Novak [66]; Biggs et al. [67] found that for well mixed activated sludge systems the typical range of floc sizes was between 10 and 70 μm . No information was found on size distribution of thickened biological sludge. To assess the possible influence of AST on the biosolids size distribution, an identical sample of 6% DS-thickened sludge was treated by the different thermal and chemical AST presented above. The experimental set-up is described in Neyens et al. [9].

Untreated and treated samples were analysed for size distribution using a Malvern Mastersizer laser diffractometer. This is a laserbeam scattering instrument that operates on the principle of Fraunhofer Diffraction Theory. The particle size detection range is between 5 and 300 μm . This size range is within the range indicated to represent most of the activated sludge flocs (Jorand et al. [25]). The particle diameters were calculated assuming spherical particles. Samples were diluted before analysis to obtain particle counts within the concentration limits of the analyser.

The results are illustrated in Table 4, and expressed as the respective particle sizes corresponding with 10, 50 and 90% of the size histogram i.e. 10% of the flocs have sizes in the range $[0 \mu\text{m}-d_{10}]$, 50% in the range $[0 \mu\text{m}-d_{50}]$ and 90% in the range $[0 \mu\text{m}-d_{90}]$.

Since larger flocs are easier to dewater than smaller flocs, and since the presence of smaller flocs tend to clog the cake during filtration thus reducing the dewaterability, the evolution of the d_{10} , d_{50} and d_{90} sizes give a clear indication of the positive or negative effects.

With the exception of thermal hydrolysis, all techniques tested increase the size of the smaller flocs being present. Thermal hydrolysis moreover significantly reduces the other characteristic particle sizes, thus pointing towards an overall reduction of flocculation performance. However, this reduced flocculation behaviour does not significantly cause reduced dewatering properties (Table 1), since the reduced flocculation is offset by the degradation of the hygroscopic EPS and subsequent disruption of the cells as explained in Section 4.1.2. This release of bound water improves the dewaterability irrespective of the decreased flocculation behaviour.

Table 4
Particle sizes and standard deviation after applying the different AST methods

| Treatment applied | d_{10} (μm) | σ_{10} (μm) | d_{50} (μm) | σ_{50} (μm) | d_{90} (μm) | σ_{90} (μm) |
|--|----------------------------|---------------------------------|----------------------------|---------------------------------|----------------------------|---------------------------------|
| Untreated (blanco) sludge sample | 32.82 | 6.11 | 106.64 | 7.12 | 169.47 | 2.71 |
| Thermal hydrolysis | 34.92 | 7.23 | 65.99 | 7.55 | 117.36 | 12.78 |
| Acid thermal hydrolysis | 54.88 | 9.84 | 106.93 | 8.10 | 169.57 | 3.11 |
| Alkaline thermal hydrolysis | 58.46 | 1.88 | 105.24 | 4.97 | 209.16 | 4.05 |
| Acid treatment at ambient conditions | 64.06 | 1.26 | 121.36 | 0.92 | 193.48 | 0.41 |
| Alkaline treatment at ambient conditions | 66.07 | 2.33 | 125.13 | 12.43 | 226.77 | 7.93 |
| Peroxidation | 47.38 | 3.84 | 91.83 | 7.53 | 161.47 | 10.59 |

Acid thermal hydrolysis causes the smaller flocs to aggregate (Table 4). This explains that the breakdown of flocs caused by thermal hydrolysis is compensated by adding acid (H_2SO_4), which is indicated in the results of acid treatment (at ambient conditions) in Table 4. This illustrates that at pH 3 the repulsive interactions and the resulting dissociation constants are minimized as stated above.

A similar compensation effect is noticed in the case of alkaline thermal hydrolysis: the thermal breakdown of flocs is compensated by the alkaline effect. The results in Table 4 also stress the importance of divalent Ca^{2+} -ions as bridging agents: divalent ions aid flocculation and dewatering by bridging negative sites on exocellular biopolymers which promotes an increase in floc size, floc density, floc resistance to shear and dewaterability (Tezuka [64]; Bruus et al. [15]; Eriksson and Alm [14]).

Peroxidation slightly reduces the value of d_{50} and d_{90} , effects counteracted by the marked increase of the d_{10} -size. This improved flocculation performance in combination with the capability of the hydroxyl radicals to degrade EPS by ORD, turns peroxidation into a promising AST method (Table 1).

5. The economy of using AST methods

Complete and detailed cost–benefit analysis of the AST methods are reported in Neyens [68], the most important results are summarized below.

Peroxidation gave the best results with respect to reducing the amounts of sludge and improving the product quality. An economic assessment is therefore made for a WWTP of 300 000 equivalent-inhabitant (IE) with sludge dewatering and incineration. For the case of a daily (d) sludge production of 60 g DS/IE d, a total annual amount of 6570 ton DS has to be dealt with. The annualised cost of operating a traditional 300 000 IE WWTP where the dewatered sludge is incinerated is 1 900 000 EUR.

The peroxidation process requires the use of corrosion-resistant construction materials. The fixed equipment costs are estimated to be 50 000 EUR per year when written off over 10 years. The maintenance costs are approximately 10 000 EUR per year. Variable costs consist of chemical costs and electricity. Chemical costs involved are rather high. Electricity is only necessary for the pumps. The sum of these variable costs is estimated to be 400 000 EUR per year. The total annualised cost for a 300 000 IE AST WWTP is therefore 460 000 EUR per year. The cost for disposal (transport and incineration) is 72 EUR/ton sludge irrespective of percentage DS of the dewatered sludge. This evidences the fact that the disposal cost per unit DS is significantly reduced for peroxidation since it causes a DS content of 45% in the dewatered sludge (disposal cost is 72 EUR for 450 kg DS thus 160 EUR/ton DS) compared to 25% DS for the traditional treatment facility (disposal cost is 72 EUR for 250 kg DS thus 288 EUR/ton DS). Implementing the peroxidation

technique, there is hence a saving of over 800 000 EUR per year on the disposal cost of the annual amount of 6570 ton DS. Considering the costs and the savings, there is a net saving of 800 000 – 460 000 EUR per year = 340 000 EUR per year or 52 EUR/ton DS for a WWTP of 300 000 IE.

6. Conclusion

Advanced sludge treatment processes such as thermal hydrolysis and peroxidation have been developed to improve sludge dewatering and to facilitate ultimate disposal. Implementing these methods in a wastewater treatment plant: (i) slightly reduces or increases the filtration rate; (ii) decreases the amounts of DS to be dewatered; and (iii) increases the DS content of the dewatered cake. The present paper made an attempt to explain these improvements in terms of the current knowledge concerning EPS which constitute up to 80% of the mass of activated sludge. It is concluded that AST methods degrade the EPS and influence the dewaterability of the sludge in two ways:

- (i) Degradation of the EPS reduces their water retention properties thereby releasing the EPS-bound water and increasing the dewatering efficiency of activated sludge. The advanced methods degrade proteins and polysaccharides of activated sludge EPS, which constitute almost 60% of EPS. The extent of the degradation varies among the AST methods from 23% for acid thermal hydrolysis to 27% for peroxidation.
- (ii) Degradation of the EPS reduces cell stability but increases the size of sludge flocs. The presence of a net surface charge on sludge surfaces creates electrostatic repulsion that prevents close contact of sludge micro-organisms. These repulsive interactions are minimized in the pH range of 2.6–3.6, near the isoelectric point, so that the dissociation constants of sludge flocs are also at a minimum. For this reason the flocculation of activated sludge improved as the pH decreased. The improved flocculation phenomena (bridging, ionic action) moreover reduce the amount of fine flocs, thus facilitating cake dewatering. This statement was underlined by conducting experiments on the size distribution of sludge samples treated by the different AST methods (Table 4).

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