Drainage properties of activated sludge

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Drainage properties of activated sludge

A dissertation submitted in partial fulfillment of the requirements for obtaining the degree of

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by

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Preface

This dissertation is submitted in partial fulfillment of the requirements for obtaining the degree of Doctor of Philosophy. The dissertation consists of an introduction, summarizing the project-related literature, and 4 scientific papers included as appendices.

This PhD project was carried out between September 2007 and August 2010 at the Section of Biotechnology in the Department of Biotechnology, Chemistry, and Environmental Engineering at Aalborg University, Denmark.

I would like to thank my supervisors, Per Halkjær Nielsen, Jeppe Lund Nielsen, Kristian Keiding, and Morten Christensen for their support and guidance in completing this study, as well as Lisbet Adrian, Flemming Andersen and Robert Hansen from Esbjerg for their help in carrying out this project. I also thank my office mates, Artur Mielczarek, Hien Nguyen, Sarita Singh, and Rikke Kristiansen, as well as other PhD fellows and technicians, for being good company to me throughout my study period. Finally, I would like to thank my lovely wife Gosia for her support and understanding.

I hereby declare this is my original work.

Dominik Dominiak Aalborg, September 2010

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1. Abstract in English

The activated sludge process for biological wastewater treatment is the most widespread wastewater treatment process in the world. The by-product of this process, the surplus activated sludge, usually contains more than 98% water, which implies a dewatering step so that transportation and handling of sludge are economically feasible. Dewatering and disposal of activated sludge is generally a problematic issue, and there is no simple solution.

Reed beds for sludge dewatering and mineralization offer an economically attractive alternative to pressure dewatering. The simplicity, low man-power and supervision requirements, and the possibility of applying the sludge residues in agriculture make this technology an attractive choice, especially for small wastewater treatment plants. However, frequent operational problems consisting of poor dewatering and mineralization performance, vegetation loss, and odor limit the widespread use of reed beds and give this technology a general reputation of being unreliable. The objective of this PhD project was to carry out a comprehensive investigation of the water-binding properties of activated sludge in relation to drainage and mineralization of sludge on reed beds. The particular aim was to solve the operational problems with handling sludge from two wastewater treatment plants in Esbjerg reed bed facility and to formulate some more general guidelines for reed bed design and operation.

A novel technique for the assessment of sludge drainability was developed and used for a comprehensive investigation of the process of gravitational sludge drainage. The specific cake resistance was used as the parameter describing sludge equality in terms of gravitational drainage. Sludge volumetric loading and sludge suspended solids concentration were found to be the two critical parameters deciding the efficiency of the drainage process. Sludge deflocculation due to shear and anaerobic conditions was found to strongly influence the quality of sludge and to be the reason behind the operational problems in Esbjerg reed bed facility. These problems were solved by upgrading the sludge quality by nitrate and calcium carbonate dosing, and by improving the basin loading schemes.

Extracellular polymers are important for floc strength and therefore determine many sludge properties. In this project focus was on extracellular DNA (eDNA) and its importance for floc strength. A new approach to quantification of extracellular DNA in mixed biofilms at microscale resolution was developed and combined with other staining techniques to assess the origin, abundance and role of eDNA in activated sludge biofilms. Most eDNA was found in close proximity to living cells in microcolonies, suggesting that most of it originated from active production. When the staining was combined with fluorescence *in situ* hybridization for identification of the microorganisms, it was found that the eDNA content varied among the different probe-defined species. The highest amount of eDNA was found in and around the microcolonies of denitrifiers belonging to the genera *Curvibacter* and *Thauera,* the ammonium-oxidizing *Nitrosomonas* and the nitrite-oxidizing *Nitrospira*. Other floc-formers also produced eDNA, although in lower amounts. The total eDNA content in activated sludge varied from 4 to 52 mg per gram volatile suspended solids in different wastewater treatment plants. Very high

local concentrations within some microcolonies were found with up to approx. 300 mg of eDNA per g of organic matter. DNase digestion of activated sludge led to general floc disintegration and disruption of the microcolonies with high eDNA content, implying that eDNA was an important structural component in activated sludge biofilms.

In conclusion, this PhD project significantly increased our understanding of sludge gravitational drainage on reed beds. The detailed mechanism of drainage was demonstrated, and the factors deciding the drainage rate and sludge quality in terms of drainage were identified. Guidelines for reed bed operation and design were proposed, taking into account the regular monitoring of sludge quality with a novel technique, capable of fast and easy sample evaluation directly after sampling. The role of extracellular DNA as an important floc- and microcolony-strengthening EPS component was demonstrated, and confirmed the intimate relationship between the microbiological composition of activated sludge, the resulting floc characteristics, and the overall sludge water-binding properties. Overall, this PhD project threw new light onto the drainage properties of activated sludge and their controlling factors, which brings in a new possibility of improving the design and operating reed bed facilities, thereby increasing the competitiveness of this simple and effective technology of surplus sludge disposal.

2. Abstract in Danish

Brug af aktiv slam til biologisk spildevandsrensning er den mest udbredte renseproces af spildevand i verden. Biproduktet af denne proces er overskudslam og det indeholder normalt mere end 98% vand, hvorfor et afvandingstrin er nødvendig for at videre transport og håndtering af slammet er økonomisk forsvarligt. Afvanding og anbringelse af overskudslam er en relativ problematisk sag og der er ingen simple løsninger.

Slambede til slamafvanding og mineralisering er et økonomisk attraktivt alternativ til traditionel afvanding ved højt tryk. Den simple proces, lille behov for mandskab og instruktion samt muligheden for at anvende slamrester indenfor jordbruget gør denne teknologi til et attraktivt valg, især for små renseanlæg. Der er imidlertid en række forhold som tyder på at denne proces kan være upålidelig, fx dårlig afvanding og mineralisering, hendøen af vegetation og lugtproblemer, hvilket forhindrer en større udbredelse af teknologien. Formålet med dette PhD-projekt var at udføre et omfattende studie af de vandbindende egenskaber ved aktivt slam i forbindelse med dræning og mineralisering af slam på slambede. Herved skabes dels et grundlag til at kunne løse driftsproblemer på to renseanlæg i Esbjerg i forbindelse med slamhåndtering af slam og dels at udvikle mere generelle guidelines til design og drift af slambede.

Der blev udviklet en ny teknik til måling og vurdering af slams dræningsegenskaber og den blev brugt til omfattende undersøgelser af afvanding ved dræning uden brug af tryk. Den specifikke kagemodstand blev brugt som parameter til at beskrive slamkvaliteten i forbindelse med dræning. Den volumetriske belastning og koncentrationen af suspenderet materiale blev fundet til at være de to mest kritiske parametre til at bestemme dræningsprocessen. Deflokkulering af slam på grund af shear og anaerobe forhold havde en kraftig effekt på slamkvaliteten og det var hovedårsagen til de driftsmæssige problemer man har haft med Esbjergs slambede. Problemerne blev løst ved at forbedre slamkvaliteten med tilsætning af nitrat og kalcium karbonat samt ved at justere selve udbringningen på bedene.

Ekstracellulære polymerer er vigtige for slamflokkenes egenskaber (fx flokstyrke) og her blev specielt tilstedeværelsen af ekstracellulært DNA (eDNA) undersøgt. En ny tilgang til at kvantificere eDNA i blandede biofilm med en mikroskala opløsning blev udviklet og kombineret med andre farvemetoder til at vurdere oprindelse, mængde, og betydning i aktivt slamflokke. Mest eDNA blev fundet tæt på levende bakterier i mikrokolonier, hvilket indikerer at det stammer fra en aktiv sekretion. Når farven blev kombineret med fluorescende *in situ* hybridisering med genprober til identifikation af mikroorganismerne kunne det ses, at eDNA indholdet varierede mellem de forskellige probe-definerede bakterier. Den største mængde blev fundet omkring mikrokolonier fra denitrificerende bakterier tilhørende slægterne *Curvibacter* og *Thauera*, de ammoniumoxiderende *Nitrosomonas* og the nitritoxiderende *Nitrospira*. Andre flokdannere producerede også eDNA omend i mindre mængde. Den totale mængde eDNA i aktivt slam varierede mellem 4-52 mg per gram organisk stof i forskellige renseanlæg. Meget høje lokale koncentrationer kunne ses med op til ca. 300 mg eDNA per gram organisk stof. Nedbrydning af eDNA i det aktive slam med DNase forårsagede en general disintegration af flokkene og specielt

mikrokolonier med højt eDNA indhold. Dette indikerede at eDNA var en vigtig struktural komponent i slamflokkene.

Sammenfattende kan det siges, at dette phd-projekt i væsentlig grad har forøget vor viden om dræning af slam på slambede. Detaljerede mekanismer blev demonstreret og faktorer, som bestemte dræningshastigheden og slamkvalitet blev identificeret. På denne baggrund blev der foreslået guidelines til design og drift af slambede inkluderende jævnlig monitering af slamkvalitet med den nyudviklede teknik, som tillader hurtig og nem undersøgelse lige efter prøvetagning. eDNA's rolle som vigtig for styrken af mikrokolonier og flokke blev vist og det understreger det tætte forhold mellem den mikrobiologiske sammensætning af slammet, de resulterende flokkarakteristika og de samlede afvandingsegenskaber. Phd-projektet har således kastet nyt lys på det aktive slams dræningsegenskaber og vigtige faktorer der påvirker disse således der åbnes nye muligheder for at forbedre design og drift af slambede hvorved konkurrenceevnen øges for denne simple og effektive teknologi til håndtering af overskudsslam.

3. Objectives of the PhD project

The objective of this PhD project was to carry out a comprehensive investigation of the water-binding properties of activated sludge in relation to drainage and mineralization of sludge on reed beds and to establish a link between the microbial composition of activated sludge, the floc properties implied by this composition, and the resulting macroscopic physico-chemical sludge characteristics, gravity drainage in particular. Formulation of this aim was inspired by the desire to help the operators of Esbjerg reed bed facility solve the operational problems they were facing when handling sludge from two local wastewater treatment plants.

A novel experimental technique for the assessment of drainage properties of activated sludge was devised and applied to unveil the factors of importance for fast and effective drainage of sludge on reed beds, and to mark new guidelines for the design and operation of safe and efficient full-scale reed bed facilities. A link between the microbial composition of activated sludge and the resulting floc properties was established through a detailed investigation of an EPS (extracellular polymeric substances) component that has so far received little attention, namely the extracellular DNA (eDNA). A method for the *in situ* detection and quantification of eDNA was developed and used to reveal the importance of this exopolymer for the floc strength and integrity in the mixed microbial community of activated sludge.

This thesis gives an introduction to the activated sludge process with special focus on the dewatering of surplus sludge, a review of the state of the art in reed bed sludge handling, an outline of methodologies used for sludge characterization in respect to dewatering, a review of floc properties with respect to dewatering and their controlling factors, an overview of gravity drainage of activated sludge and factors controlling this phenomenon, and four supporting papers. Three of these papers investigate the phenomenon of sludge gravity drainage and factors controlling this process, and one paper describes eDNA as an important component of EPS in relation to floc strength and integrity.

4. List of supporting papers

Papers included

Christensen, M.L., **Dominiak, D.M.**, Nielsen, P.H., Sedin, M., Keiding, K. (2010) Gravitational drainage of compressible organic materials. *Journal of the American Institute of Chemical Engineers*, DOI: 10.1002/aic.12222.

Dominiak, D.M., Christensen, M.L., Keiding, K., Nielsen, P.H. (2010) Gravity drainage of activated sludge: new experimental method and considerations of settling velocity, specific cake resistance and cake compressibility. *Water Research* (submitted).

Dominiak, D.M., Christensen, M.L., Keiding, K., Nielsen, P.H. (2010) Sludge quality aspects of full-scale reed bed drainage. *Water Research* (submitted).

Dominiak, D.M., Nielsen, J.L., Nielsen, P.H. (2010) Extracellular DNA is abundant and important for microcolony strength of bacteria in mixed microbial biofilms. *Environmental Microbiology* (accepted).

Papers not included

Christensen, M.L., **Dominiak, D.M.**, Adrian, L., Hansen, R.B., Keiding, K., Nielsen, P.H. (2010) Slambede – hvordan håndterer vi dem? *Vand og Jord*, February 2010

Dominiak, D.M., Larsen, P., Givskov, M., Nielsen, P.H., Nielsen, J.L. (2010) Influence of the quorum sensing inhibitor furanone C-30 on the integrity of complex microbial consortia. *Biofouling* (submitted)

5. The activated sludge process

5.1. Overview of biological wastewater treatment with the activated sludge process

The activated sludge process for biological wastewater treatment is the most common means of water treatment and is the most widespread wastewater treatment process in the world (Lindrea and Seviour, 2002). Although many varieties of the process exist, it is essentially composed of three basic steps: pre-treatment, the actual biological step, and the solid-liquid separation (Fig. 1).

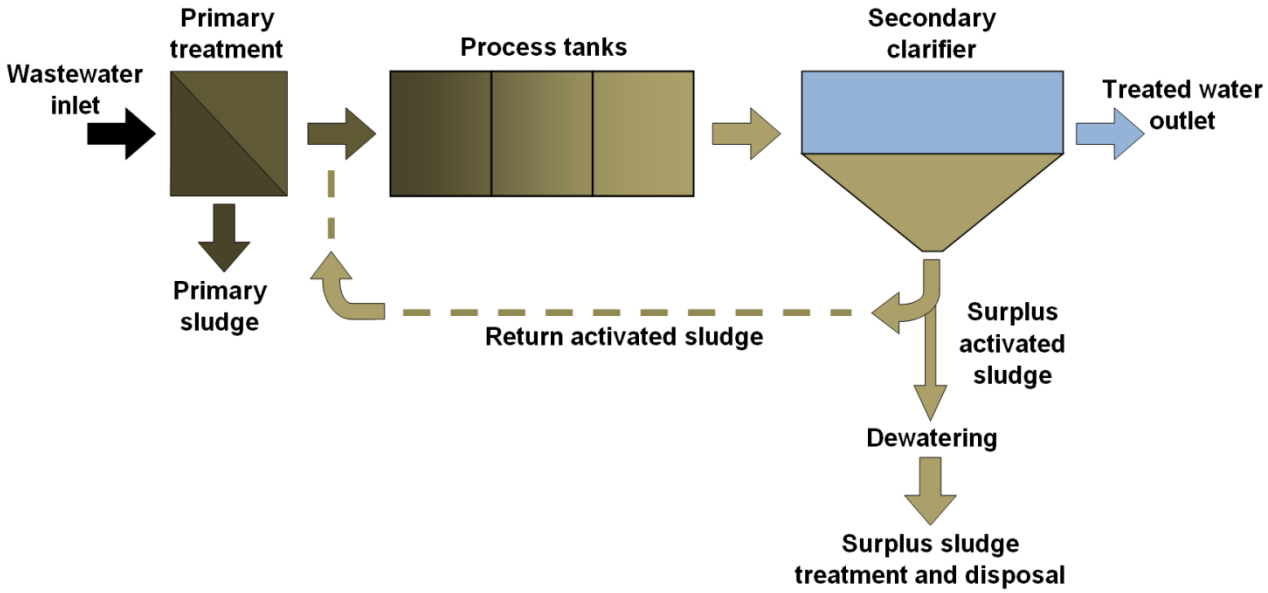

Figure 1. General layout of a wastewater treatment plant applying the activated sludge process.

The primary treatment is designed to remove large objects by means of sieving, and both floating (oils, fats) and sinking (sand, etc.) materials by flotation and settling inside the primary clarifier. Wastewater treated by these means is directed to the process tanks, where the biological removal of carbon, nitrogen, and phosphorus takes place. Depending on the actual plant design and configuration, the process tanks can be compartmented and selectively aerated in order to take advantage of certain biological phenomena, e.g. enhanced biological phosphorus removal (Fuhs and Chen, 1975). In the process tanks the bacterial biomass, referred to as the mixed liquor suspended solids (MLSS), is kept at the concentration range of 3 to 5 g of suspended solids per liter (Henze *et al*., 2002). This mixture is then directed to the secondary clarifier, where the separation of sludge and clear, treated water occurs thanks to sludge gravity settling. Treated water is either directly discharged to receiving waters or directed to tertiary treatment (the so-called effluent polishing), depending on the actual quality and local regulations. The settled sludge is recycled to the process tanks.

Since activated sludge microorganisms feed on carbon and nutrients in the influent wastewater, biomass accumulation would take place in the system if a constant stream of sludge was not taken

away from the secondary clarifier. This stream, called the surplus activated sludge and comprising 3 to 20% of the settled sludge, needs to be removed from the plant and handled (Nielsen, 2002).

5.2. Dewatering, surplus sludge treatment and disposal strategies

The water content of the surplus activated sludge usually exceeds 98%, which implies a dewatering step so that transportation and handling of sludge are economically feasible. Since the dewatering is usually performed by mechanical devices like filter presses, belt presses, or centrifuges (Chu *et al*., 2005), this unit operation is the most expensive one, constituting 30-50% of the annual operating costs of a wastewater treatment plant (Sørensen, 1996). The disposal of dewatered sludge is another expensive problem, where no fixed strategy exists. Some of the solutions are incineration, landfill disposal, and application to fields as a fertilizer, but the latter is rarely used in Denmark due to regulations (Lundin *et al*., 2004). These high costs inspire the search for inexpensive alternatives to mechanical dewatering. One such alternative is the application of surplus sludge to reed beds, or constructed wetlands, which depend on gravitational drainage and mineralization of sludge in specially designed ditches planted with vegetation. It is especially attractive to small wastewater treatment plants, because it requires smaller investment and operating costs than incineration plants, especially in small scale.

6. Sludge dewatering and mineralization on reed beds

Reed beds for sludge dewatering and mineralization offer an economically attractive alternative to pressure dewatering and centrifugation. Many reed bed facilities have been built in Denmark (approx. 95 until 2002), and most of our experience comes from the Danish systems, but this technology is also successfully applied in many European countries (Haberl *et al.*, 1995; Cooper *et al*., 2004) and in the USA (Kim and Cardenas, 1990). Their primary advantage is that they are simple in design and operation, require low manpower (Cooper *et al*., 2004), and since processed sludge residue complies with agricultural standards, they are suitable for field or forest application as a fertilizer (Nielsen and Willoughby, 2007). Sludge volume reduction occurs due to the combination of water gravity drainage, evapotranspiration through the leaves of the reeds, and mineralization of sludge residues (Aagot *et al*., 2000). An additional advantage, justifying the field application of sludge residues, is the high pathogen removal rate and the mineralization of hazardous organic compounds typically omitted by the activated sludge process (Nielsen, 2005).

6.1. Design and operation of reed beds

Reed bed facilities typically consist of several basins (often 8-10) and additional infrastructure for sludge storage and handling (Fig. 2). Sludge is pumped to the facility by a pipeline and stored in a storage tank, which allows mixing, aeration, and adjustments of sludge suspended solids (Nielsen, 2003).

Figure 2. Reed bed facility located in Esbjerg, Denmark. It is composed of 24 basins, each of approx. 2200 m^2. Storage tanks are visible left of the basins.

A basin is typically a rectangular ditch, approx. 2 m deep, with a special multi-layer design for efficient water flow (Fig. 3).

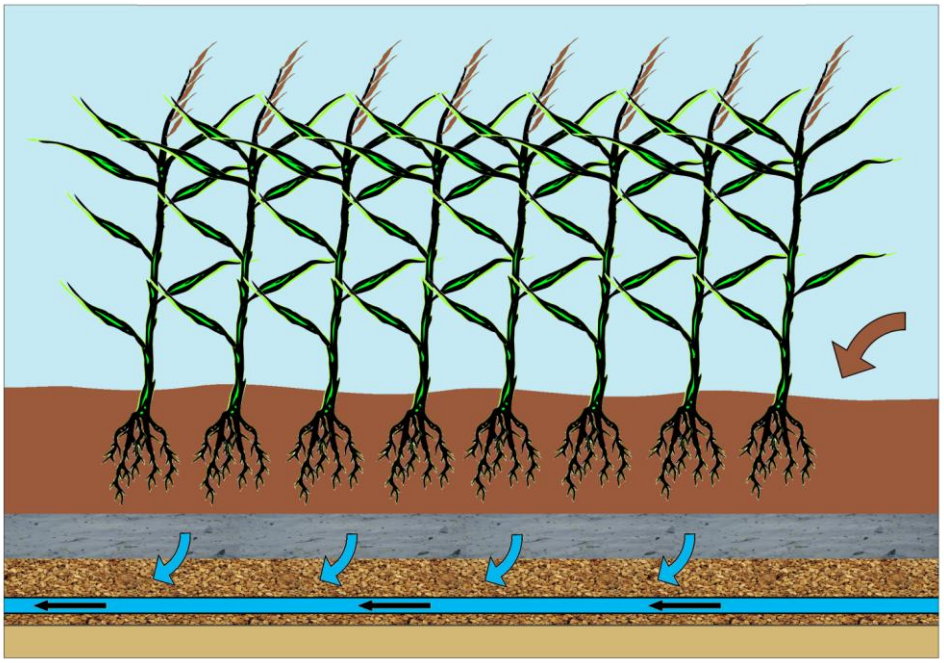

Figure 3. Multi-layer design of a reed bed basin. Water from the sludge residues poured from the top drains down through the sand and gravel filter layers and is collected by a pipeline system.

The bottom of the ditch is sealed by a plastic membrane, which is covered by a gravel layer with a water collection system. This system, composed of drilled piping, collects water draining down and transports it back to the treatment plant. It also contributes to aeration of the sludge layer from the bottom. Gravel layer is typically covered by a sand layer, onto which sludge is applied, and the total filter layer thickness is usually 0.55 to 0.6 m. Vegetation (typically *Phragmites australis*) grows out of the sludge layer and contributes to sludge volume reduction through evapotranspiration as well as to sludge layer aeration by creating tortuous cracks in the sludge cover (Nielsen and Willoughby, 2007). The depth of the basin usually exceeds 1.8 m from the filter to the top, allowing for a build-up of sludge of at least 1.5 m.

Reed bed systems are usually designed to operate for at least 30 years with at least 3 operational cycles of 10 years (Nielsen, 2003). Each operation cycle begins with a 2-year commissioning phase following the planting of the reeds. It is designed to create good growing conditions for the reeds and to gradually increase sludge loading until the maximum loading is achieved at the beginning of the next phase. In year 3, the phase of normal operation begins, where periods of loading and resting alternate, and the beds are operated at design capacity. The loading/rest period ratio length depends on sludge concentration, sludge layer thickness, and the age of a particular basin. The emptying phase typically begins in year 8 and includes several basins, while the other basins continue normal operation. After emptying, basins are re-established and subjected to similar loadings as in the initial commissioning phase. Each basin follows the 10-year cycles, and the phases alternate between basins such that the overall performance of the reed bed facility remains constant.

Reed bed facilities are usually designed for loading with approx. 60 kg dry matter/m^2/year. Guidelines formulated by Steen Nielsen (Nielsen 2002; 2003) prescribe a maximum of 60 kg DM/m^2/year for surplus activated sludge and a maximum of 50 kg DM/m^2/year for digested sludge, sludge with high fat content or low sludge age (Nielsen and Willoughby, 2007). The final dry matter content of the residues is expected to reach 30 to 40%, but a typical value for Danish systems is approx. 20% (Nielsen 2002; 2003). However, experience shows that these figures are rarely attained, and reed bed sludge treatment has a general reputation of being unpredictable (De Maeseneer, 1997).

6.2. Common problems and shortcomings of reed bed sludge treatment

Many reed bed facilities, especially when this technology was first applied, suffered from operational problems such as poor dewatering performance and mineralization, vegetation loss, and odor (Nielsen, 2005b). According to Steen Nielsen, this was most often caused by overloading, insufficiently long resting periods and improper construction of the filter layer. The latter was most often caused by poor capillary connections between the layers of the filter, which created obstacles for water drainage. Overloading and insufficiently long resting periods are, however, an operational issue, most often caused by under-dimensioning of the systems. If too few basins were constructed, but the facility operates at design loading, the loading periods are too long with respect to the resting periods, which can cause water accumulation in the basins, leading to anaerobic conditions and methane generation. These conditions tend to lower the basins' performance in the long term, causing the overall loss of facility's performance (Nielsen, 2005b).

Under-dimensioning and overloading of reed beds during their operation are, to some extent, caused by the lack of a good and reliable means of assessing the drainability of activated sludge. Although Steen Nielsen recommends dimensioning the facilities based on sludge quality assessed by the capillary suction time (CST), it would be an oversimplification to believe that the sludge dewatering properties remain constant in the long term. Reed bed operators generally only follow instructions given by the plant designer, rather than monitor the sludge quality and re-adjust loadings according to the current needs. The accurate determination of sludge characteristics with respect to dewatering and drainage is difficult and often requires sophisticated equipment or training, which prevents its everyday use. A simple and reliable test of sludge drainability, supported by a set of simple-to-follow guidelines, could help maintain the high performance of reed bed facilities and improve the performance of this attractive technology.

7. Sludge characterization in relation to dewatering and drainage

The dewatering characteristics of activated sludge can be assessed with several techniques. Some were developed for the general determination of compound dewatering characteristics, others specifically for activated sludge. Since most wastewater treatment plants employ mechanical sludge dewatering with pressure devices, e.g. belt presses and filter presses, most research focused on pressure dewatering of sludge with the aim of increasing the performance of these technologies. Even though gravitational sludge dewatering is economically much more attractive, it has received little attention in the literature, most probably because pressure dewatering tends to produce drier cakes (Novak *et al.*, 1999), and extensive literature is available on pressure dewatering of inorganic suspensions.

Two techniques have gained the most popularity for the characterization of sludge dewaterability, the specific resistance to filtration (SRF) and the capillary suction time (CST). During the course of this PhD project, a new technique was developed so as to account for phenomena and sludge characteristics omitted by the SRF and CST. Besides these direct estimates of dewaterability and drainability, other sludge characteristics are widely used to determine sludge quality in relation to solid-liquid separation processes in wastewater treatment plants. The most common one is the sludge volume index (SVI), which is measured daily in most facilities. The particle size distribution (PSD) and floc strength determination are limited to laboratory use, but they provide valuable information allowing the predictions of sludge dewatering performance.

7.1. Specific Resistance to Filtration (SRF)

The specific resistance to filtration (SRF) has been developed by Carman (1938) and Ruth (1946) as a general measure of filterability of slurries for industrial use. This technique employs pressure filtration under high, constant pressure applied by a specialized apparatus. The apparatus measures the filtrate expression rate and uses this information, along with the pressure information and solid and liquid phase characteristics, to estimate the specific cake resistance. The method, traditionally criticized for being difficult to perform and time-consuming, was improved and automated by Christensen and Dick (1985).

The application of SRF to activated sludge was challenged by Sorensen *et al*. (1996), because SRF results are pressure-dependent for compressible materials, and SRF devices operate with pressures far above the critical pressure for activated sludge. Expression of water from activated sludge produces compressible cakes (Underwood *et al.*, 1928) which collapse and decrease their porosity under pressures far below those found in mechanical dewatering devices (Tiller *et al.*, 1998; Sørensen *et al.*, 1997), hence the SRF cannot be used to describe the drainability of sludge under gravity drainage conditions correctly, where pressures on the cake are orders of magnitude lower than those found in SRF tests and usually lower than the critical pressure for activated sludge.

7.2. Capillary Suction Time (CST)

The CST test is a commonly used alternative to SRF, especially because it is much easier and cheaper to perform. It was developed by Baskerville and Gale (1968) and is nowadays one of the most widely used compound dewatering characteristics. This technique depends on the measurements of the speed of wetting front travelling between two points on a filter paper under suction of approx. 50 cm H_2O (Tiller *et al*., 1983).

The CST method was used by Steen Nielsen as a measure of sludge dewaterability for the purpose of reed bed dimensioning and design (Nielsen, 2003). Although the CST employs much lower pressures than SRF, it has a weak point with regard to sludge gravity drainage characterization, namely it does not take sludge compressibility into account. For this reason, it cannot be used to describe the gravity drainage process and the behavior of sludge under gravity drainage conditions correctly.

7.3. Specific Resistance to Drainage (SRD)

The SRD technique, developed during this PhD project, aimed at describing the gravity drainage taking all the important phenomena into account. It was originally developed with dextran-MnO_2 particles, as a representation of a compressible organic slurry (Christensen *et al.*, 2010), and then successfully applied to describe the gravity drainage of activated sludge (Dominiak *et al*., 2010a). The method revealed the importance of settling velocity of particles, low pressure filtrate expression, cake compressibility and cake collapse due to capillary forces (Bierck *et al.*, 1988).

The test itself is an accurate simulation of the actual gravity drainage process. A sample of mixed liquor activated sludge is introduced into a transparent glass tube and drained through a filter closing the bottom of the tube (Fig. 4). The process is recorded by a web camera acquiring images at a specified frame rate, and the data are recorded by a personal computer. The latest evolution of the test setup employs only a digital camera, capable of taking pictures at pre-defined time intervals, the glass tube with a filter-closed bottom, and a beaker for filtrate collection. The simplicity of this technique and the portability of the apparatus allows for easy determination of sludge drainage properties directly after sampling in wastewater treatment plants or in reed bed facilities.

The raw data from the experiment are the level of sludge-water interface and the level of water-air interface. The difference between these two, i.e. the thickness of the clear water phase, produces a specific pattern when plotted against time and allows for the calculation of the settling velocity of particles, the specific cake resistance and cake compressibility. A detailed description of the gravity drainage phenomenon and the method itself is given in Chapter 9.

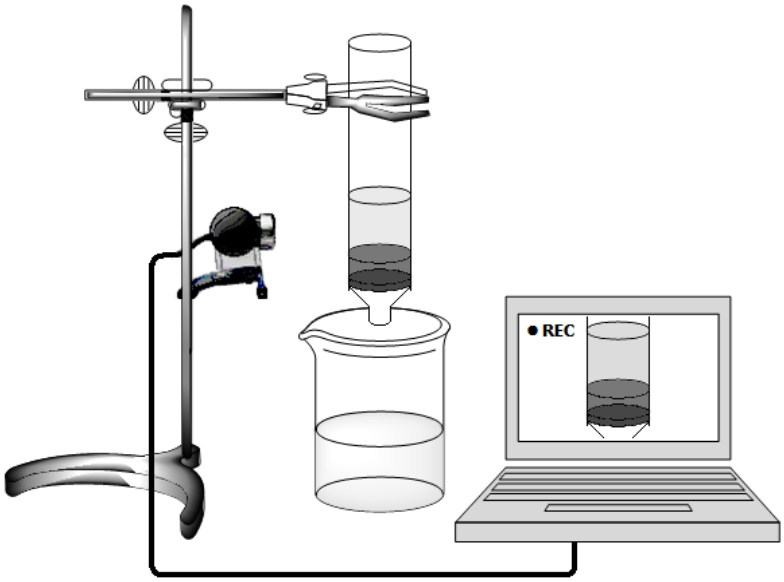

Figure 4. The experimental setup used for the determination of sludge drainage properties with the SRD method. The raw data curve is presented in Chapter 9, Figure 10.

To my knowledge, the SRD methodology is the most accurate description of sludge gravity drainage and the phenomena involved in this complicated process. It emphasizes the importance of settling and particle settling velocity, the compressibility of the cake and its final collapse due to capillary drag forces. It appeared to be very precise and sensitive and was used in this project to determine the factors of greatest importance to fast and efficient drainage of activated sludge in reed bed basins. These findings allowed for the formulation of a set of advice and guidelines for reed bed operators with the hope of substantial improvement of the overall performance of these facilities.

7.4. Sludge Volume Index (SVI)

Sludge volume index (SVI) is the most common measure of sludge settleability and compactability used by wastewater treatment plant operators, both because it is very easy to perform and because it gives an immediate idea of the performance of secondary clarifiers. The method consists of settling a 1 L sample of sludge in a graduated cylinder and reading the volume of settled sludge after 30 min. The final volume of the settled sludge, divided by the sludge suspended solids (SS) concentration, gives the SVI value in ml/g SS. This method is sometimes complemented by the initial settling rate (ISR) measurements, which describe the maximum velocity of particle settling at the beginning of the process.

The characteristics of both the solid and liquid phase of activated sludge, like density difference, liquid viscosity, or solid phase characteristics, are not directly measured or considered, but they obviously influence the process. The mathematical description of sludge settling including these characteristics was given by Canale and Borchardt (1972), who used Stoke's law to describe the free settling of sludge particles, and by Tiller (1981), who described the thickening process of compressible sludge sediment. Due to the high concentration of particles in sludge,

the consolidation thickening, proposed by Tiller, is a closer approximation of the actual phenomenon, and it can be used to calculate the final sludge sediment volume.

Although the SVI measurement illustrates and, to some extent, quantifies sludge settleability and consolidation, it cannot be directly used to describe the drainage characteristics of sludge during gravity drainage because the sole driving force in an SVI experiment is gravity, and there is no liquid movement. However, sludge exhibiting a low SVI value can be expected to offer less resistance to filtration due to larger particles and smaller degree of deflocculation (Karr and Keinath, 1978; Barber and Veenstra, 1986; Mikkelsen *et al*., 1996).

7.5. Particle Size Distribution (PSD)

Particle size distribution (PSD) does not directly measure the quality of sludge in terms of dewatering or drainage, but it can provide valuable information and help predict the behavior of sludge during drainage or dewatering. PSD can be determined by light scattering techniques, employing lasers and visible light, or by direct microscopic observation and measurements of flocs. Many trials have been made to correlate the PSD with other dewatering characteristics of sludge, especially the CST and SRF (Karr, 1976; Sørensen *et al*., 1996; Friedrich *et al*., 1993), but direct and general correlations were not very strong. However, the PSD is a measure of the degree of sludge flocculation, and it can therefore be useful in linking the loss of drainability to the deflocculation of sludge, which seem to follow each other closely (Dominiak *et al*., 2010b).

7.6. Floc strength determination

Floc strength is not a measure of dewatering characteristics of sludge, but its determination is useful when describing the stability of activated sludge floc under the action of shear forces. Many approaches have been developed to measure the floc strength, both at the macroscopic and microscopic (single floc) levels (Jarvis *et al*., 2005). One of the most popular macroscopic methods is the standardized shear reactor test. It is performed by stirring a sludge sample in a baffled reactor, and the level of shear is controlled by changing the agitation speed. The measured parameter can be the floc (particle) size distribution (Biggs and Lant, 2000) or turbidity of the supernatant produced by controlled centrifugation of mixed liquor (Mikkelsen and Keiding, 1999). Although floc strength data from experiments performed with different techniques cannot be directly compared, these measurements can be used to assess the effect of different conditions on activated sludge floc strength and integrity (Jarvis *et al*., 2005). Higher floc strength can be linked to larger and more stable flocs, and this can be related to lower resistance during sludge gravity drainage (Karr, 1976; Dominiak *et al*., 2010b).

Floc size and strength, two important floc properties influencing the drainability and dewaterability of sludge in full-scale situations, are a consequence of floc composition and architecture, decided by microorganisms living inside the flocs. This link was of particular interest in this PhD project and the reason for the choice of research directions. In the next chapter, the influence of bacteria and their activity on the microscopic floc properties and the resulting large-scale sludge characteristics will be unveiled.

8. Floc properties in relation to dewatering and drainage

8.1. Floc morphology and composition

Activated sludge is a complex mixture of flocs, smaller cell aggregates, and both organic and inorganic particles suspended in water. The activated sludge floc is a complicated structure composed of biotic and abiotic components. The general structure of a floc is a result of the selective pressure in the wastewater treatment plant, favoring dense aggregates with good settling properties. The biotic community of an activated sludge floc is composed of both prokaryotes – *Bacteria* and some *Archaea* – and eukaryotes – protozoan and often metazoan organisms (Eikelboom, 2000). The actual community composition is dynamic and is a net result of the influent wastewater composition and the conditions inside the treatment plant.

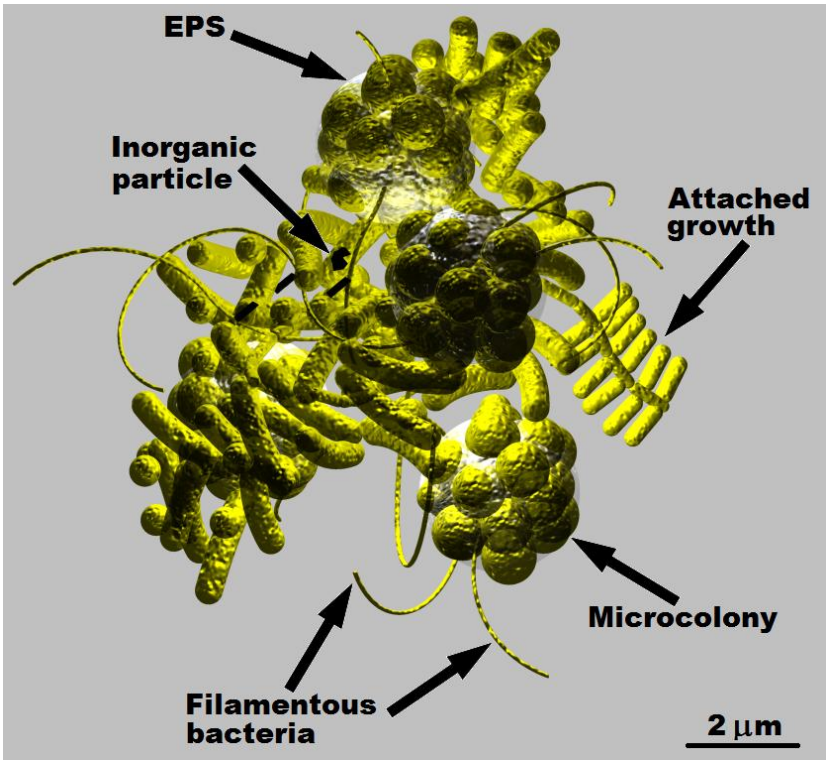

Figure 5. Morphology of a typical activated sludge floc.

A typical activated sludge floc is presented in Fig. 5. It is composed of bacterial cells growing in dense, grape-shaped microcolonies, as filaments or as single cells embedded in the matrix of extracellular polymeric substances (EPS) or attached to filamentous organisms (Jorand *et al*., 1995; Snidaro *et al*., 1997; Jenkins *et al*., 2003). Filamentous bacteria are generally recognized as 'backbones' of a floc, responsible for its mechanical strength, as well as settling properties (Ekama *et al*., 1997). The EPS matrix, composed of several fractions, is a dense and sticky, glue-like material, responsible to a large degree for floc and microcolony integrity. In the EPS matrix many holes, cavities and channels are present, which make up for the large surface area of flocs and facilitate water and nutrient transport to the cells growing deep in the floc structure

(Liss *et al*., 1996; Daims *et al*., 2001; Chu and Lee, 2004). The EPS matrix can be regarded as a typical gel because of its swelling/deswelling properties and divalent cation bridging (Keiding *et al*., 2001). This is extremely important for the floc properties, which determine the behavior of activated sludge in full-scale processes like settling, dewatering and gravity drainage.

The EPS matrix, which constitutes approx. 40 to 60% of the total organic matter of activated sludge, is composed of several groups of polymers. The most important are the proteins (40-60% of EPS), humic compounds (20-30%), polysaccharides (10-20%), extracellular nucleic acids (2-5%), as well as lipids and other compounds (5-10%) (Nielsen, 2002; Watnick and Kotler, 2002). Since the EPS matrix is the largest organic fraction of activated sludge, possessing many characteristics influencing sludge physico-chemical properties, it has been the object of many research studies focusing on its effect on settling and dewaterability (see Section 8.3.1).

The size of activated sludge flocs is typically 40 to 125 μm, but values down to 25 μm and up to 1000 μm have been reported (Frølund *et al*., 1996; Ekama *et al*., 1997; Eikelboom, 2000; Jenkins *et al*., 2003). The floc size is a net result of the floc strength and the mechanical stresses that the floc is subjected to, whereas the floc strength results from a range of chemical and biological factors. All in all, the floc size is a very dynamic floc characteristic with many implications on sludge macroscopic properties and sludge behavior in large-scale processes.

8.2. Floc size and strength as important parameters for sludge drainage and dewatering

The effective dewatering of activated sludge by gravity drainage depends on a number of physico-chemical and microbiological factors, the most important of which seems to be the particle size distribution, similarly to the case of dead-end filtration of abiotic suspensions. The advantage of larger particles over smaller particles during gravity water drainage can be easily demonstrated if one imagines a cake composed of particles of two different sizes (Fig. 6). Larger particles would form a cake with larger voids, i.e. larger porosity, and would therefore offer less resistance to water flow than smaller particles forming a more compacted cake with narrower channels and smaller porosity. Moreover, the presence of smaller particles results in the increased internal surface area of the cake, which also translates into higher hydraulic resistance. These simple considerations can be directly projected to activated sludge flocs, however particle compressibility has to be kept in mind in the case of sludge. Larger flocs are more beneficial to gravity drainage due to the fact that they impose lower specific cake resistance values and promote faster drainage. Deflocculation, a process of floc disruption into smaller fragments, is especially damaging to the drainage process. Small floc fragments can easily penetrate the cake voids and close the pores (process known as blinding), which leads to increased drag, slower drainage and progressing cake compression (Fig. 6). This example illustrates the importance of floc strength, which determines the floc size distribution under a certain set of conditions and therefore defines the drainage properties on a large scale.

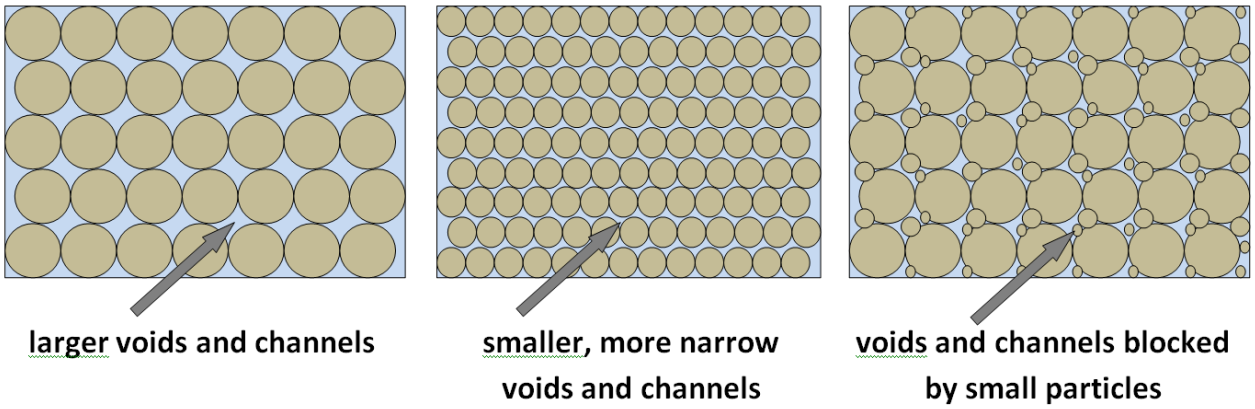

Figure 6. Illustration of water flow through a cake composed of larger and smaller particles, and a cake blocked by small particles.

The presence of small particles in the activated sludge suspension has been shown to decrease dewaterability many times (Karr and Keinath, 1978; Barber and Veenstra, 1986; Mikkelsen *et al.*, 1996). Since deflocculation of activated sludge flocs is a direct result of reduced floc strength (Mikkelsen and Keiding, 1999), the knowledge of floc strength and factors affecting it can be effectively used to investigate the phenomena behind the quality of sludge in terms of gravity drainage.

Floc strength can be regarded as a sum of all the interactions that bind bacteria and floc constituents together. The four most commonly cited floc-binding interactions are the DLVO-type interactions (Hermansson, 1999), bridging of EPS with divalent (Eriksson and Alm., 1991) and trivalent cations (Nielsen and Keiding, 1998), hydrophobic interactions (Urbain *et al.*, 1993), and physical entanglement of floc entities (Rijnaarts *et al.*, 1995). All these interactions can be affected by both physico-chemical properties of bulk liquid and biological activity of bacteria inhabiting the flocs, which makes the floc strength a continuously changing parameter, the magnitude of which can be managed with a number of strategies.

According to the DLVO theory, bacterial adhesion to floc surface can be increased by increasing the ionic strength of the solution. This effect is expected to result from decreasing the double layer thickness and decreasing the surface potential, which would eventually act against the electrostatic repulsive forces (Hermansson, 1999). EPS bridging mechanisms are facilitated by the presence of di- and tri-valent cations, especially calcium, iron and aluminum. However, the reduction of Fe(III) to Fe(II) by anaerobic bacteria (Nielsen, 1996; Nielsen *et al.*, 1997), or Fe(III) precipitation as iron sulphide (Nielsen and Keiding, 1998), results in immediate decrease in floc strength leading to deflocculation and, subsequently, to problems with sludge settling and dewaterability. Hydrophobicity of cells and floc surfaces has been shown to be a very important selective force in a wastewater treatment plant, capable of leaving the hydrophilic species unattached and, as a consequence, removing these species with effluent (Zita and Hermansson, 1997). All these mechanisms are a combination of chemical and microbiological processes and stand between these two worlds. It is therefore very important to remember that any action,

designed to interact with one process, will most probably affect other processes, and the overall effect can be different than initially assumed.

8.3. Factors controlling the floc size and strength

8.3.1. Extracellular Polymeric Substances (EPS) and cations

EPS matrix of activated sludge flocs constitutes 80 to 90% of organic matter in activated sludge and therefore determines the integrity of flocs to a very high extent (Frølund *et al.*, 1996; Münch and Pollard, 1997; Liu and Fang, 2002). The work of Novak and Park resulted in the fractionation of activated sludge extracellular polymers into three major groups according to the distinct cations responsible for attachment of these polymers: (1) polymers composed of lectin-like proteins bound to polysaccharides, bridged by Ca^{2+} and Mg^{2+} and extractable by a sodium-rich cation exchange resin (CER), (2) protein-rich biopolymers bound to Fe cations and extractable by sulfide, and (3) biopolymers bound to Al cations, extractable with bases (Novak *et al.*, 2003; Park and Novak, 2007; Park *et al.*, 2008; Park and Novak, 2009). Each EPS fraction was found to impart different characteristics on the activated sludge floc, including different digestion characteristics and different dewatering properties. The amount of CER-extractable EPS was found to correlate negatively to sludge settling properties (Liao *et al.*, 2001; Wilén *et al.*, 2003), but simultaneously to improve the dewaterability (Mikkelsen and Keiding, 2002; Jin *et al.*, 2004), while the EDTA-extractable EPS was shown to correlate negatively with both settling and dewatering properties (Eriksson and Alm, 1991). Furthermore, the role of lectins, the carbohydrate-binding proteins, has recently been recognized as an important mechanism for activated sludge bioflocculation (Park and Novak, 2009). These differing influences of EPS on floc overall characteristics are most often attributed to EPS proteins, which have been reported to be a predominant organic component of EPS (Urbain *et al.*, 1993; Frølund *et al.*, 1996). Park and Novak found that each cation-bound fraction of EPS produced a unique SDS-PAGE protein fingerprint, suggesting a different protein composition and therefore accounting for different characteristics conveyed by each fraction (Park *et al.*, 2008). As the pool of EPS proteins is augmented by incoming proteins from influent stream, by proteins originating from sludge cell lysis, and by proteins actively secreted by sludge microorganisms (Park *et al.*, 2008), the actual role of EPS proteins is most probably very significant, but also very complex. Extracellular DNA is another EPS component of potential significance to the floc strength, if one takes into account the fact that it is a long-chained, negatively-charged polymer resembling commercial polymers used for sludge conditioning and potentially capable of taking part in cation bridging.

Di- and tri-valent cations are important to the floc strength and size because the surface charge of activated sludge flocs is negative at neutral pH (Mikkelsen, 2003). This is caused by the ionization of functional groups of different EPS components (Rijnaarts *et al.*, 1995), especially important of which seem to be the proteins and humics (Wilen *et al.*, 2003), but also eDNA. Cation bridging is therefore an important mechanism responsible for the floc strength and size as well as for the dynamics of sludge flocculation and deflocculation.

8.3.2. Extracellular DNA (eDNA)

Extracellular DNA (eDNA) is an EPS component traditionally regarded as a product of cell lysis and decay and therefore a short-lived remnant of dead cells. However, as eDNA was discovered in many pure cultures in significant amounts, far exceeding those expected from cell lysis (Hara *et al.*, 1981; Dillard and Seifert, 2001; Qin *et al*., 2007; Lorenz *et al*., 1991), the research of its origin and suspected functions started. The presence and abundance of this polymer in activated sludge was first reported by Palmgren and Nielsen (1996), and the amount detected in that study (20 mg/g of organic matter) created a suspicion that it might be purposefully excreted by sludge microorganisms.

The research on extracellular DNA in various pure cultures and natural environments provided the hints of various roles assigned to eDNA by different bacteria. The extensive investigation of *Pseudomonas aeruginosa* documented the importance of this polymer for cell surface attachment and biofilm strengthening. Steinberger (2002) and Whitchurch (2002) have reported large eDNA production by *P. aeruginosa* during the alginate biosynthesis, which suggests its important role in cell attachment and the early stages of biofilm formation. Similar discoveries have been made for *Staphylococcus epidermidis* (Qin *et al*., 2007) and *Streptococcus* (Petersen 2004; 2005). Flocculating properties of marine photosynthetic bacterium *Rhodovulum sp*. are ascribed to the extracellular DNA produced by this organism (Watanabe *et al*., 1998). The importance of eDNA for the establishment of biofilms, and as a biofilm structural component, appears huge since it has been reported that most bacterial species are capable of binding to free DNA (Dubnau, 1999) and that many bacterial biofilms can be disrupted by a DNase. The structural role of eDNA in activated sludge flocs appeared likely, since DNA is composed of long, negatively-charged molecules theoretically capable of bridging the EPS components in a fashion similar to that of the commercial conditioning polymers. The investigation of the origin and role of eDNA in activated sludge is presented in the supporting paper entitled 'Extracellular DNA is abundant and important for microcolony strength of bacteria in mixed microbial biofilms' (Dominiak *et al*., 2010c).

In this project, a new method for the detection and quantification of eDNA at a microscale resolution was developed and, combined with other techniques, used to investigate the abundance and function of this exopolymer in the EPS matrix of activated sludge. Extracellular DNA was mostly found around living cells and inside microcolonies, suggesting the active and purposeful secretion (Fig. 7). Especially certain types of microcolony-forming bacteria, such as the denitrifiers *Curvibacter* and *Thauera*, the ammonium-oxidizing *Nitrosomonas*, and the nitrite-oxidizing *Nitrospira*, were found to exhibit very high concentrations inside the microcolonies (up to 300 mg eDNA/g of organic matter).

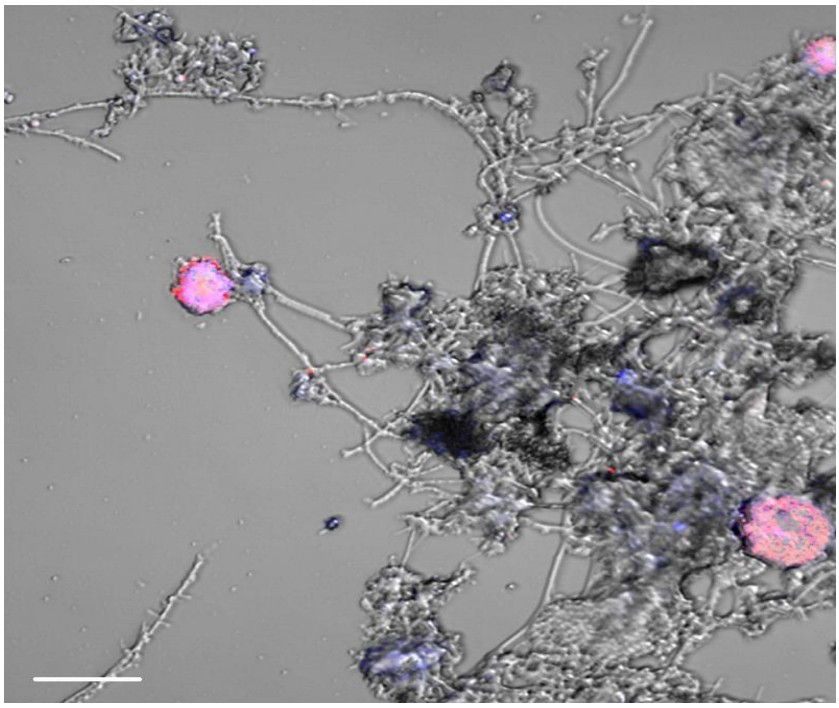

Figure 7. Activated sludge floc with eDNA stained blue with DDAO and microcolonies of *Curvibacter* stained red by FISH. Blue and red colors overlap, suggesting the presence of eDNA in the microcolonies of *Curvibacter*.

Digestion of eDNA with DNase I affected the integrity of both the microcolonies and the whole flocs. Disruption of certain eDNA-rich microcolonies, as well as entire flocs, was revealed by particle size distribution analysis before and after the digestion. This discovery confirmed an important structural role of eDNA in activated sludge flocs and suggested the importance of this exopolymer for floc size and strength, the floc characteristics critical to dewatering and drainage of sludge.

8.3.3. Microbial composition and activity

Behind the macroscopic physico-chemical properties of activated sludge flocs, and the EPS matrix composition and function, stand the sludge microorganisms. Even though bacterial cells only make up from 10-20% of the total sludge organic matter (Nielsen and Nielsen, 2002), the composition of sludge microbiota determines the amount and composition of EPS and therefore influences the overall floc characteristics. It has been shown that different groups of bacteria influence the floc strength to a different extent, i.e. that *Beta-*, *Gamma-*, and *Deltaproteobacteria* form relatively strong microcolonies, while colonies of other bacteria like *Alphaproteobacteria* and *Firmicutes* are rather weak (Klausen *et al.*, 2004). This claim is supported by the findings that sludge supernatant and the settled floc differ in microbial composition (Morgan-Sagastume *et al.*, 2008) and that sludge flocs generally have loosely and strongly attached fractions of cells and EPS (Keiding and Nielsen, 1997; Liao *et al.*, 2002; Sheng *et al.*, 2006). The easily detachable fraction of approximately 5-15% of cells can be removed from flocs by shear forces alone, the strongly attached fraction of further 15-40% of cells requires certain physico-chemical

treatments in addition to shear forces in order to deflocculate, and the remaining 50-75% of cells cannot be removed from flocs (Larsen *et al.*, 2008). Therefore, it becomes clear that the bacterial community composition determines how a given sludge reacts to a given set of factors and therefore how a given treatment influences floc strength, floc size distribution and, as a consequence, sludge dewaterability and draining characteristics (Klausen *et al.*, 2004).

There is a huge body of evidence that microbial activity is in fact determining the floc strength to the highest degree. The general rule of thumb states that anaerobic conditions, inhibiting aerobic microbial metabolism, lead to reduced floc strength and deflocculation (Wilén *et al.*, 2000a, Wilén *et al.*, 2000b), which can be reverted to some extent by aeration (Mikkelsen and Keiding, 1999; Wahlberg *et al.*, 1994; Biggs and Lant, 2000). The degree of deflocculation can be increased if shear forces are present (Wilén *et al.*, 2000a). The reasons behind the anaerobic deflocculation can be the reduction of Fe(III) to Fe(II) by anaerobic bacteria (Nielsen *et al.*, 1997) mentioned above, the anaerobic hydrolysis of EPS floc matrix by bacterial exoenzymes (Rasmussen *et al.*, 1994; Nielsen *et al.*, 1996), or simply the lack of aerobic microbial activity, responsible for EPS formation and floc strengthening. Some of the strategies applied to prevent the deflocculation due to microbial activity loss are the nitrate addition in order to supply an electron acceptor (Wilén *et al.*, 2000a) and the addition of an organic substrate (like ethanol) to stimulate microbial activity (Wilén *et al.*, 2000b; Wilén *et al.*, 2004).

8.4. The link between microscopic floc properties and macroscopic effects on drainage and dewatering

The objective of this PhD project was to carry out a comprehensive investigation of the water-binding properties of activated sludge in relation to drainage and mineralization of sludge on reed beds, and to establish a link between the microbial composition of activated sludge, the floc properties implied by this composition, and the resulting macroscopic physico-chemical properties, gravity drainage in particular. Sections 8.2 and 8.3 present a top-down approach to the problem by first describing the floc properties of importance for drainage and subsequently demonstrating the importance of EPS, chemical interactions, and revealing the role of sludge microbial population. These interactions can be shown by a flow chart (Fig. 8).

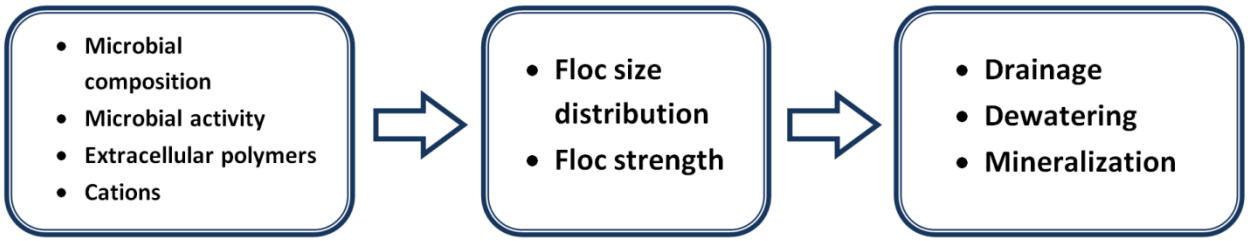

Figure 8. The influence of microbial composition and activity on floc properties, which determine the large-scale physico-chemical sludge characteristics.

Understanding how particular microorganisms shape their environment under different conditions, how these changes influence the floc properties, and how these properties manifest themselves in real-life engineering applications is an important environmental engineering and biotechnological challenge - and the object of interest in this PhD project.

9. Gravity drainage of activated sludge on reed beds

The very high water content of activated sludge (over 90% by weight) imposes a dewatering step in order to reduce the volume and weight of sludge and thus make transportation and handling more economical. Sludge gravity drainage is a widely spread technique due to its simplicity and low costs. Besides, all the mechanical sludge dewatering technologies employ pressures far greater than the critical pressure for activated sludge. The critical pressure values for biological sludge range from 5-50 kPa (Novak *et al*., 1999), and exceeding this value does not increase the filtrate production rate. Operating above the critical pressure compresses the filtration cake, and the only advantage associated with this is the lower final water content of the cake, which is the reason why belt and filter presses are often used to remove most water at low pressures and then compress the sludge to decrease its final water content. Gravity drainage of activated sludge is therefore an attractive and economical process which is, however, very poorly covered in scientific literature. Gravity drainage of inorganic materials (Nenniger *et al*., 1958; Wakeman and Vince, 1986) and activated sludge (Severin and Grethlein, 1996; Severin *et al*., 1999) were investigated in the context of industrial belt presses, but the models omitted cake compressibility, which is expected to appear even at the very low pressures found in gravity drainage. This knowledge gap inspired the need for comprehensive characterization of activated sludge gravity drainage, with a specific goal of applying this knowledge to improve the performance of sludge drying reed beds.

9.1. Overview of activated sludge gravity drainage phenomenon

When activated sludge is drained by gravity, sludge particles settle forming a cake, water is filtered through the cake and cake becomes compressed. All these processes occur simultaneously, so in order to understand and correctly describe this process, all these phenomena need to be observed, recorded, and included in calculations. Figure 9 illustrates the three stages that can be identified during sludge gravity drainage.

During stage A (cake formation stage) sludge particles settle until a filtration cake of constant thickness is formed. As the particles settle, they leave a clear water phase behind, which allows the estimation of sludge settling velocity. Stage B (pure filtration stage) begins when the cake is fully formed (time t_1) and lasts until no more free water is present above the cake (time t_2). This stage provides the data used for the calculation of the specific cake resistance. When the air-water interface touches the cake's surface, stage C begins (cake collapse stage). During this stage, the cake collapses slightly due to capillary forces in the cake's pores, and the data from this stage allow the calculation of the cake compressibility.

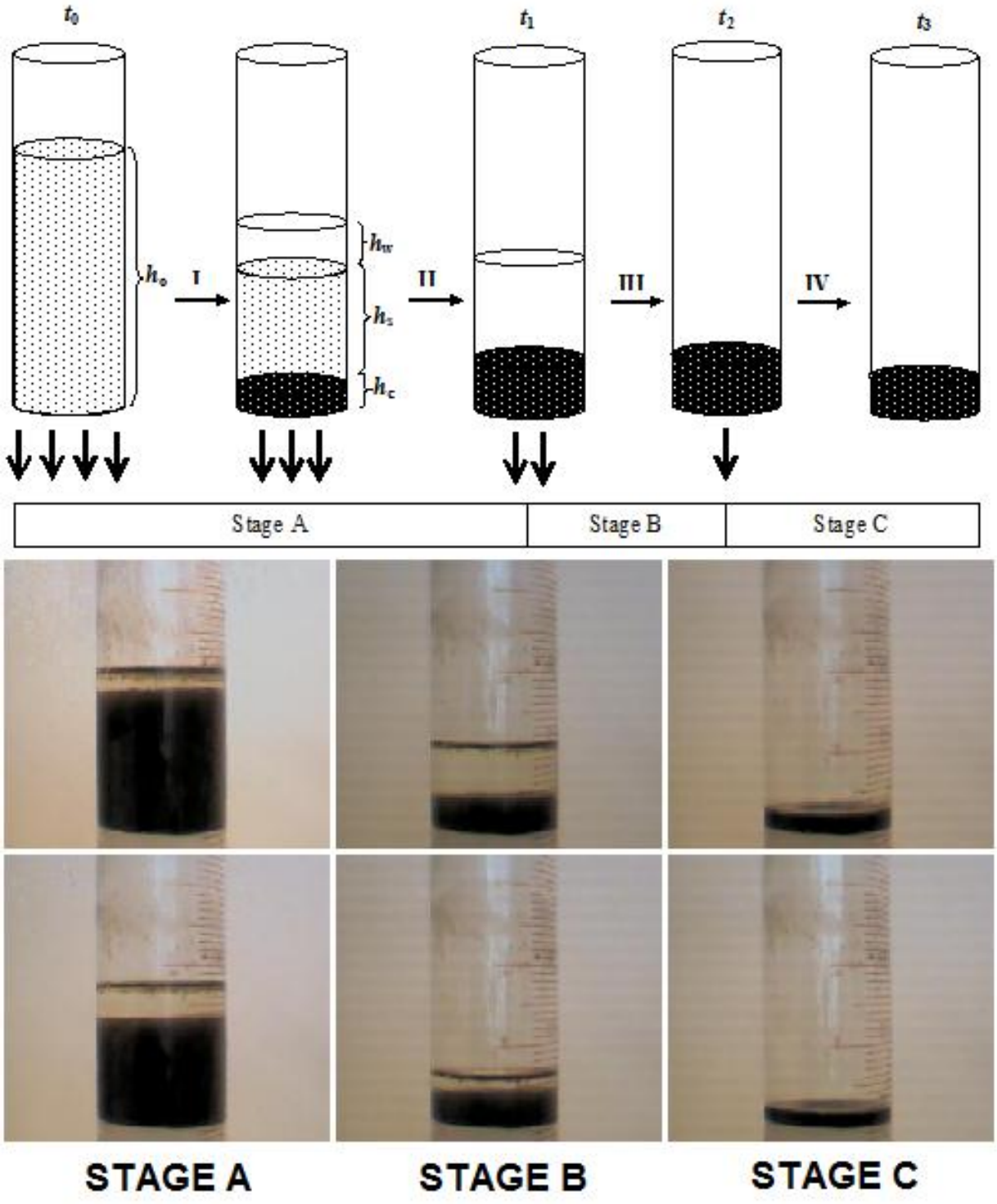

Figure 9. The three stages in gravity drainage of activated sludge and description of the symbols used in calculations, referring to water, sludge suspension, and cake levels.

Eq. 1 (Severin and Grethlein, 1996) describes the drainage rate through a filter cake.

$$v_d = \frac{\Delta P}{\eta\left(\alpha_{cake}\omega + R_m\right)} \quad (1)$$

Notice that the term 'specific cake resistance' is used to describe the average specific cake resistance of the entire cake and not the local values. It is assumed that the average specific cake resistance is constant throughout the drainage experiment. In fact, resistance decreases during the initial part of the drainage process because the pressure difference across the cake decreases with sample level. However, this hardly influences the average specific cake resistance and can therefore be neglected (Christensen *et al.*, 2010).

The pressure difference, defined as stress on medium surface, is given in Eq. 2.

$$\Delta P = \frac{Mg}{A} = \varrho g h_t + c g h_0 \left(1 - \frac{\varrho}{\varrho_s}\right) - \omega g \quad (2)$$

Settling velocity influences the drainage rate because it regulates the deposited amount of solids (ω). The amount of deposited cake has a direct influence on the drainage rate and can be calculated according to Equation 3.

$$\omega = \begin{cases} c(h_0 - h_t) + c v_s t \\ c h_0 \end{cases} \quad (3)$$

where v_s is the settling rate.

Cake compression has an equally important indirect influence on the drainage rate, as the specific cake resistance increases with the cake solid volume fraction.

Cake formation stage (A)

During the initial phase of drainage, when filtration cake develops, three phases can be distinguished, i.e. the cake itself, the sludge particle suspension, and the clear liquid above the suspension. The total level of the sample in the cylinder is given as $h_t = h_w + h_s + h_c$. Since the particles settle, the clear water phase above the suspension develops, and the height of this water phase at any given time depends on settling velocity (Eq. 4).

$$h_w = v_s t \quad (4)$$

The height of the clear water phase, which is the difference between the total level of the sample and the level of the sludge blanket, is measured during the experiment and can be used to determine the settling velocity by revealing the speed at which the particles move away from the liquid surface.

Pure filtration stage (B)

At the time described as t_1, settling stops since all sludge particles are deposited on the filter medium forming a cake, the thickness of which remains constant during stage B. The amount, and consequently the height, of deposited solids depend on the feed concentration and the volume of the sample used (as well as the filter area). Assuming that the weight of the dry cake is low compared to the sample, and that the density of particles is equal to the density of the filtrate, it is possible to derive an equation describing the level of the sample during the entire stage of pure filtration, as in Christensen *et al*. (2010).

$$h_t = h_t(t_1)e^{-\tau(t-t_1)} \quad (5)$$

where

$$\tau = \frac{\rho g}{\mu(\alpha c h_0 + R_m)} \quad (6)$$

Once t_1 and t_2 are identified from Figure 10, and Eq. 5 is fitted to experimental data from stage B, τ can be determined from Eq. 5, providing α - the specific cake resistance – according to Eq. 6. The resistance of the filtration medium itself is neglected since it was found that it accounts for less than 1% of total resistance (Dominiak *et al*., 2010a). The compression of the cake, which takes place at the beginning of stage B when the pressure is the highest, is irreversible. This has been shown by recording the cake height throughout the drainage experiments and experiments employing settling followed by drainage, as well as in analytical centrifugation experiments (Dominiak *et al*., 2010a).

Cake collapse stage (C)

At the time described as t_2, the liquid level reaches the cake surface, and the cake collapse stage begins. Since air enters into contact with the cake surface, liquid menisci form between solid particles, and the cake starts to collapse due to the capillary pressure (Barr and White, 2006). Eq. 7 was used to describe the cake compression of sludge at low pressure (Curvers *et al.*, 2009).

$$\varphi = \varphi_0\left(1+\frac{p_s}{p_a}\right)^{\beta} \quad (7)$$

The compressive pressure p_s is a function of wet cake weight and thus a function of initial solids concentration and volume of the sample. Hence for practical use, Eq. 8 can be used.

$$\varphi = \varphi_0\left(1+\frac{M}{Ak}\right)^{\beta} \quad (8)$$

The final dry matter content is expected to increase with initial solids concentration and initial load, as the wet cake weight increases with solids concentration and load.

The actual calculations of the settling velocity and specific cake resistance are based upon the changes of clear water phase height (h_w) throughout the drainage process, which initially increases linearly until time t_1, and then decreases exponentially until time t_2 (Fig. 10). The settling velocity is determined as the slope of the line fitted to data from stage A, according to Eq. 4, and the specific cake resistance is calculated based on data from stage B and Eq. 5.

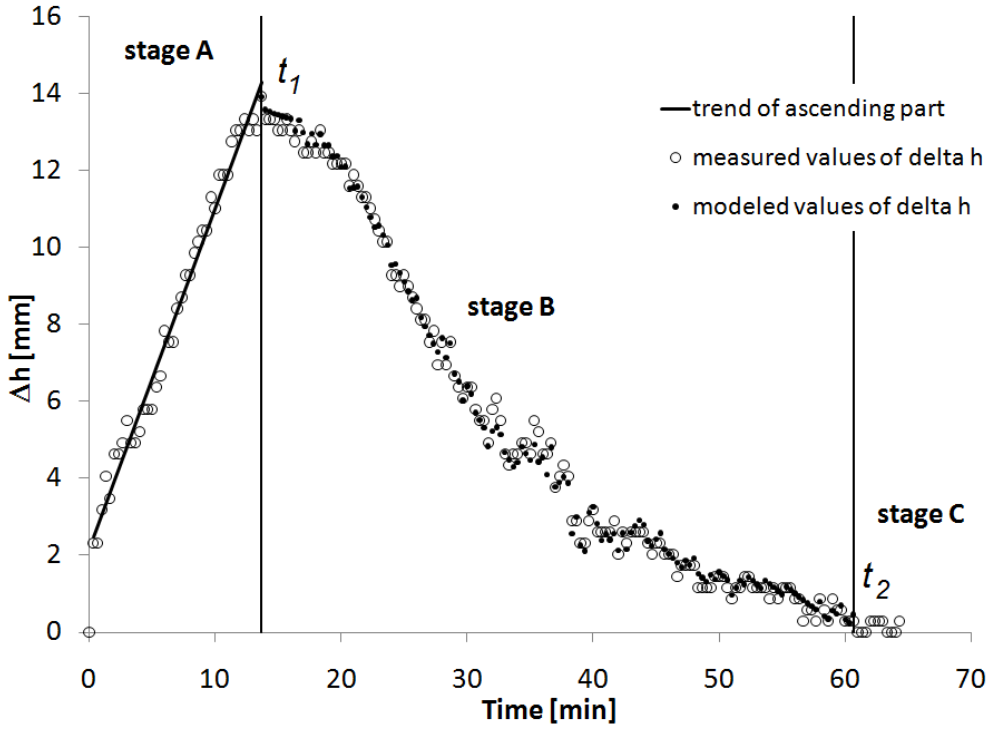

Figure 10. Water phase height (h_w) as a function of time in a typical sludge gravity drainage experiment.

The typical values of the specific cake resistance for activated sludge, determined by the SRF methodology employing piston pressure filtration, are of the order of 10^{12} m/kg (Rasmussen *et al.*, 1994). The technique described in this thesis allows the determination of specific cake resistance during low pressure gravity drainage. In a representative experiment, the specific cake resistance during gravity drainage was $4.2 \cdot 10^{10}$ m/kg (Dominiak *et al.*, 2010a), which is much lower than usually found in SRF experiments. This means that for activated sludge, which is a highly compressible material, it is not possible to increase the dewatering rate by applying higher pressures, because the specific cake resistance increases almost proportionally with pressure.

9.2. Physical, chemical, and biological factors influencing sludge gravity drainage

The highlights of a successful gravity drainage process for activated sludge are the fast water drainage and the low water content of the final filtration cake. Many factors are responsible for the actual outcome of this complex process, including physical, chemical, and biological factors.

A thorough knowledge of these factors is indispensable for the successful operation of gravity drainage-based processes, like reed bed drainage.

9.2.1. Physical factors

The physical factors of importance for sludge gravity drainage are, according to Eq. 1, the differential pressure running the process (ΔP), the amount of solids deposited in the cake (ω), and the specific cake resistance (α). The driving pressure of this process is the hydrostatic pressure of the liquid, which practically means that it depends on the volumetric sludge loading and the resulting liquid height. The amount of solids forming the cake depends on the concentration of the feed, i.e. the suspended solids content of the sludge being drained. The magnitude of the specific cake resistance, due to the highly compressible nature of activated sludge solid fraction, depends on many factors, including the above-mentioned volumetric loading, but also the floc properties, such as floc size and floc strength. The importance of these physical factors was investigated in the study described in the supporting paper entitled 'Gravity drainage of activated sludge: new experimental method and considerations of settling velocity, specific cake resistance, and cake compressibility' (Dominiak *et al*., 2010a).

Volumetric loading

The effect of sludge loading on the gravity drainage process is very strong, and this factor appears to be critical to the outcome of the process. Increasing load causes the increase in the total drainage time, and the relationship between these two parameters is exponential (Fig. 11 A). The specific cake resistance increases linearly with increasing pressure (Fig. 11 B), which is a clear manifestation of cake's compressibility. The more pressure acts on the filtration cake, the more compressed it will become and the more resistance to flow will appear in the cake's pores, which translates to increased values of specific cake resistance. Since increased loading causes the cake to become thicker (as more solids are added), the relationship between the loading and the total drainage time is exponential.

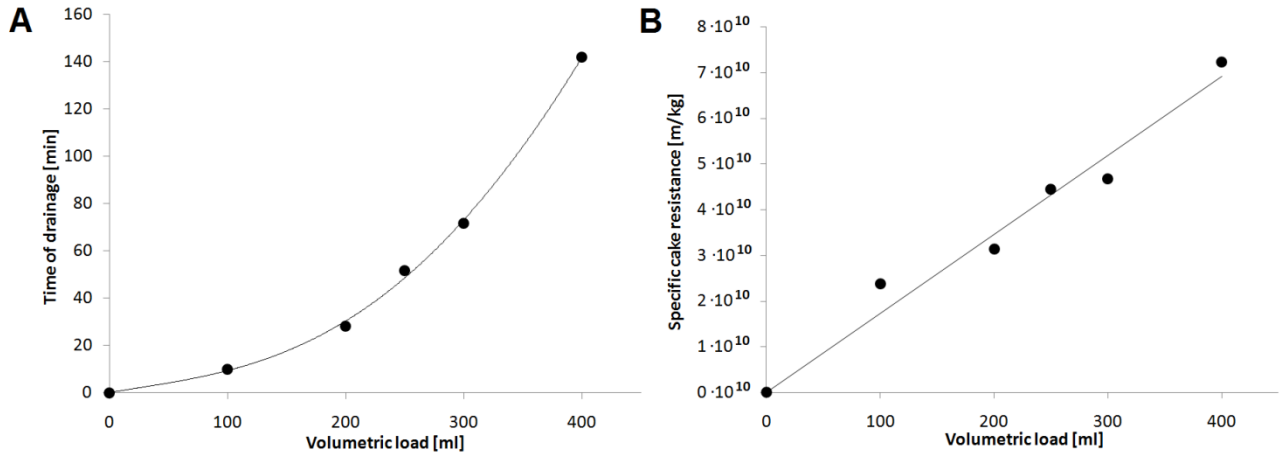

Figure 11. The effect of volumetric sludge loading on the time of drainage (A) and the specific cake resistance (B).

Due to the cake compressibility, the final dry matter content of the cake increases with solids load. A more compact cake has smaller water-filled voids, which translates to a drier cake.

Sludge concentration

The concentration of suspended solids in activated sludge also has a strong effect on the overall process outcome, yet this effect is not as strong as that of volumetric loading. As the concentration of sludge increases (at constant loading), the total drainage time also increases, but the increase is linear rather than exponential (Fig. 12 A). The specific cake resistance is independent of sludge SS concentration (Fig. 12 B). The increase in the total drainage time is solely caused by the increasing cake thickness causing increasing cake total resistance. The fact that the specific cake resistance remains constant as the SS content of sludge increases is caused by the constant pressure drop across the cake during the drainage. In other words, even though the amount of solids increases as SS increases, the pressure on the cake is constant, and the cake is compacted to the same extent in each case.

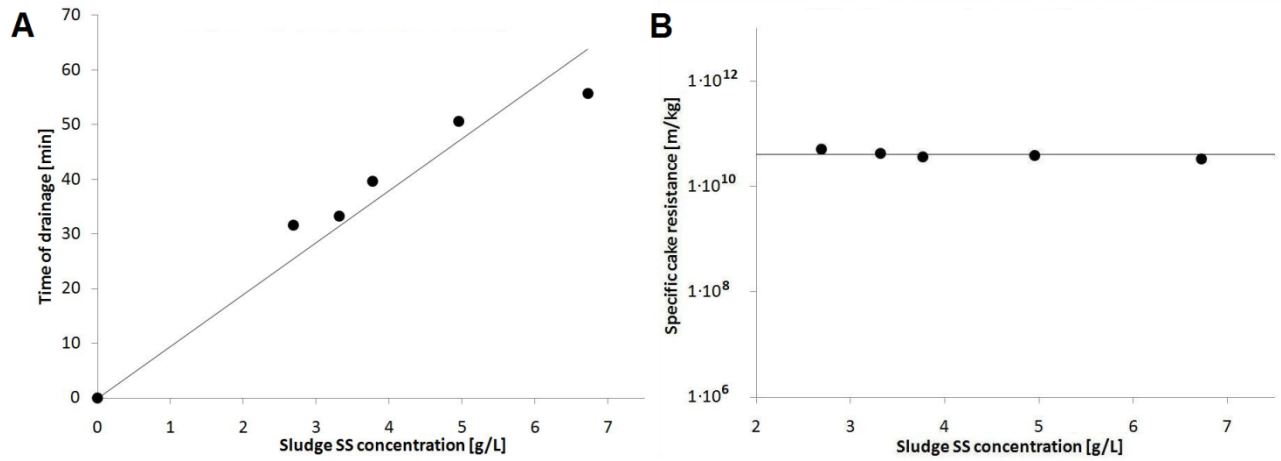

Figure 12. The effect of sludge suspended solids concentration on the time of drainage (A) and the specific cake resistance (B).

The dry matter content of the cake can be increased by increasing the SS concentration of sludge. The compaction of cake under its own weight takes place in the final stage of drainage (stage C), when the pure water phase above the cake disappears. As the SS concentration increases, the cake weight also increases and thus the cake final dry matter content raises.

The drainage process of sludge is successful when the drainage is fast and the final residue is dry. The final dry matter content of the cake can be increased by increasing both the sludge loading and the sludge concentration, but increasing these two parameters increases the total drainage time. Proper adjustment of these two parameters is therefore critical in order to achieve sufficient drainage rate and the satisfactory final dry matter content of the residues.

Sludge condition

Floc size distribution plays an important role in the determination of the drainage rate, and sludge deflocculation can lower the drainage rate considerably. Anaerobic conditions and mechanical shear are some of the well-described deflocculating factors (Wilen *et al*., 2000), and the impact of these factors on the drainability of sludge was investigated (Dominiak *et al*., 2010b).

Anaerobic sludge storage and mechanical shear at different shear rates all lower the drainage rate by increasing the specific cake resistance due to deflocculation. The relationship between the sludge loading and the specific cake resistance is linear (as indicated in Fig. 11 B), and the slope of this relationship will be defined as sludge drainability. The drainability depends on the condition of sludge after a certain treatment (Fig. 13).

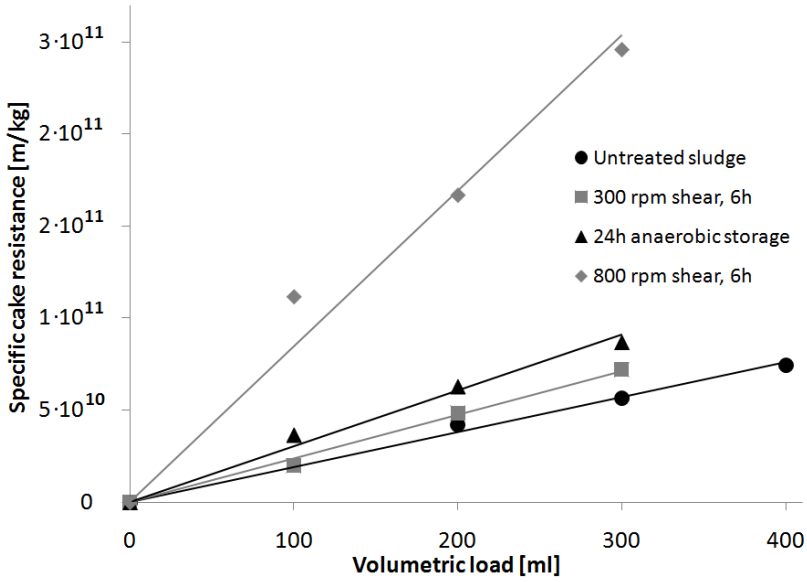

Figure 13. Results of the drainage experiments comparing the effect of 24h anaerobic storage of activated sludge, shearing at 300 rpm and shearing at 800 rpm on the drainage properties of activated sludge from a single sample batch.

Shearing at the rate of 300 rpm for 6 hours caused a mild effect, consisting of the decrease in drainability. A stronger effect was brought about by 24 hours of anaerobic storage, but the most significant drop in drainability was, as predicted, a result of shearing at 800 rpm. The most probable reason for these phenomena is that both shear and anaerobic conditions have a deflocculating effect on activated sludge, resulting in floc fragmentation and liberation of small cell aggregates and single cells (Rasmussen *et al.*, 1994). These small particles would then clog the pores inside the cake, causing slower water flow and more significant cake compression due to liquid pressure.

From our work a general relationship between the sludge load and specific cake resistance can be derived, as depicted in Figure 13:

$$\alpha = \frac{kV}{A} \quad (11)$$

where *V* is volume of sludge, *A* is area of the filter, and *k* is a proportionality coefficient, whose size depends on sludge quality, and which can be influenced by various treatments applied to sludge. The harsher the treatment the sludge was subjected to, the higher the *k* value, and the more difficult the sludge drainage process.

9.2.2. Chemical factors

The role of chemical factors in the process of gravitational drainage of activated sludge consists in their influence on floc strength and floc size. As demonstrated in Section 8.2 the floc strength, which directly determines the floc size, is a combination of several mechanisms such as the DLVO interactions, bridging with di- and tri-valent cations, and hydrophobic interactions (Larsen *et al*., 2008). For this reason, addition of chemicals can have either a stabilizing or destabilizing effect. The mechanism that is most commonly targeted with the goal of increasing the floc strength and promoting flocculation is the cation bridging. The importance of this mechanism for floc stability was demonstrated many times by the removal of cations (Bruus *et al*., 1992; Liao *et al*., 2002) and by showing the beneficial effect of cations on floc characteristics like the floc settling velocity (Jin *et al*., 2003). Addition of calcium, magnesium, aluminum, and iron salts as well as positively charged polyelectrolytes is a common practice in wastewater treatment plants aiming at improving the sludge settleability and dewaterability. The effect of sludge flocculation with calcium carbonate prior to its application on reed beds was demonstrated in the supporting paper entitled 'Sludge quality aspects of full-scale reed bed drainage' (Dominiak *et al*., 2010b).

9.2.3. Biological factors

Microbial composition of activated sludge and the activity of microorganisms were described in Section 8.3.3 as factors of great importance for floc strength and the resulting floc size, so they also strongly affect the drainage properties of activated sludge (Klausen *et al.*, 2004). The control of microbial composition of sludge is now possible to a certain extent, especially in relation to filamentous bacteria (Eikelboom, 2000). The general objectives of population control in wastewater treatment plants, aiming at good settling properties of sludge, agree with the desires of reed bed operators, who need large and strong flocs with just the right amount of filamentous organisms. The freedom of full sludge population control and design is still beyond our reach, but the knowledge of microbial activity and the related sludge behavior is sufficient to successfully control the outcome of solid-liquid separations by the adjustments of microbial activity.

As stated in Section 8.3.3, the general rule of thumb states that anaerobic conditions, inhibiting aerobic microbial metabolism, lead to reduced floc strength and deflocculation (Wilén *et al.*, 2000a; Wilén *et al.*, 2000b), which can be reverted to some extent by aeration (Mikkelsen and Keiding, 1999; Wahlberg *et al.*, 1994; Biggs and Lant, 2000). Figure 13 illustrates the damaging effect of anaerobic conditions on sludge drainability. More evidence is supplied in the supporting paper

entitled 'Sludge quality aspects of full-scale reed bed drainage' (Dominiak *et al*., 2010b) which, based on a case study, describes the operational problems on reed beds caused by the development of anaerobic conditions during sludge pumping. The way to solve the operational problems caused by decreased aerobic microbial activity is to try to maintain that activity or regain it once it has been lost. Maintaining the aerobic microbial activity can be achieved by aeration or, if aeration is impossible, by nitrate dosing to ensure anoxic conditions, which tend to conserve a substantial part of floc strength in the absence of oxygen and in the presence of shear (Wilen *et al*., 2004; Dominiak *et al*., 2010b).

9.3. Recommendations for the design and operation of reed beds for sludge drying and mineralization

Traditionally, the design and operation of sludge drying reed beds have been based on fixed guidelines describing the average yearly sludge loading per unit area of basins, such as those proposed by Nielsen (Nielsen, 2002; 2003; 2005b). He prescribes 60 kg dry matter/m^2/year for surplus activated sludge and 50 kg dry matter/m^2/year for surplus sludge blended with anaerobically digested sludge. However, as revealed in one of the supporting papers, entitled 'Sludge quality aspects of full-scale reed bed drainage' (Dominiak *et al*., 2010b), the quality of sludge in relation to gravity drainage differs substantially between different wastewater treatment plants. This finding illustrates the importance of regular sludge quality monitoring and operating the reed bed basins based on the current sludge characteristics.

The novel SRD method for the accurate determination of sludge characteristics in relation to gravity drainage is described in Section 9. It assures fast and easy assessment of any sludge on site, immediately after sampling, and therefore the precise determination of drainability, which can be directly projected to full-scale drainage process. The value of the specific cake resistance, obtained with the SRD technique, and a basic sludge parameter – SS concentration – are sufficient to successfully operate the reed beds and minimize the risk of operational problems, based on the guidelines presented in the supporting papers. The importance of the operating parameters – sludge loading and sludge SS concentration – is presented in Section 9.2.1. Since these parameters are critical to the effective drainage of activated sludge, their adjustments, based on the current sludge characteristics, should be made regularly if the performance of the reed beds is to be kept constant and high.

The determination of sludge drainage characteristics by means of the specific cake resistance measurement with the SRD technique should always be the starting point. This value allows for the estimation of sludge condition and permeability, based on Fig. 14.

One measurement of the specific cake resistance is enough to determine the slope of the relationship presented in Fig. 14, hence to predict the specific cake resistance for any loading. This information, coupled with the knowledge of the SS content of sludge, enables the prediction of the drainage time, according to Fig. 15.

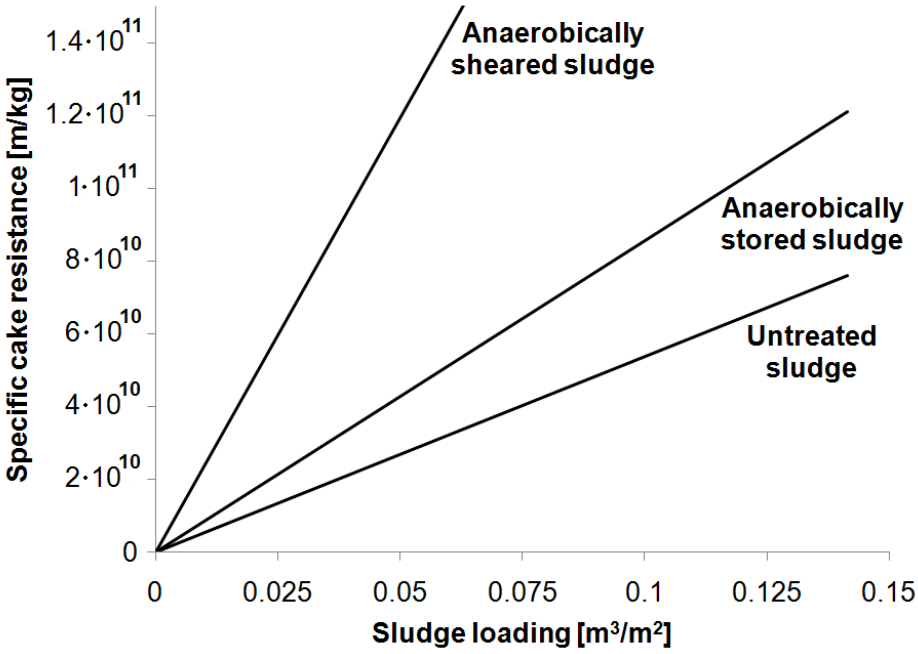

Figure 14. The relationship between the specific cake resistance and the sludge loading for sludge samples of different permeability caused by different deflocculating conditions.

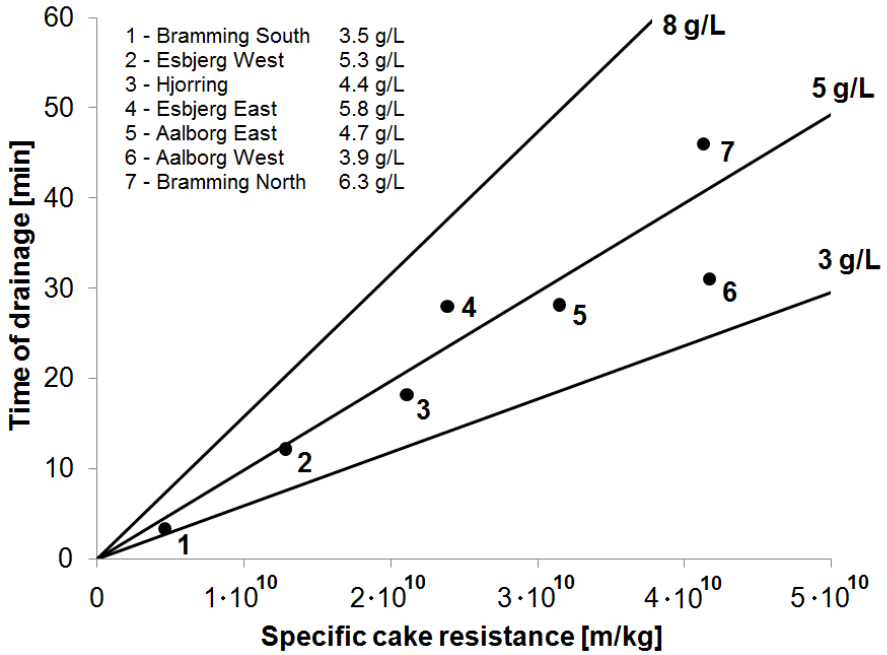

Figure 15. The relationship between the specific cake resistance and the time of drainage at different sludge SS concentrations. Points show the measured values for seven Danish wastewater treatment plants.

Knowledge of the drainage time is highly beneficial because it helps select the sludge dosing program so that a new portion of sludge is not applied before the previous one drains completely.

It can therefore help to find a balance between the loading and resting period so that both complete drainage of sludge and air penetration take place.

Generally, it is always better to apply smaller sludge portions more frequently than to flood the basins with one large portion and wait for the water to drain. This remark is based on the fact that activated sludge forms very compressible cakes, and the specific cake resistance increases proportionally to the pressure. Therefore, keeping the load low assures faster drainage and minimizes the risk of anaerobic layer formation. Anaerobic conditions lead to sludge deflocculation and formation of the so-called 'skins', which are dark and dense sludge layers of very low water permeability. Since the residues in reed bed basins keep accumulating as the basin is exploited, such skins persist in the residues and have a strong negative effect on the overall basin permeability for the rest of the basin's life cycle. It is especially important not to overload the bed during the commissioning period, because the impermeable skin may remain at the bottom of the basins for the entire operation of the bed, i.e. up to ten years.

The findings of this PhD project were applied on a large scale by Esbjerg reed bed facility operators. The SS concentration in the aeration tanks of both plants has been lowered from 4-6 to 3.5-4 g/L. Nitrate is continuously dosed to the sludge transportation pipeline, and calcium carbonate is continuously used to flocculate sludge prior to its application to the basins. Finally, the sludge application program has been changed for all basins, and sludge is now applied in smaller portions, but with higher frequency. It is now 2000 m^3/basin every 6^{th} week, and this volume is divided into 5 batches on each basin. Each batch is pumped out for 1 hour with 25 hours to drain before the next batch is added. In this way, the problems with the operation of basins handling sludge from plant Esbjerg West have been eliminated, and the overall performance of the reed bed facilities has been significantly improved after 1 year.

Some general guidelines for reed bed design can be formulated. The most important operational parameter for a reed bed is the average yearly sludge loading per unit area of a basin. The benchmark value for the design of reed beds is the 60 kg DM/m^2/year proposed by Nielsen (Nielsen 2002; 2003). However, the drainability of sludge differs strongly between wastewater treatment plants (points in Fig. 15), and designing a reed bed to receive 60 kg DM/m^2/year in a case where sludge quality is actually poor would most probably lead to overloading if the presumed amount of solids was indeed loaded.

The starting point in the design process should be an assay of sludge drainability with the SRD methodology (Fig. 16). The obtained value of the specific cake resistance should then be compared to the range presented in Fig. 15. If the value appears high compared to those for other wastewater treatment plants, it is reasonable to try to improve the drainability by sustaining the microbial aerobic metabolism during transportation or by flocculating the sludge by aeration or cation/polymer conditioning. The final specific cake resistance value could then be compared to that for plant Esbjerg East ($2.4 \cdot 10^{10}$ m/kg - point 4 in Fig. 15), which reports consistent and predictable operation at 40 kg DM/m^2/year without significant problems. The results of this

comparison should help choose a reasonable value of the average yearly sludge loading so that the operation of the reed bed is flawless in the long term.

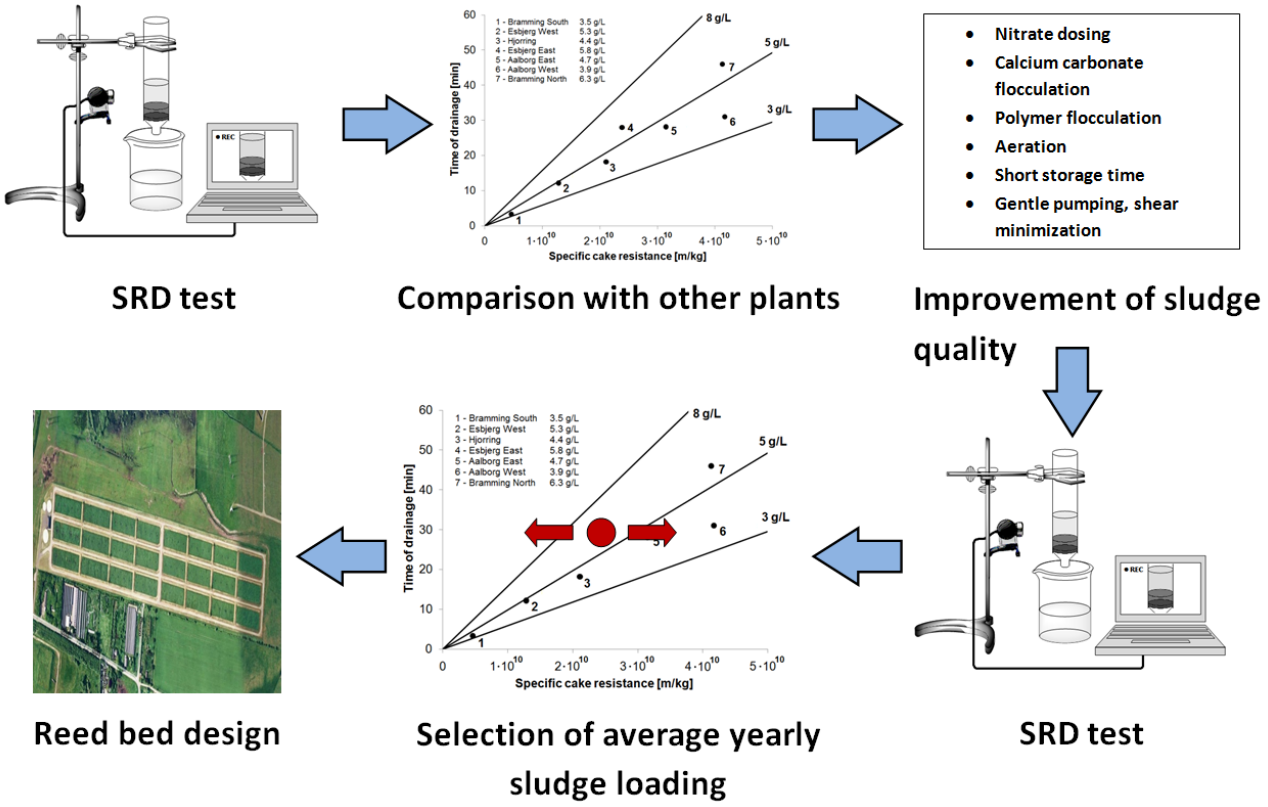

Figure 16. Flow chart for the reed bed design process.

10. Conclusions and perspectives

The objective of this PhD project was to carry out a comprehensive investigation of the water-binding properties of activated sludge in relation to drainage and mineralization of sludge on reed beds and to establish a link between the microbial composition of activated sludge, the floc properties implied by this composition, and the resulting macroscopic physico-chemical properties, gravity drainage in particular. Understanding how particular microorganisms shape their environment under different conditions, how these actions influence the floc properties, and how these properties manifest themselves in real-life engineering applications is an important environmental engineering and biotechnological challenge.

The link between sludge microbial population, the resulting floc properties, and the overall sludge drainage characteristics was established by demonstrating the important role played by eDNA for floc and microcolony strength and integrity and, further, by showing the importance of floc size and durability for the drainage process.

A new method for the detection and quantification of eDNA at microscale resolution was developed and, combined with other techniques, used to investigate the abundance and function of this exopolymer in the EPS matrix of activated sludge. Extracellular DNA was mostly found around living cells and inside microcolonies, suggesting the active and purposeful secretion. Especially certain types of microcolony-forming bacteria, such as the denitrifiers *Curvibacter* and *Thauera*, the ammonium-oxidizing *Nitrosomonas*, and the nitrite-oxidizing *Nitrospira*, were found to exhibit very high concentrations inside the microcolonies (up to 300 mg eDNA/g of organic matter). Digestion of eDNA with DNase I affected the integrity of both the microcolonies and the whole flocs. Disruption of certain eDNA-rich microcolonies, as well as entire flocs, was revealed by particle size distribution analysis before and after the digestion. This discovery confirmed an important structural role of eDNA in activated sludge flocs and suggested the importance of this exopolymer for floc size and strength, the floc characteristics critical to dewatering and drainage of sludge.

The importance of strong, big flocs for sludge drainage properties was demonstrated in a case study of two wastewater treatment plants in Esbjerg, sharing a reed bed facility. Deflocculation due to shear forces and anaerobic conditions was shown to be responsible for increased specific cake resistance, which is a direct demonstration of sludge quality in terms of gravity drainage. Re-flocculation of activated sludge by aeration, calcium carbonate and nitrate dosing proved to be an effective strategy for improving the drainability of sludge.

A novel technique for quick and easy sludge quality determination on site, immediately after sampling and at low pressure, was developed and used to investigate the process of sludge gravitational drainage. Sludge volumetric loading and suspended solids concentration were identified as the factors of greatest importance for the effective operation of reed bed basins. A method for the determination of reed bed loading schemes, based on direct and regular sludge quality monitoring, was proposed along with a new approach to reed bed facility design.

The findings of this project were used in full scale and improved the operation of reed bed facility in Esbjerg, previously suffering from frequent operational problems and process failures.

This PhD project significantly increased the understanding of phenomena taking place during sludge gravitational drainage on reed beds and revealed the link between sludge microbiology, floc properties, and macroscopic sludge characteristics. These findings were successfully transferred to full-scale and used to improve the operation of an existing reed bed facility. The perspectives for further research may assume the application of the guidelines formulated in this project for the construction and subsequent operation of a full-scale reed bed facility with the aim of achieving a high and sustainable performance and improving the competitiveness of sludge reed bed disposal.

11. Nomenclature

List of symbols

A	cross-sectional area of the cylinder (m^2)
c	particle concentration in the feed (kg/m^3)
h_0	initial level of the suspension (m)
h_c	height of the cake (m)
h_s	distance between cake surface and sample-water interface (m)
h_t	actual level of the suspension (m)
h_w	height of the clear water phase (m)
m	final mass of the wet filtration cake (kg)
M	mass of the sample (kg)
P	applied pressure (Pa)
p_a	fitting parameter in Eq. 7
p_s	solids pressure (Pa)
R_m	media resistance (m^{-1})
V	volumetric load of the sample (m^3)
k	treatment-dependent proportionality factor (1/kg)

Greek symbols

α	specific filter cake resistance (m/kg)
β	fitting parameter in Eq. 7 and 8
ρ	density of the filtrate (kg/m^3)
ρ_s	density of the particles (kg/m^3)
v_d	drainage rate (m/s)
v_s	settling velocity (m/s)
μ	filtrate viscosity (Pa s)
φ	solids volume fraction
φ_0	solids volume fraction for p_s equal to 0
ω	amount of deposited material per unit area of media (kg/m^2)

Abbreviations

CER	cation exchange resin
CST	capillary suction time
DDAO	7-hydroxy-9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one)
DM	dry matter
DLVO	Derjaguin Landau Verwey Overbeek (theory)
eDNA	extracellular deoxyribonucleic acid
EPS	extracellular polymeric substances
ISR	initial settling rate
MLSS	mixed liquor suspended solids
PSD	particle size distribution
SDS-PAGE	sodium dodecyl sulphate – polyacrylamide gel electrophoresis
SRD	specific resistance to drainage
SRF	specific resistance to filtration
SS	suspended solids
SVI	sludge volume index

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Research paper 1

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Gravitational Drainage of Compressible Organic Materials

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A model was developed to simulate drainage of compressible particle suspensions, and study how cake compression and volumetric load influence the process. The input parameters were settling velocity, cake resistance and compressibility. These parameters were found using a new experimental method. Dextran-MnO_2 particle suspensions were drained as these resemble organic waste slurries with respect to settling and compressibility. It was demonstrated that cake compressibility must be taken into account to obtain adequate simulations. This implies that pressurized filtration resistances cannot be used for drainage simulations. In the filtration step, a distinct increase of dry matter from top to bottom of the cake was observed. During the subsequent consolidation, the cake compressed and a uniform dry matter profile was found. The final dry matter content of the cake increased with feed concentration and volumetric load. The drainage time increased proportionally with feed concentration and, more importantly, proportionally with squared *volumetric load.* © 2010 American Institute of Chemical Engineers *AIChE J,* 00: 000–000, 2010
Keywords: dewatering, settling, manure, sludge, specific cake resistance

Introduction

Organic waste products such as biological sludge and animal manure can be used for heat and power generation, used as fertilizer in agricultural systems, or converted to transportation biofuels.[1,2] Furthermore, it is possible to convert organic waste into substances that can be used in various industrial products.[2] As the concentration of dry material is low in sludge and manure, solid-liquid separation is an important part of pretreatment in order to lower transportation costs, minimize the need for storage capacity, or increase the energy output from incineration. There are several methods for solid-liquid separation, including gravity drainage; for example, organic slurries can be drained using belt filter presses[3] or sludge drying reed beds.[4] The critical pressure above which the dewatering rate is constant has been determined to be in the range of 5–50 kPa for biological sludge.[5] Operation at pressures above the critical pressure have only a limited effect on separation performance, because cake porosity decreases and hydraulic resistance, therefore, increases with pressure. Gravity drainage, is, thus, an energy-efficient means of dewatering organic waste products as the pressure is close to the critical pressure, so little energy is wasted on cake compression.

In designing and optimizing gravity drainage processes, a mathematical model and laboratory-scale measurements of settling velocities, cake compressibility and cake resistance would be helpful. In the case of cake resistance, most methods described in the literature measure the resistance at pressures higher than 100 kPa.[6] These pressures are higher than

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the critical pressure of organic materials and higher than the pressures usually observed during gravity drainage. Applying such high pressures leads to the problem of transforming and applying the obtained cake resistances to the low-pressure conditions encountered in gravity drainage experiments. Furthermore, studies that describe the gravity drainage processes of inorganic particles[7,8] and biological sludge,[3,9] for example, do not include cake compression in the model. Cake compression may influence the drainage processes, but it is not known whether cakes are compressible at the low pressure achieved during drainage. However, most organic cakes are highly compressible at high pressure (>100 kPa), so it might be expected that organic cakes are compressible at lower pressures as well. In that case, existing drainage models must be modified before application to organic slurries.

The settling velocity is usually determined from sedimentation experiments and decreases with particle concentration.[10] However, it is not known whether the settling velocity changes with drainage rate or to what extent settling influences the drainage process. Thus, to predict how, for example, feed concentration, load, and media resistance influence the drainage process, it is necessary to know whether or not settling is important for the drainage process and, if so, how the settling velocity can be determined.

Several experiments are required to find out how cake resistance, cake compressibility and settling influence the gravity drainage of organic slurries. However, organic slurries are often complex mixtures, so it is difficult to use organic slurries for such studies. Alternatively, simpler model compounds can be used; for example, dextran-MnO_2 particles have been used in pressure filtration studies.[11] The compressibility of dextran-MnO_2 particles is comparable to that of other organic materials, such as biological sludge.[11] Moreover, it is easy to measure the porosity profile through dextran-MnO_2 cakes due to the higher γ-ray attenuation of MnO_2 than of water or ethanol. For that reason, dextran-MnO_2 particles are a good candidate for studying separation processes in organic compounds.

This study aims (1) to develop an experimental method for measuring settling velocity, cake resistance and cake compressibility, (2) to clarify how media resistance, cake compression and settling influence the drainage process, and (3) to simulate the drainage process to determine how load influence the final dry matter content of the drained cake. Dextran-MnO_2 particles will be synthesized and drained; local cake porosity will be monitored online at high-spatial resolution to investigate cake inhomogeneity and cake compression during the experiments.

Experimental

Dextran-MnO_2 particles

Dextran-MnO_2 particles were synthesized according to Hwang et al.[11] Before particle production, 0, 1, 2, and 3 g of 400–500 kDa *Leuconostoc mesenteroides* dextran (Sigma-Aldrich, St. Louis, MO) were suspended in 100 mL of 99.9% v/v ethanol. Particles were then prepared by adding 100 mL of the dextran suspension to 100 mL of 1.5% w/w $KMnO_4$ solution. The dextran suspension was added gradually (5 mL of dextran suspension per addition), and after each addition, the mixture was stirred at 600 rpm for 20 s and then at 400 rpm for 40 s. An overhead stirrer (RZR 2041; Heidolph Instruments, Schwabach, Germany), and an impeller (BR 13; Heidolph) were used. After preparing the dextran-MnO_2 particles, the suspension was analyzed using a LSM510 confocal scanning laser microscope (CLSM; Carl Zeiss, Oberkochen, Germany) at 10× magnification. Large dextran-MnO_2 particles (up to 500 μm) were formed, but the suspension also contained nonflocculated MnO_2 particles (1–10 μm). The produced particles settled overnight at room-temperature and supernatant was withdrawn until the desired concentration was achieved (8–45 g/L). The suspension was then gently mixed and used in the dewatering experiments.

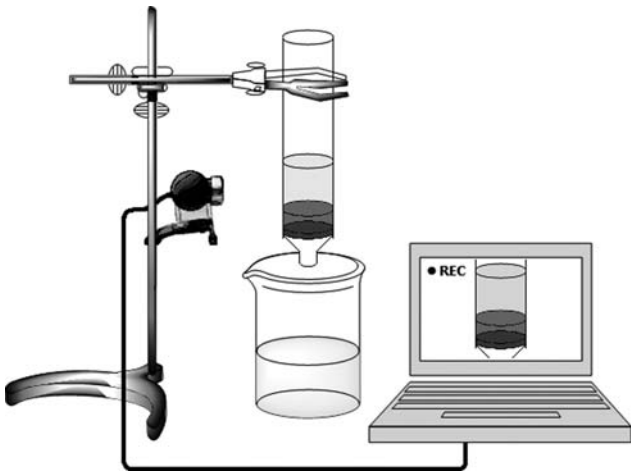

Figure 1. Sketch of drainage equipment used.

Gravity drainage and sedimentation

A series of gravity drainage experiments was performed. During drainage, a cake was formed on the filter media. It was observed that the particles settled during drainage. For that reason, a sedimentation experiment was setup, as well as to compare the settling velocity determined during the gravity drainage and sedimentation experiments.

Samples of 100 or 200 mL were drained using a 40-cm-high-transparent glass cylinder fitted with a Whatman no. 41, 20–22 μm cut-off, filter article (Whatman, Maidstone, U.K.) placed at the bottom of the cylinder (Figure 1). The internal diameter of the cylinder was 6 cm, more than 1,000 times larger than the dextran-MnO_2 particles. If the dextran-MnO_2 particles behaved like individual particles, the reduction in settling velocity due to wall effects would then be less than 5%.[12] A digital camera was placed roughly 10 cm away from the cylinder and used to monitor the drainage process. The images were analyzed to determine the distance between (1) the filter media and the interface between the suspension and the clear liquid phase ($height_I$), as well as (2) the filter media and the interface between the clear liquid and the surrounding air ($height_{II}$). Sedimentation experiments were performed using the same setup, but with an impermeable media at the bottom of the glass cylinder. Hence, datasets of time, $height_I$, and $height_{II}$ were obtained.

The local solid-mass fraction was measured at different positions in the cake using a ^{241}Am source that emits γ-rays

at 59.54 keV. The attenuation was measured using an NaI(T1) scintillation detector and integrating the number of counts between 58 and 83 keV. The calibration curve was acquired by measuring the attenuation of the feed suspension, as well as the attenuation of the final cake, the solid-mass fraction of which was known.[13] It was found that $\mu_{\gamma,\text{filtrate}} = 17.7\ \text{m}^{-1}$ and $\mu_{\gamma,\text{particles}} = 56.0\ \text{m}^{-1}$.

The filtrate viscosity was measured to be 2.4×10^{-3} Pa s using a KPG no. 100 capillary viscometer (Cannon-Fenske, State College, PA), and the filtrate density was measured to be 940 g/L by weighting the filtrate volume during the experiments. The measured values were similar to the viscosity and density of 50% v/v ethanol. The resistance of the media was found by filtering 200 mL of 50% v/v ethanol through a clean Whatman no. 41 filter paper and was calculated to be $9.7 \times 10^7\ \text{m}^{-1}$. After one of the experiments, 50% v/v ethanol was filtered through the used filter paper, and no increase in R_{mem} was observed afterwards. The cakes were easily removed from the filter media.

Analysis of cake

The dry matter content of the cake was measured by weight loss after drying at 104°C overnight. The amount of organic material was determined by measuring the loss on ignition after 4 h at 500°C. The determined ratio between organic and inorganic materials nicely fit the ratio between added dextran and MnO_2, assuming that all $KMnO_4$ was reduced to MnO_2 (slope 0.99 ± 0.06, intercept 0.0 ± 0.1). The suspension changed color from purple to brown when dextran was added to $KMnO_4$, which confirmed the reduction of MnO_4^- to MnO_2.

The solid-volume fraction of the filter cake was calculated from the measured dry matter content, as follows

$$\phi = \frac{\varphi}{\varphi + (1-\varphi)\frac{\rho_s}{\rho_L}} \tag{1}$$

The particle density was measured by producing 25 g of the particles. The particles were filtered and dried overnight at 104°C. The dried cake was crushed and dissolved in 200 mL of demineralized water in a 500-mL pycnometer. Air bubbles were removed by placing the pycnometer *in vacuo* for 1 h. The pycnometer was then filled with degassed demineralized water and weighted. After that, the dry content of the sample was found by drying the sample at 104°C overnight. The density of dextran-MnO_2 particles (dextran/MnO_2 = 2.7 g/g) was determined to be 1950 ± 10 g/L.

Theory of the Drainage Process

The drainage process can be simulated if average specific cake resistance, cake compressibility, and settling velocity are known. To do this, the drainage rate v_d, must be calculated, which can be done using Eq. 2[3]

$$v_d = \frac{\Delta P}{\eta(\alpha_{\text{cake}}\omega + R_m)} \tag{2}$$

where the pressure difference is given as

$$\Delta P = \frac{Mg}{A} = \rho g h_t + c g h_0 \left(1 - \frac{\rho}{\rho_s}\right) - \omega g \tag{3}$$

As can be seen in Eq. 2, the drainage rate is directly related to the average specific cake resistance. Furthermore, the drainage rate is indirectly related to cake compressibility and settling velocity. Cake compressibility describes how the solid-volume fraction of the cake changes with pressure; the compressibility influences the drainage rate indirectly because the average specific cake resistance increases with solid-volume fraction. Settling is important because the cake grows faster if the settling velocity is high. This affects the drainage process because the drainage rate decreases with cake thickness.

Average specific cake resistance, settling velocity, and cake compressibility can be determined from the introduced laboratory experiments discussed here. To estimate the three parameters, it is practical to divide the drainage process into three stages: (A) cake formation, (B) pure filtration, and (C) cake collapse (Figure 2). Settling velocity can only be determined using data obtained during cake formation (stage A). Average specific cake resistance is most easily determined using data obtained during pure filtration (stage B), and cake compressibility is most easily determined using data obtained after final cake compression (stage C). The procedure used will be described in the following sections. When all parameters are determined, the level of the sample, i.e., the drainage rate, can be simulated numerically using Eq. 2, and Euler's method as $dh_t/dt = v_d$. Such simulation can be used to study the impact of different input parameters on the drainage process.

F2

Cake formation (Stage A)

During the initial part of the process when $t < t_1$, a cake builds up on the media (stage A), and the cylinder consists of cake, suspension, and clear liquid phases (Figure 2); the total level of the sample is $h_t = h_w + h_s + h_c$. Furthermore, cake height is a function of the amount of cake deposited on the media and of the dry matter content of the cake

$$h_c = \frac{\omega}{\phi \rho_s} \tag{4}$$

and

$$\omega = S(h_0 - h_t) + c v_s t \tag{5}$$

where S is given as[6]

$$S = \frac{1}{\frac{1}{\rho_s}\left(\frac{\rho_s}{c} - 1\right) - \frac{m-1}{\rho}} \tag{6}$$

If the settling velocity is zero, ω increases proportionally with the specific filtrate volume ($h_0 - h_t$). If particles settle, the cake builds up faster and a clear liquid phase develops above the suspension. The height of clear water can be calculated using Eq. 7

$$h_w = v_s \cdot t \tag{7}$$

As h_w is measured during the drainage experiments, it is possible to calculate the settling velocity by plotting h_w as a function of time and using linear regression. The same method can be used to estimate the settling velocity from the results of the settling experiment.

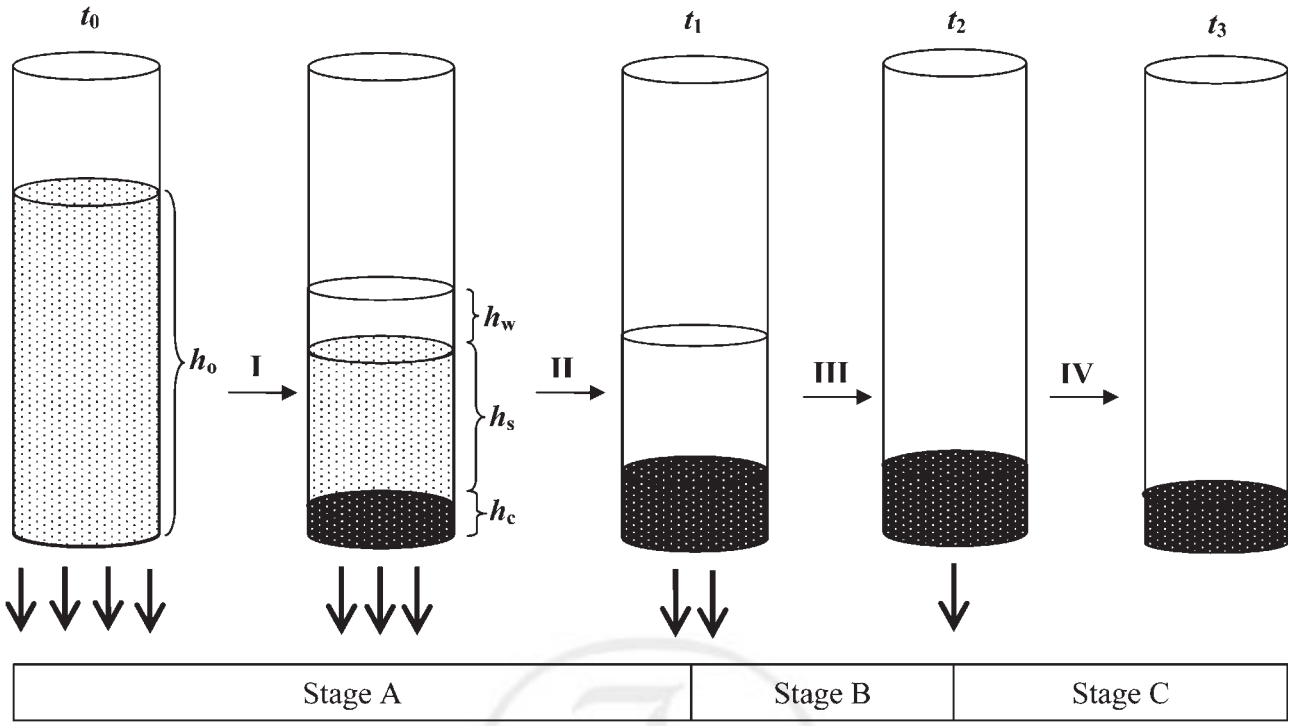

Figure 2. Principle of drainage experiment.

Pure filtration (Stage B)

All particles are deposited on the media and the liquid above the cake is filtered through the cake when $t_1 \leq t < t_2$ (Figure 2). The amount of deposited cake is constant and equals $\omega = ch_0$. It is possible to derive an equation for the sample level h_t, throughout the stage. Equation 8 has been derived assuming that $\rho h_t \gg c(1 - \rho/\rho_s)h_o - \omega$, inserting $\omega = Sh_0$ into Eq. 2 and integrating

$$h_t(t) = h_t(t_1) \cdot e^{-\chi(t-t_1)} \tag{8}$$

where

$$\chi = \frac{\rho g}{\eta(\alpha c h_0 + R_m)} \tag{9}$$

Furthermore, h_w is given as the level of the sample minus the cake height

$$h_w = h_t - h_c = h_t(t_1) \cdot e^{-\chi(t-t_1)} - \frac{ch_0}{\phi\rho_s} \tag{10}$$

It, is, therefore, possible to estimate χ by fitting Eqs. 8 or 10 to experimental data. When χ is known, Eq. 9 can be used to calculate the average specific cake resistance.

Cake collapse (Stage C)

All liquid has been drained through the cake when $t \geq t_2$. Thus, air reaches the cake surface and menisci are formed between the solid particles (Figure 2). This generates a capillary pressure, and, thereby, a drag on the cake structure.[14] Organic materials form highly compressible cakes, so the cake porosity is higher at the top of the cake than at the bottom. Thus, the cake will collapse because the cake cannot withstand the drag on the cake structure.[14] The compression starts at the cake surface, while the underlying cake compresses when the capillary pressure exceeds the local compressible yield stress. The compressible yield stress increases with the solid-volume fraction; hence, cake collapse and final dry matter content are derived from the relationship between the solid-volume fraction and the compressible yield stress. An often used constitutive equation for the compressible yield stress is given in Eq. 11[15,16]

$$p_y = p_a\left(\left(\frac{\phi}{\phi_0}\right)^{1/\beta} - 1\right) \tag{11}$$

The process stops when the liquid pressure at the sample-media interface is zero. The liquid pressure can be calculated using Eq. 12

$$p_l^{mem} = \rho g h_t + g\omega\left(1 - \frac{\rho}{\rho_s}\right) - p_s^{mem} \tag{12}$$

and is zero when

$$p_s^{mem} = \rho g h_c + \Delta\rho g h_c \phi \tag{13}$$

as $h_c = h_t$ at the end of the drainage experiment, and $\omega = h_c\phi\rho_s$ according to Eq. 4. The drag at the top of the cake, which results from the capillary forces, then equals $\rho g h_c$, and the effective pressure increases from $\rho g h_c$ at the top of the cake to $\rho g h_c + \Delta\rho g h_c \phi$ at the bottom. If $\Delta\rho\ \phi \ll \rho$, it can be assumed that the effective pressure and solid-volume fraction are constant throughout the thickness of the cake. The final cake height can then be obtained by combining Eqs. 11 and 13 setting $p_s^{mem} = p_y$

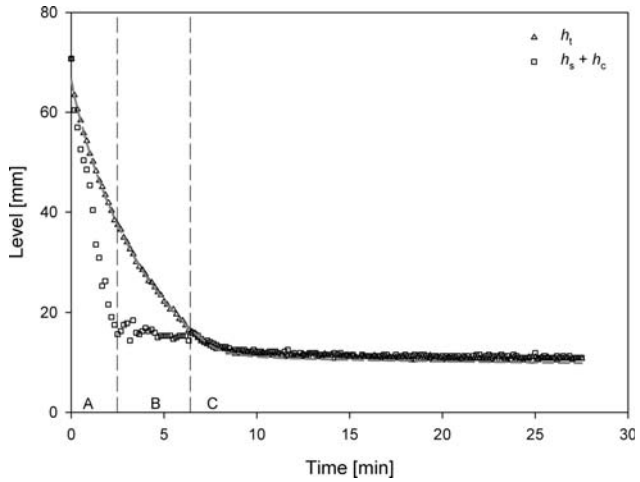

Figure 3. Drainage of a suspension of 12 g/L dextran-MnO_2 particles.

The actual level of the suspension is shown (h_t), as well as the level of the water-suspension interface ($h_c + h_s$).

$$h_{c,\infty} = \frac{p_a}{\rho g} f(\phi) \tag{14}$$

where

$$f(\phi) = \frac{(\phi/\phi_0)^{1/\beta} - 1}{1 + \phi \frac{\Delta\rho}{\rho}} \tag{15}$$

Both the cake height and the solid-volume fraction can be measured at the end of the experiment. From a series of experiments in which different amounts of solid materials are drained it is, therefore, possible to determine p_a, β, and ϕ_0 by fitting Eq. 14 to the experimental data. To lower the number of adjustable parameters ϕ_0 has been determined separately; ϕ_0 is the solid-volume fraction where particles just form a continuous network—the so-called gel point. An estimate of ϕ_0 can therefore be made from the results of settling experiments in which the effective pressure is low, i.e., $p_s^{mem} = \omega g(1 - \rho/\rho_s)$, and the solid-volume fraction of the formed cake is close to, but greater than ϕ_0.

Results and Discussion

Description of gravity drainage setup

F3 Figure 3 shows data from a typical gravity drainage process. Three different stages (cf. Figure 2) can be identified: stage A in which particles settle and the cake builds up, stage B in which liquid is filtered through the nongrowing cake, and stage C in which the liquid-air interface reaches the cake surface and the cake collapses. The transition between stages A and B is the time at which $h_s = 0$, i.e., when the suspension vanishes. Furthermore, the transition between stages B and C is found at the time at which $h_w = 0$, i.e., when the pure water vanishes. The pressure at the sample-media interface was calculated from the sample level, and from these calculations it was found that the pressure decreased from roughly 650 Pa at the onset of the experiment to 90 Pa at its end.

Determination of specific cake resistance and settling velocity

The data presented in Figure 3 were used to estimate the specific cake resistance and the settling velocity. This was done by measuring the height of the clear water phase, which increased during cake formation (stage A) and disappeared again during the pure filtration stage (stage B). Figure 4a shows the calculated values of h_w. F4

The settling velocity was determined during cake formation (stage A), and the result was compared with the settling velocity determined from a simple sedimentation experiment. For the gravity drainage experiment, the settling velocity was determined from the initial increase in h_w (Figure 4a). It was observed that h_w increased linearly until $t_1 = 2$ min and 20 s. Equation 7 was fitted to the measured data obtained during stage A (Figure 4b), and the settling velocity was calculated to be 1.6×10^{-4} m/s. Data from the sedimentation

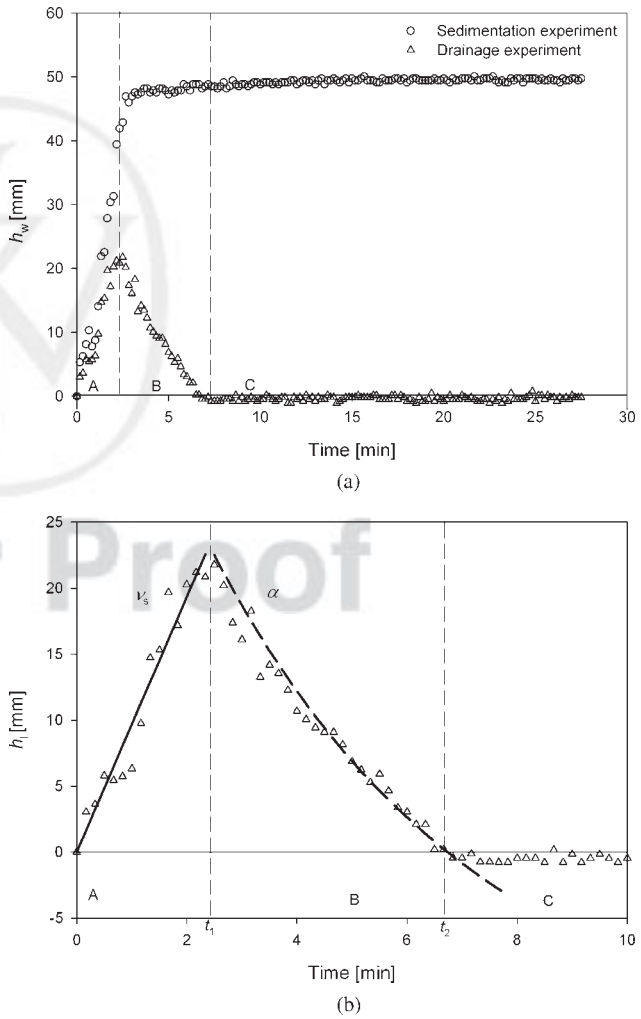

Figure 4. The height of the clear liquid phase (h_w) in the gravity drainage experiment shown in Figure 3, i.e., a suspension of 12 g/L dextran-MnO_2 particles.

(a) Data from the drainage experiment, as well as a sedimentation experiment, and (b) data from the drainage experiment and simulated data for stage A (solid line), and stage B (dotted lines).

Table 1. Experimental data for drainage of dextran-MnO_2 suspensions of varying feed concentrations

Concentration [g/L]	Settling velocity [m/s]	Resistance [m/kg]	Cake height [mm]	Dry matter content
8	1.9×10^{-4}	3.2×10^{9}	15.5	0.084
19	1.2×10^{-4}	1.7×10^{9}	19.7	0.10
29	1.2×10^{-4}	1.5×10^{9}	31.3	0.11
45	3.1×10^{-5}	1.5×10^{9}	42.6	0.12

experiment are included in Figure 4a. The settling velocity was almost identical during both drainage and sedimentation. Thus, simple sedimentation experiments can be performed to determine the settling velocity relevant to drainage experiments. Settling can influence the drainage process, because settling affects cake buildup. The impact of settling on the drainage rate will be discussed in section "Simulation of Gravity Drainage Processes."

Specific cake resistance is a parameter that describes how difficult it is to dewater a given material. Using the method presented here, it is possible to calculate the specific cake resistance obtained during gravity drainage processes and compare this value with the resistance determined during pressure filtration processes. The specific cake resistance was determined during pure filtration (stage B). The total resistance was calculated to be 1.2×10^{9} m^{-1} by fitting Eq. 8 to the measured values of h_w obtained during stage B (Figure 4b). The total resistance was given as the sum of the cake resistance and the media resistance. Using the measured media resistance of the clean filter paper, the average specific cake resistance was calculated to be 1.2×10^{9} m/kg. This resistance was lower than the resistance obtained from piston filtration experiments (approximately 4×10^{12} m/kg at 1 bar).[11] Thus, the cake was more loosely packed after the drainage experiment than were cakes formed during piston filtration. The measured resistance indicates that it is impossible to increase the dewatering rate using higher pressure because the specific cake resistance increases almost proportionally with pressure. Drainage is, therefore, a good method for initially dewatering organic material. If high-dry matter content is needed, drainage must be combined with cake consolidation.

The settling velocity and specific cake resistance were calculated from four drainage experiments in which the feed concentration of dextran-MnO_2 particles in the sample was varied between 8 and 45 g/L (Table 1). The settling velocity decreased with particle concentration. This has often been observed and explained as a result of hindered settling, i.e., hydrodynamic interaction between particles.[17] Different mathematical expressions exist for estimating the relative settling velocity from the volume fraction of the particles, i.e., $\upsilon_s(\text{relative}) = (1 - \phi_p)^{4.65}$.[10] However, the particles are water swollen in this case, and if the volume fraction is calculated from the mass and the density of the *dry* materials, the relative settling velocity is underestimated. At 45 g/L, the estimated relative settling velocity has been calculated to be 0.9, whereas it has been *measured* to be <0.2. The conclusion is that the settling velocity must be determined experimentally as a function of concentration, i.e., from simple sedimentation experiments or drainage experiments.

The apparent average specific cake resistance has been estimated as well (Table 1). The resistance was highest for the sample with the lowest particle concentration. This could be because the filter media resistance was underestimated and the average specific cake resistance, therefore, overestimated. This had a considerable impact on the calculated resistance when the formed cake was thin and the total cake resistance low. At higher particle concentrations, the resistance was constant and independent of concentration. This was reasonable because the initial height of the suspension, and, therefore, the pressure drop across the cake, was the same in all four experiments.

Cake compressibility

The dextran-MnO_2 cakes were highly compressible. This was, for example, observed from the data shown in Figure 3, where the cake collapsed during the final stage of the drainage process. During this part of the process, the dry matter content of the cake increased from 6.4% g/g to 9.0% g/g. Moreover, the dry matter content of the cake formed during the sedimentation experiment was estimated to be 4.6% g/g (i.e., $\phi_0 = 0.023$). This dry matter content was lower than that of the cake formed during drainage. Thus, the extra drag on the cake structure from the liquid flow contributed to the cake compression. The effective pressure at the bottom of the cake was calculated to be $p_s^{mem} = \omega g(1 - \rho/\rho_s) = 4$ Pa during sedimentation, compared with 90–650 Pa during drainage. The effective pressure at the bottom of the cake is a function of both feed concentration and load. As cake compression increases both cake porosity and specific cake resistance, both the drainage rate and the final dry matter content increases with load and feed concentration.

To study cake compressibility in more detail, the local solid-mass fractions were measured online. Figure 5 shows the local solid-mass fractions measured at four different positions in the cake, i.e., 2, 3, 5, and 7 mm from the top of the filter media. Notice that after 28 min, the cake height was lower than 7 mm, which is why no more measurements were made at this depth. The data measured 3 mm above the filter media will now be discussed. During initial cake buildup (stage A), the solid-mass fraction increased to 8% g/g; during

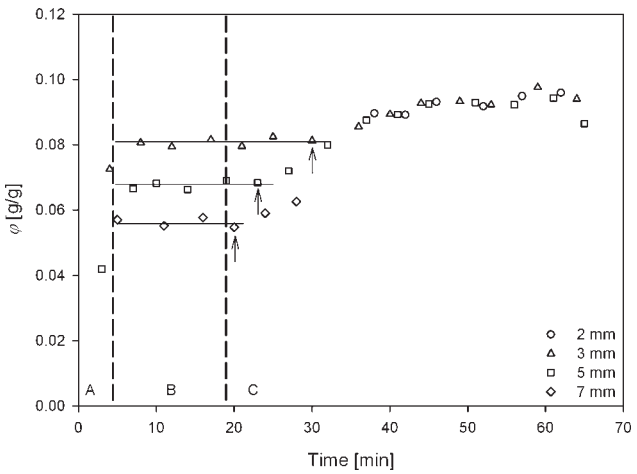

Figure 5. Local solid-mass fraction of the cake formed when draining dextran-MnO_2 particles.

The solid-mass fraction was measured online at different positions above the filter media.

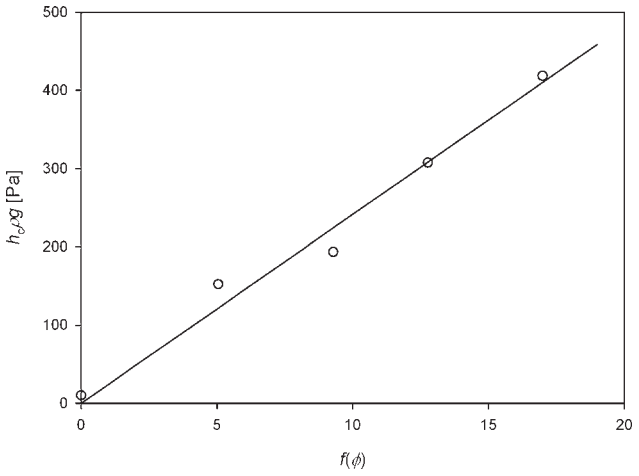

Figure 6. Cake compressibility. Data from four different drainage experiments (Table 1), and a sedimentation experiment.

The final dry matter content was measured at the end of the experiments and Eq. 15 was used to calculate $f(\phi)$. The final structural pressure was calculated and plotted as a function of $f(\phi)$.

stage B, the mass fraction was constant. The solid-mass fraction remained constant during stage C, until the solid-mass fraction of the cake exceeded 8% g/g; the solid-mass fraction then increased to 9.5% g/g. Thus, during stage A, a cake first formed and then became compressed. The solid-mass fraction was higher at the bottom of the cake than at the top, which is as expected for compressible cakes. During stage B, when water was filtered through the cake, the cake neither became compressed nor swelled. Finally, during stage C, the cake became compressed from the top until a constant solid-volume fraction was obtained throughout the cake. After roughly 40 min, the drainage process ended and the solid-mass fraction became constant and was measured to be 9.5% g/g. Hence, the final collapse resulted in a cake with a constant structural pressure throughout its thickness, indicating a cake with homogeneous water distribution. If the cake is consolidated after drainage, the best analytical models of the consolidation will, thus, be one in which it is assumed that the initial structural pressure is constant throughout the cake. Such a model has been derived in Shirato et al.[18]

The static compressibility of the dextran-MnO_2 cake was determined from drainage experiments in which the feed concentration was varied. As the solid-mass fraction was constant throughout the cake at the end of the experiment, it was possible to determine the cake compressibility by measuring the dry matter content at the end of the drainage experiment and compare it with the cake weight. Using data from the experiments shown in Table 1, it was found that the dry matter content increased with feed concentration. The sample volume was the same in all four experiments, and the increase in dry matter content was due to the higher weight of the formed cake. Equation 15 was used to calculate $f(\phi)$, setting $\phi_0 = 0.023$ as found in the previous presented sedimentation experiment. Figure 6 shows the final structural pressure throughout the cake as a function of $f(\phi)$. Equation 14 was fitted to the experimental data using linear regression; β was thereby estimated to be 0.33, and P_a estimated to be 24 Pa. In the literature, β is regarded as a compressibility coefficient and a cake is termed highly compressible if $\beta > 0.25$.[19] Furthermore, P_a was found to be lower than the pressure drop across the cake, i.e., 90–650 Pa. Thus, the estimated parameters indicate that the dextran-MnO_2 particles formed highly compressible cakes even at very low structural pressure. This has also been demonstrated in the case of biological sludge,[20] although the situation is very different from that observed for minerals. Hence, organic particles are also highly compressible at low pressure, and this must be incorporated into the drainage model as cake compression affects both drainage time and final dry matter content. Cake compression is a function of media resistance, feed concentration, and load.

Simulation of gravity drainage processes

The determined settling velocity, specific cake resistance and cake compressibility were then used as input parameters in simulating the entire drainage process. The simulations were performed numerically using Eqs. 2 and 3.

As the cake was compressible, the structural pressure in the cake was of particular interest because it influences the solid-mass fraction and, subsequently, the specific cake resistance. From the model, it is possible to simulate the structural pressure at the membrane-cake interface as $p_s = \eta R_{mem} \upsilon_f A^{-1}$. Figure 7 shows the simulated data. Different values for the media resistance have been used to demonstrate that the maximum structural pressure is strongly dependent on the media resistance. Consequently, the cake compression at the membrane is also expected to be strongly dependent on the media resistance. The initial pressure drop across the membrane is 650 Pa, and the calculated maximum structural pressure is shown in Table 2 at different membrane resistance values. For example, if R_{mem} is set to 8.1×10^8 m^{-1}, the pressure at the bottom of the cake never exceeds the pressure throughout cake at the end of the experiment. The resistance of the media used was 9.7×10^7 m^{-1} and the

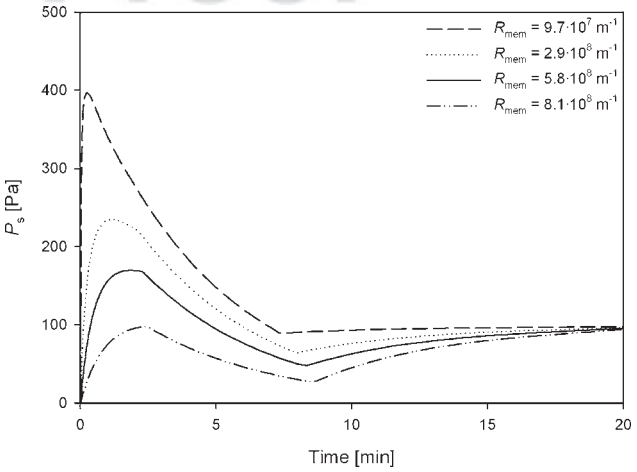

Figure 7. Simulated structural pressure at the sample-media interface for different media resistances.

Feed concentration has been set to 12 g/L. The specific cake resistance and compressibility data measured for cakes consisting of dextran-MnO_2 particles were been used in the simulation model.

Table 2. Simulated maximum structural pressure at the sample–media interface at different media resistances. The relative cake thickness at time of maximum pressure is also shown

Media resistance [m^{-1}]	Max pressure [Pa]	Relative cake thickness
9.7×10^7	397	31%
2.9×10^8	234	66%
5.8×10^8	170	86%
8.1×10^8	98	100%

structural pressure at the bottom of the cake declined during drainage. Consequently, (1) the solid-mass fraction at the bottom should be higher than throughout the rest of the cake, or (2) cake compression should be reversible, so that the solid-mass fraction at the bottom of the cake decreases during the final part of the drainage process. This was not observed when the local solid-mass fraction was measured (Figure 5). According to the theory of highly compressible cakes, most of the pressure drop happens toward the very bottom of the cake.[21] The resolution of the solid-mass fraction measurements was roughly 1 mm, so it was impossible to measure the solid-mass fraction just above the filter media. This could explain why it was impossible either to detect a solid-mass fraction gradient at the end of the process or to measure a decreasing solid-mass fraction near the cake bottom.

F8 Figure 8 shows simulated values of the actual level of the suspension, h_t, together with the experimental data from Figure 3. The following input data were used in the model (solid line): $\alpha = 1.2 \times 10^9$ m/kg, $v_s = 1.6 \times 10^{-4}$ m/s, $h_{c,\infty} = 10.7$ mm, and $R_{mem} = 9.7 \times 10^7$ m^{-1}. The drainage rate is overestimated during the initial part of the experiment, so h_t decreases more rapidly than was observed during the experiment. This could be because the average specific cake resistance decreased during the drainage experiment due to cake compressibility and decreasing pressure across the cake. Initially, the structural pressure at the bottom of the cake was high, and the average specific cake resistance was high as well. After a while, the structural pressure declined

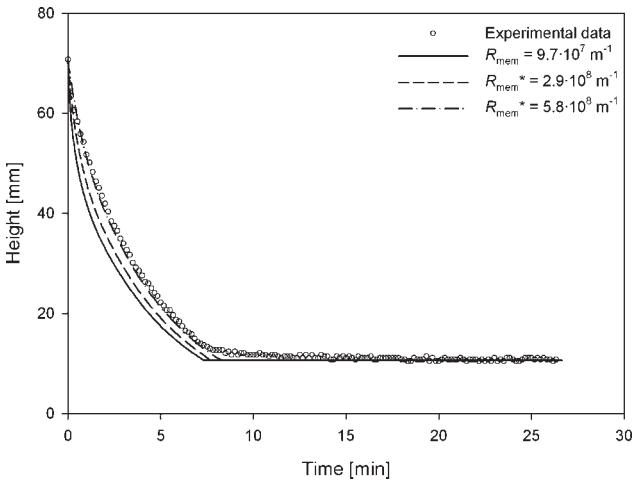

Figure 8. Experimental and simulated data for drainage of a suspension of 12 g/L dextran-MnO_2 particles (cf. Figure 3).

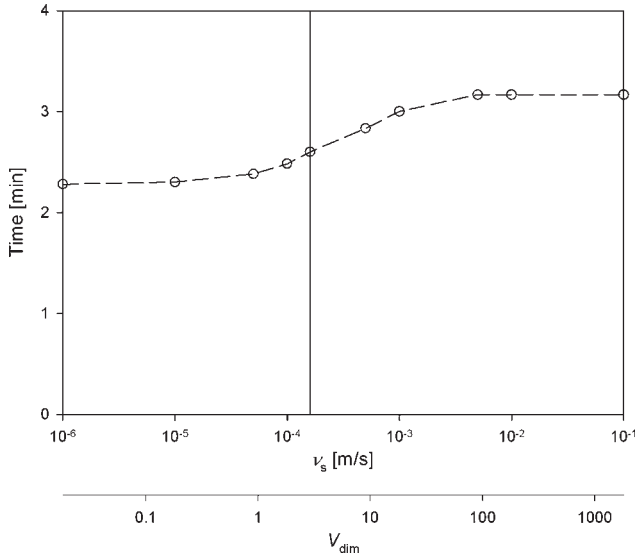

Figure 9. Impact of settling on the drainage process.

Nine simulations were carried out in which only the settling velocity was varied. The required time for draining 100 mL of the sample was found as a function of settling velocity. The vertical line indicates the actual measured settling velocity of dextran-MnO_2 particles.

and the newly formed cake structure was more loosely packed than it would have been if the pressure difference across the cake were constant during the experiment. This resulted in a decreasing average specific cake resistance. An apparent media resistance (R^*_{mem}) can be used to obtain a better fit (Figure 8). During the final cake collapse, a small deviation between the simulated and experimental data was observed, because the drag in the cake from the increasing capillary pressure was not incorporated into the model. To do so, it would be necessary to know the empirical parameters in Eq. 11, and model the local resistance and solid-mass fractions in the cake using a more complicated model. However, the error is small and the model is useful for evaluating how different input parameters influence drainage.

The model is used to study how the settling velocity influences the process. The largest difference between the simulated curves of h_t with and without settling is observed when approximately half the sample is drained. Thus, in Figure 9, F9 the time required to drain 100 mL of the sample is plotted as a function of settling velocity. The required drainage time is short at a low-settling velocity, but increases when the settling velocity becomes comparable to the drainage rate. The time required to drain 100 mL increases by a maximum of 40% if the particles settle. As seen in Figure 9, settling becomes important for the drainage process if $v_s h_t$ is of the same order of magnitude or more than $v_d h_c$. Hence, the settling velocity v_s can be determined from a simple sedimentation experiment, and if $v_s h_t \ll v_d h_c$, settling does not influence the drainage model. In this situation, the settling velocity can be omitted from the simulation model, i.e., it can be assumed that cake height growth is proportional to filtrate volume. If the settling velocity is unknown, the maximum drainage rate can be calculated, assuming that the cake height grows proportionally with filtrate volume ($v_s = 0$), i.e., stage B will not appear. The minimum drainage rate can be calculated, assuming that the cake

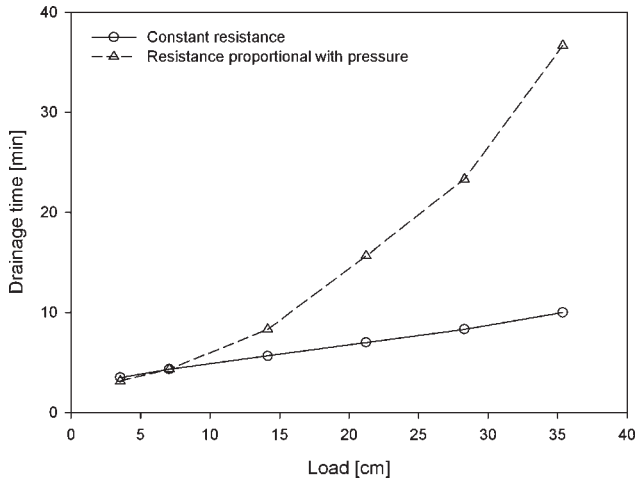

Figure 10. Impact of sample load on the drainage process.

Two series of six simulations were performed in which the load was varied. In series 1, it is assumed that the resistance was constant; in series 2, it is assumed that the resistance increases proportionally with pressure. The required time for draining 90% of the maximum possible drainage volume was then calculated as a function of load.

is fully builtup when the drainage process is started ($\upsilon_s \rightarrow \infty$), i.e., stage A will not appear.

The model simulation indicates that the required drainage time increases almost linearly with feed concentration; furthermore, the impact of the load has been studied as well. Figure 10 shows simulated data assuming a constant average specific cake resistance and a resistance that increases linearly with pressure. All input parameters are the same as used in Figure 8 where the load was 7.07 cm; at this load, the average specific cake resistance was set to 1.2×10^9 m/kg. It can be seen from Figure 10 that the required drainage time increases proportionally with load if the resistance is constant (i.e., in the case of noncompressible cakes); however, for highly compressible material, the required drainage time increases with load raised to the second power (highly compressible cakes). Thus, for highly compressible materials such as organic slurries, the load is very important for the drainage result. If the load is doubled, the drainage time increases by a factor of four assuming that the media resistance is negligible. At the end of the drainage process, a higher dry matter content of the cake will be observed if the load is doubled. Thus, there exists an optimum load, for example, on a filter press depending on drainage time (i.e., belt length and velocity) and media resistance. The feed concentration also influences the drainage rate and final dry matter content. The drainage rate increases proportionally with feed concentration, because a thicker cake is formed at a higher feed concentration. For the same reason, a higher dry matter content is obtained at the end of the drainage process if the feed concentration is increased. Now two alternative methods exist for increasing the final dry matter content of the cake: (1) increase the load, or (2) concentrate the feed. If possible, the best solution is to concentrate the feed before drainage, because the drainage time only increased by a factor of two when the feed *concentration* is doubled, not by a factor of four as observed when the *load* is doubled.

Conclusion

A mathematical model was developed to simulate drainage processes, and a new experimental method was used to determine needed input parameters: settling velocity, cake resistance and cake compressibility. Dextran-MnO_2 particle suspensions were drained, and it was found that the mathematical model fitted the experimental data well. Dextran-MnO_2 particles settled during drainage. However, settling only had a small impact on the drainage process. The drainage time was underestimated with less than 10% if settling was neglected during the simulation. The cake resistance was 1,000 times lower during drainage than during pressure filtrations. Thus, pressurized filtration resistances cannot be used for drainage simulations. Moreover, the formed cake was highly compressible also at the low pressure obtained during drainage. In the filtration step of the drainage process, a distinct increase of dry matter from top to bottom of the cake was observed. During the subsequent consolidation step, the cake compressed and a uniform dry matter profile was found. This is important. Due to cake compression, the dry matter content of a fully drained cake increases with both feed concentration and volumetric load. Furthermore, for highly compressible cake, the drainage time increased proportionally with *squared* volumetric load and not only linearly with load as for noncompressible materials. Thus, the effect of volumetric load on drainage time will be greatly underestimated if it is incorrectly assumed that the material is noncompressible.

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Notation

A = cross-sectional area of cylinder, m^2
c = solid concentration in feed, kg/m^3
h_0 = initial suspension level, m
h_c = cake height, m
h_s = distance between cake surface and sample-water interface, m
h_t = actual suspension level, m
h_w = height of clear water phase, m
L = sample volume divided by cross-sectional area of cell, m
M = sample mass, kg
m = ratio of wet and dry cake mass, kg/kg
P = applied pressure, Pa
R_m = media resistance, m^{-1}
S = ratio between dry mass of cake and total filtrate volume, kg/m^3
V_f = filtrate volume; equals $A(h_t - h_0)$, m^3

Greek letters

α = specific filter cake resistance, m/kg
ρ = filtrate density, kg/m^3
ρ_s = particle density, kg/m^3
$\Delta\rho$ = density difference between solid particle and liquid, kg/m^3
φ = cake solid-mass fraction, kg/kg
ϕ = cake solid-volume fraction, m^3/m^3
η = filtrate viscosity, Pa s
v_d = drainage rate, m/s
v_s = settling velocity, m/s
μ = attenuation coefficient, m^{-1}
ω = amount of disposed material per unit media area, kg/m^2

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Research paper 2

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Gravity drainage of activated sludge: New experimental method and considerations of settling velocity, specific cake resistance and cake compressibility

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ABSTRACT

A laboratory scale setup was used for characterization of gravitational drainage of waste activated sludge. The aim of the study was to assess how time of drainage and cake dry matter depended on volumetric load, SS content and sludge floc properties. It was demonstrated that activated sludge forms compressible cakes, even at the low pressures found in gravitational drainage. The values of specific cake resistance were two to three orders of magnitude lower than those obtained in pressure filtration. Despite the compressible nature of sludge, key macroscopic parameters such as time of drainage and cake solid content showed simple functional dependency of the volumetric load and SS of a given sludge. This suggests that the proposed method may be applied for design purposes without the use of extensive numerical modeling. The possibilities for application of this new technique are, among others, the estimation of sludge drainability prior to mechanical dewatering on a belt filter, or the application of surplus sludge on reed beds, as well as adjustments of sludge loading, concentration or sludge pre-treatment in order to optimize the drainage process.

1. Introduction

The activated sludge process is the most widespread technology of biological wastewater treatment. One of the most important challenges is the handling of surplus activated sludge, which is a side product of the process. The water content of this sludge exceeds 90% by weight, which requires dewatering it in order to make the handling and transportation of sludge physically and economically feasible. Several techniques of activated sludge dewatering exist, some employ mechanical devices like filter presses or centrifuges, others, like reed beds or belt presses, depend on gravitational water drainage. Although the gravitational drainage of sludge is an economically attractive alternative to sludge pressure dewatering, it has received little attention from researchers so far. Most research has been conducted on pressure dewatering of activated sludge in devices such as filter presses (Novak et al., 1999).

The works of Severin et al. (Severin and Grethlein, 1996; Severin et al., 1999), and of Olivier et al. (2004) lead to the development of models describing the gravity drainage of activated sludge on belt presses, aiming at improved design of these devices. However, in both cases the cake compressibility was omitted. Most of the work carried out on sludge drying reed beds has been purely empirical and based on indirect sludge quality estimation using a capillary suction time (CST) method (Nielsen, 2003). It is the claim of the present authors,

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that design models for reed beds are insufficient, hence a more fundamental modeling approach has been taken. In a recent report (Christensen et al., 2010) we presented a comprehensive characterization of gravity drainage of compressible organic materials with a novel technique, revealing the importance of settling velocity of particles, low pressure filtrate expression, cake compressibility and cake collapse due to capillary forces. The model system used in that study was a suspension of dextran–MnO_2 particles, which had been reported as a good representative of compressible organic slurries and resembles physico-chemical properties of activated sludge (Hwang et al., 2006). A novel technique for the assessment of drainage properties of compressible organic materials was created. It was reported that pressures exceeding the critical pressure do not accelerate drainage, and load and feed concentration were identified as the key parameters deciding the drainage time and the final dry matter content of the cake.

The aim of this study was to adapt this new test method to the assessment of drainability of full-scale activated sludge in an easy, fast and repetitive manner and to assess the relationship between macroscopic variables such as the volumetric load and suspended solids on the parameters characterizing the drainability of the sludge, namely the time of drainage and cake dry matter content.

2. Theory of the drainage process

Gravity drainage process differs from constant pressure filtration in that the pressure decreases as the filtrate is expressed and that the cake is partly built up due to the settling process, so the rate of cake formation is not proportional to filtrate volume. In order to correctly describe the gravity drainage process, knowledge of settling velocity, specific cake resistance and cake compressibility is necessary.

Eq. (1) (Severin and Grethlein, 1996) describes the drainage rate through a filter cake.

$$v_d = \frac{\Delta P}{\mu(\alpha_{cake}\omega + R_m)} \quad (1)$$

It is important to remember that throughout this paper the term 'specific cake resistance' is used to describe the average specific resistance of the entire cake, and not its local values. Cake resistance values differ across a compressible cake and are the highest at the cake/filter interface. This inhomogeneity problem was solved by the introduction of the average specific cake resistance term defined as in the pressure filtration literature (Tiller and Yeh, 1987; Teoh et al., 2002). When filtering activated sludge in a constant pressure apparatus, the average specific cake resistance is denoted specific resistance to filtration (SRF) (Sørensen et al., 1996). In this study it is assumed that the average specific cake resistance is constant throughout the drainage experiment. In fact, resistance decreases during the initial part of the drainage process because the pressure difference across the cake decreases with sample level. In our previous report (Christensen et al., 2010), the compression of the MnO_2–dextran cake exerted by the high hydrostatic pressure present at the beginning of the drainage process was however demonstrated to be irreversible and, for this reason, changes in the average specific cake resistance as drainage takes proceeds can be neglected (Christensen et al., 2010).

The pressure difference, defined as stress on the medium surface, is given in Eq. (2).

$$\Delta P = \frac{Mg}{A} = cgh_0\left(1 - \frac{\rho}{\rho_s}\right) + \rho g h_t + \omega g \quad (2)$$

Settling velocity is not included in Eq. (2), but it still influences the drainage rate, because it regulates the deposited amount of solids (ω), and thereby the drainage rate. In order to determine the settling velocity and the specific cake resistance in the easiest way, the drainage process was divided into three stages, i.e., cake formation, pure filtration and cake collapse (Fig. 1). During the first stage, sludge particles settle and form a cake on the filter medium surface, leaving the clear water–air interface behind (stage A). This provides information on the settling velocity. When the settling is complete (time described as t_1), the sludge blanket level remains constant and the level of pure water decreases as filtrate is expressed (stage B), which reveals the data necessary to calculate the specific cake resistance as described below. At t_2 – *denoted as the time of drainage* – all the free water above the cake disappears, the cake starts to collapse slightly (stage C). It is important to remember that Eq. (2) can only be used to describe drainage until time t_2, when the drag forces not included in Eq. (2) start to rise to the cake surface.

The amount of deposited cake has a direct influence on the drainage rate and can be calculated according to Eq. (3).

$$\omega = \begin{cases} c(h_0 - h_t) + cv_s t & \text{stage A} \\ ch_0 & \text{stage B} \end{cases} \quad (3)$$

As indicated in Eq. (3), the settling velocity influences cake buildup and therefore indirectly influences the drainage process.

2.1. Cake formation

During the initial phase of drainage, when filtration cake develops, three phases can be distinguished, i.e., the cake itself, the sludge particle suspension and the clear liquid above the suspension. The total level of the sample in the cylinder is given as $h_t = h_w + h_s + h_c$. Since the particles settle, the clear water phase above the suspension develops, and the height of this water phase at a given time depends on settling velocity (Eq. (4)).

$$h_w = v_s t \quad (4)$$

The height of the clear water phase, which is the difference between the total level of the sample h_t, and the level of the sludge blanket h_s, is measured during the experiment and can be used to determine the settling velocity by revealing the speed at which the particles move away from the liquid surface. The settling velocity turned out to be constant during stage A in every experiment performed in this study.

2.2. Pure filtration

At the time described as t_1, settling is over and all sludge particles have formed a cake, the thickness of which remains

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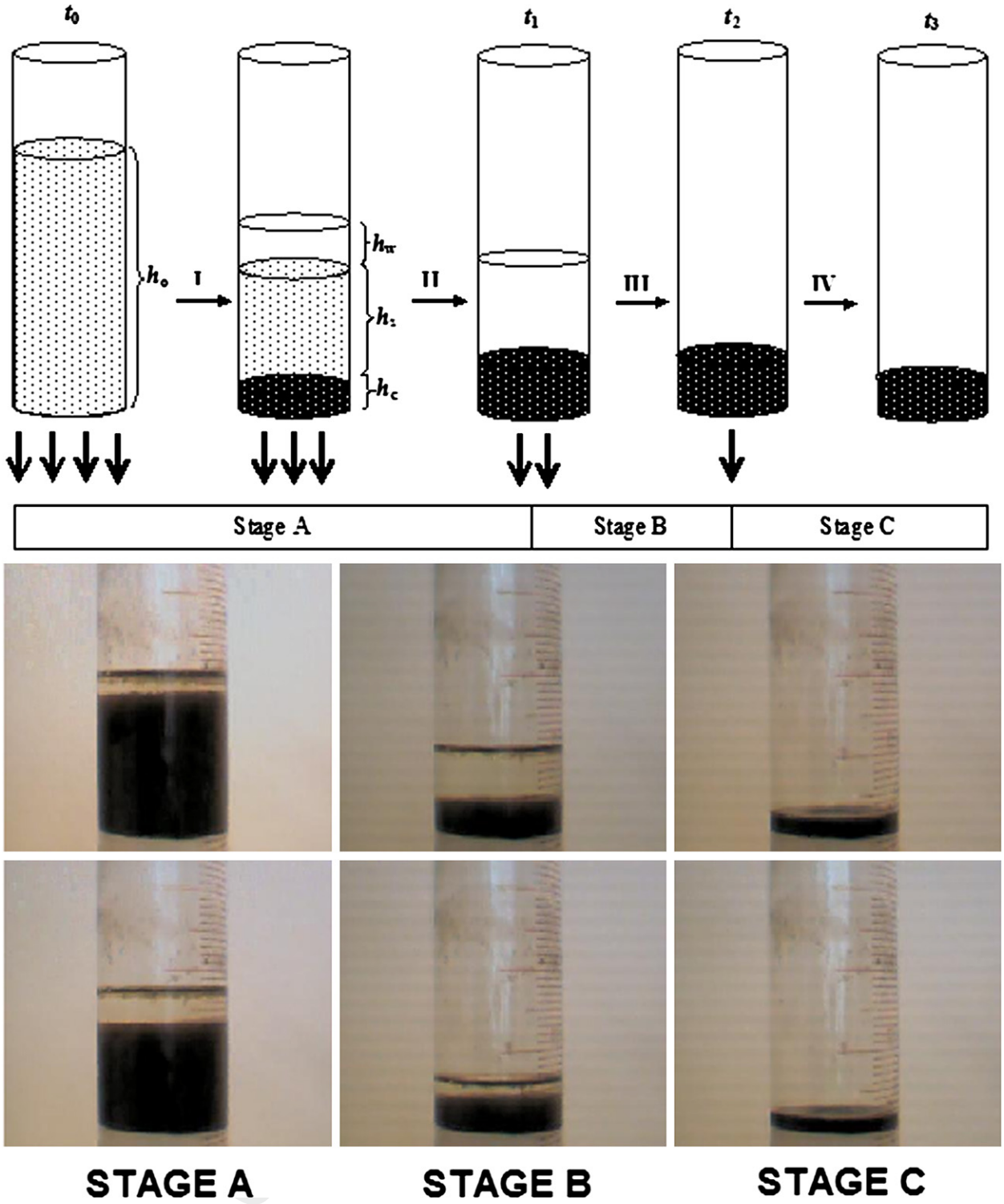

Fig. 1 – The three stages in gravitational drainage of activated sludge and other compressible organic slurries: cake formation (A), pure filtration (B), and cake collapse (C).

constant during stage B. This is a highlight of irreversible compression which took place in the beginning of stage A, when the pressure was the highest. The amount and consequently the height of deposited solids depend on the feed concentration and the volume of the sample used. Assuming that the pressure on the medium/cake interface is primarily an effect of the hydrostatic pressure component (simplified Eq. (2)), and that $\rho h_t >> ch_0(1 - \rho/\rho_s) - \omega$, it is possible to derive an equation describing the level of the sample during the entire stage of pure filtration, as in Christensen et al. (2010).

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$$h_t = h_t(t_1)e^{-\tau(t-t_1)} \quad (5)$$

where

$$\tau = \frac{\rho g}{\mu(ach_0 + R_m)} \quad (6)$$

Once t_1 and t_2 are identified from Fig. 2, and Eq. (5) is fitted to experimental data from stage B, τ can be determined from Eq. (5), providing α – the specific cake resistance – according to Eq. (6). The resistance of the filtration medium itself was neglected, since it was found that it accounted for less than 1% of total resistance in a separate experiment with pure water running through a clean filter medium (data not shown).

2.3. Cake collapse

At the time described as t_2, the air reaches the cake surface. Menisci are formed at the cake surface, which results in a drag on the cake surface, and the cake starts to collapse (Barr and White, 2006). This behavior has been documented by using dextran–MnO_2 particles instead of sludge whereby it is possible to measure the porosity profile through the cake during drainage (Christensen et al., 2010). Eq. (7) has been used previously to describe the cake compression of sludge at low pressure (Curves et al., 2009).

$$\varphi = \varphi_0\left(1 + \frac{p_s}{p_a}\right)^{\beta} \quad (7)$$

The compressive pressure p_s is a function of wet cake weight and thus a function of initial solids concentration and volume of the sample. At the end of the drainage process, the structure pressure (p_s) is mainly a function of the drag at the cake surface because the cake dry weight is low compared with weight of the wet cake. For that reason, the porosity is constant through the cake as also shown experimentally (Christensen et al., 2010). The structure pressure is proportional with the wet mass of the cake because the drag at the surface arises from the weight of the liquid within the cake. Hence for practical use Eq. (8) can be used.

$$\varphi = \varphi_0\left(1 + \frac{M}{Ak}\right)^{\beta} \quad (8)$$

The final dry matter content is expected to increase with initial solids concentration and initial load, as the wet cake weight increases with solids concentration and load.

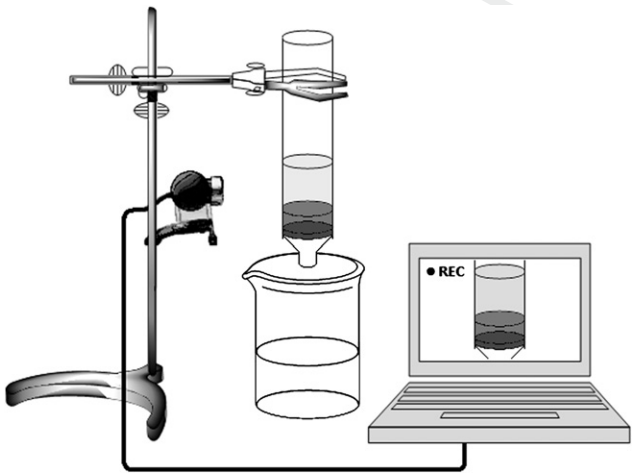

Fig. 2 – Experimental setup used in drainage experiments. Beaker collects the filtrate drained from the tube through the filtration medium. A web camera records a film illustrating the drainage and settling processes. Data from the web camera is recorded by a laptop computer.

3. Materials and methods

3.1. Samples of activated sludge

All the experiments were performed on samples of mixed liquor activated sludge from the aeration tank of the Aalborg East Wastewater Treatment Plant in Aalborg, Denmark. It is a Biodenipho plant with biological N and P removal. The suspended solids (SS) contents of the samples were adjusted by dilution with supernatant originating from the same batch of activated sludge, or by the removal of supernatant after 30 min of settling. The dry matter contents (SS) of activated sludge and filtration cakes were determined according to standard methods (APHA, 2005) by overnight weight loss at 104 °C.

3.2. Filter medium choice

Four different filtration media of different cut-off values were tested in drainage experiments in order to assess the influence of filtration medium on the experimental outcome. The media used were the following: Kemira cloth (Kemira, Denmark, cut-off – 200 μm), polyester fabric (cut-off – 40 μm), Whatman 41 paper (Whatman, UK, cut-off – 20–22 μm) and Whatman 40 paper (Whatman, UK, cut-off – 8 μm). In each case a 200 ml sample of activated sludge originating from the same batch was used (source – Aalborg East WWTP, SS – 4.9 g/L). Due to its high cut-off value, Kemira cloth turned out to let sludge particles through in the initial phase of the experiment (9–10 s) until the cake buildup was complete. For this reason the filtration cake was thinner and comprised larger particles. This resulted in lower resistance values as compared to other media, for which this phenomenon was not observed. The other three media behaved very similarly and yielded cakes of similar resistance values. Whatman 41 paper was chosen for further experiments, due to the fact that it is routinely used for the examination of activated sludge in SRF experiments (Christensen and Dick, 1985), and that it is capable of retaining nearly all particles, thus creating a complete cake.

3.3. Gravity drainage and sedimentation

The experimental setup (Fig. 2) was the same as described in Christensen et al. (2010) and consisted of a transparent glass cylinder (inner diameter of 60 mm), a filter medium closing one end of the pipe and supported by a funnel located inside the tube, and a laptop computer connected to a web camera. The filter was mounted on one end of the cylinder, which was then mounted vertically above a beaker. A sludge sample of a given

volume was introduced into the cylinder and gravitationally drained of water into a beaker, while the web camera filmed the process at a specified frame rate. After completion of the experiment, the film sequence was divided into single frames, and each frame was analyzed separately with ImageJ software (http://rsbweb.nih.gov/ij). The positions of sludge blanket and liquid surface (Fig. 3) as functions of time were recorded and imported to a spreadsheet for further data handling.

Evaluation of the repetitiveness of the results provided by the experimental technique was carried out by measuring the specific cake resistance (α) five times on the same sample of untreated activated sludge from Aalborg East WWTP (SS of 5.1 g/l). The mean specific cake resistance turned out to be 4.8×10^{10} m/kg, with a standard deviation equal to 5% of the mean value, showing that the experimental technique is repetitive and reliable.

3.4. *Analytical centrifugation*

Analytical centrifugation was used to study compressibility of sludge cakes (Lumiziser 613 Dispersion Analyser from L.U.M. GmbH, Berlin, Germany). For each test, 2 ml of activated sludge were added to rectangular cuvettes (10 mm). Five samples with varying amounts of dry matter content were analyzed. The raw sludge sample was diluted by filtering the sludge – the filtrate was used to vary the dry matter content of the samples from 5 to 25 g/L. The experiment was operated at 1000 rpm for 2000 s, 4000 rpm for 2000 s and then lowering the rotation speed to 1000 rpm for 2000 s. The height of the formed cake was measured during the experiment and the structure pressure within the cake was calculated using the method described in Sobisch et al. (2006). The cake height depended on both the rotation speed and the dry matter content of sludge.

3.5. *Determination of settling velocity and specific cake resistance*

Calculations of the settling velocity and specific cake resistance were based upon the changes of clear water phase height (h_w) over the drainage process, which initially increases linearly until time t_1, and then decreases until time t_2 (Fig. 4). The settling velocity was determined as the slope of the line fitted to data from stage A, according to Eq. (4), and it turned out to be 1.8×10^{-5} m/s for a representative experiment. This value was identical in a comparative settling experiment on the same sample of sludge, when draining was absent, and therefore the concept of zone settling, assuming the equal settling velocity of all particles, was applied.

Cake compression was irreversible, no cake swelling was observed during the drainage experiment even through the pressure declines during stage B as confirmed from the analytical centrifugation experiment. Further, two drainage experiments were performed, one with simultaneously settling and drainage, and one where all particles were settled before the drainage was started. No significant difference in the determined average specific cake resistance was found. Thus, cake compression was irreversible and the average specific cake resistance was constant during stage B. This implies that the assumption hitherto stated was validated, and changes in the average specific cake resistance as drainage takes proceeds can be neglected.

Specific cake resistance is a parameter describing the difficulty in dewatering a given sample. Typical values of specific cake resistance for activated sludge, determined by constant pressure filtration with pressure of 1–2 bar, are of the order of 10^{12} m/kg (Rasmussen et al., 1994). The technique described in this study allows for the determination of specific cake resistance during low pressure gravity drainage. In a representative experiment, the specific cake resistance during gravity drainage was 4.2×10^{10} m/kg, which is much lower than those usually found in SRF experiments. The much lower values of average specific cake resistance originate from much lower pressures encountered in gravity drainage resulting in lower cake compression and lower cake permeability loss.

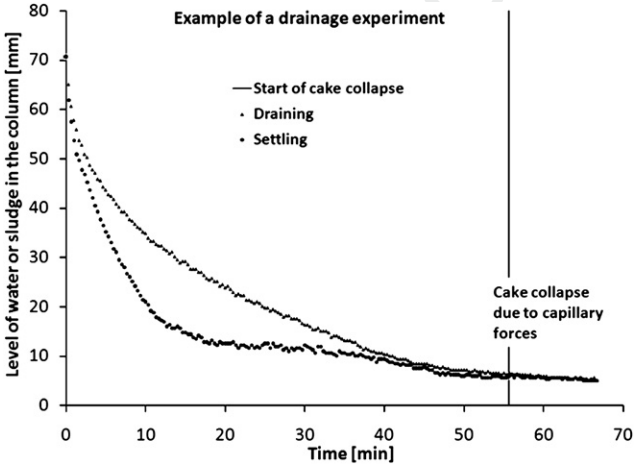

Fig. 3 – Settling and draining curves for a typical drainage experiment. Sludge sampled in Aalborg East WWTP, SS – 4.8 g/L.

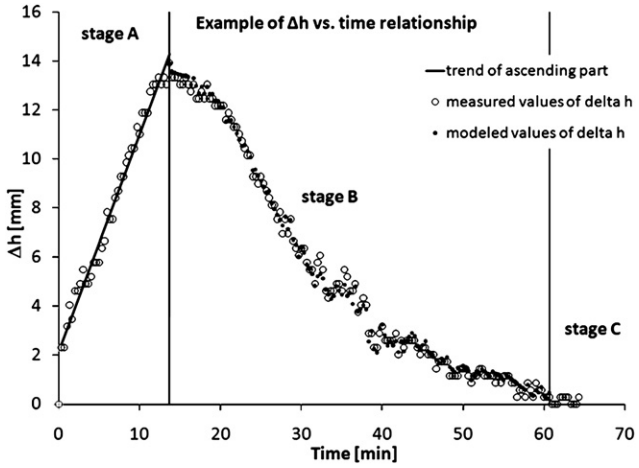

Fig. 4 – Example of a relationship between the time and the difference between liquid level and sludge blanket level. The slope of the ascending part provides the settling velocity of sludge, and the function fitted to the descending part provides information on the specific resistance of the filtration cake.

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3.6. Shear experiments

Shear experiments were performed in order to investigate the influence of different treatments on the quality of activated sludge in terms of drainability, as described by the specific cake resistance. A batch of fresh activated sludge from Aalborg East WWTP was divided into four samples. One sample was used directly for measurements of specific cake resistance in fresh sludge, the second was stored anaerobically at room temperature for 24 h, the third was sheared for 6 h at the rate of 300 rpm in an aerated reactor and the fourth sample was sheared for 6 h in an identical reactor, but at the rate of 800 rpm. Shear experiments were performed according to Klausen et al. (2004).

4. Results

4.1. The effect of volumetric load on drainage

Five different volumes (100–400 ml) of mixed liquor activated sludge, originating from the same sample batch, were tested for their drainage properties. The settling velocity, the specific cake resistance, water content of the filtration cake and the time necessary for the liquid above the cake to drain were determined for each sample (Fig. 5). The relationship between the volumetric load of the sample and the time of drainage showed that t_D depends on the volumetric load squared. (Fig. 5a). Specific cake resistance, given by the α value, increased linearly with the volume of sample drained (Fig. 5b). Settling velocity was independent of the volumetric load (Fig. 5c). The total solids content of the cake increased when the volumetric load increased, which is discussed below.

4.2. The effect of sludge concentration on drainage

The influence of sludge SS concentration on drainage dynamics was assessed in an experiment with five different SS contents (2.7–6.7 g/L). The specific cake resistance, settling velocity, water content of the filtration cake and the time necessary for the liquid above the cake to drain were determined for each sample (Fig. 6). The relationship between the SS content and the resulting time of drainage appeared clearly linear (Fig. 6a). The values of the specific cake resistance (α) tended to remain constant as SS was changed (Fig. 6b). An interesting behavior could be noticed in the case of settling velocity values, which significantly dropped as the SS concentration was increased (Fig. 6c). This was attributed to the hindered settling effect. The Vesilind equation (Vesilind, 1968) was fitted to the relationship between the SS concentration and the settling velocity, providing the Vesilind maximum settling velocity of 1.75 m/h and the Vesilind parameter of 0.58 m^3/kg.

4.3. The effect of various sludge treatments on drainage characteristics

Specific cake resistance is a parameter which describes the quality of sludge with respect to dewaterability. In pressure filtration studies, the average specific cake resistance correlates with floc size and strength. The objective of this investigation was to determine whether the same effect can be observed in gravity drainage. Some of the well-described deflocculating factors are shear and anaerobic conditions

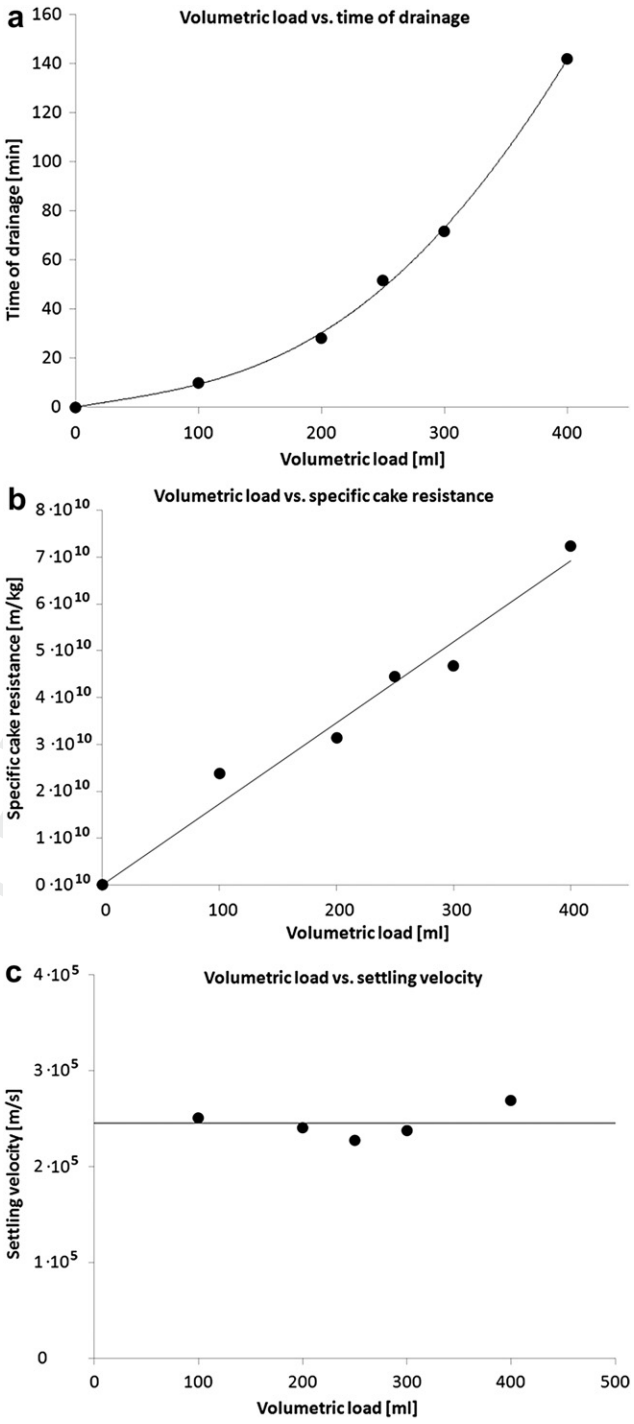

Fig. 5 – Results of the drainage experiments with five different volumes of activated sludge (source – Aalborg East WWTP, SS – 4.7 g/L). (a) Relationship between volume of sludge sample tested and the time of drainage. (b) Relationship between the volume of sludge sample tested and the specific cake resistance. (c) Relationship between the volume of sludge sample tested and the settling velocity of sludge particles.

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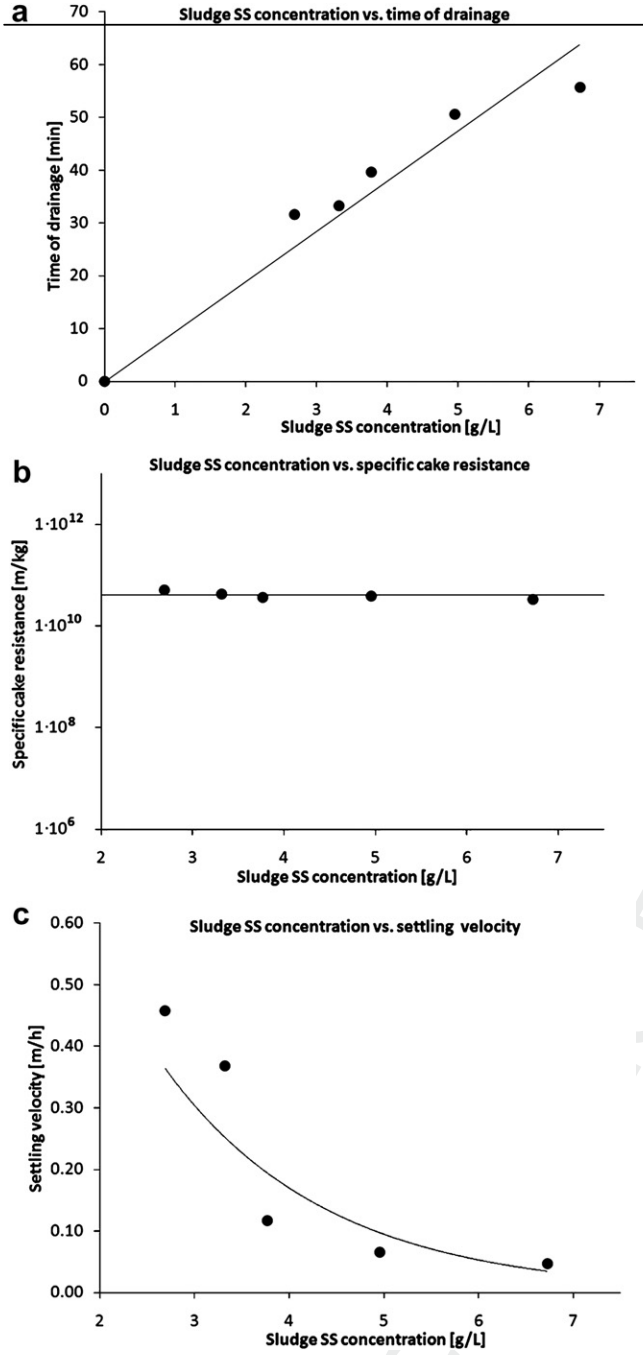

Fig. 6 – Results of the drainage experiments with five samples of sludge with different SS contents (source – Aalborg East WWTP). (a) Relationship between the SS content of the sludge sample tested and the time of drainage. (b) Relationship between the SS content of the sludge sample tested and the specific cake resistance. (c) Relationship between the SS content of the sludge sample tested and the settling velocity.

(Wilen et al., 2000; Nielsen et al., 2004). In order to assess the effect of shear and anaerobic conditions on the quality of sludge in terms of drainability, which is reflected by the specific cake resistance, a series of experiments was run. A batch of fresh activated sludge from Aalborg East WWTP (SS content – 5.1 g/L) was divided into four samples. For each of the samples three volumes were drained, which resulted in four distinct linear relationships between volumetric load and specific cake resistance, one for each sample subjected to different treatments (Fig. 7).

Shearing at the rate of 300 rpm caused a mild effect, consisting in the decrease in drainability, described as the slope of load vs. specific cake resistance relationship. A stronger effect was observed by a 24 h anaerobic storage, but the most significant drop in drainage speeds resulted from vigorous shearing at 800 rpm. The most probable reason for these phenomena is that both shear and anaerobic conditions have a deflocculating effect on activated sludge, resulting in floc fragmentation and liberation of small cell aggregates and single cells (Rasmussen et al., 1994). Higher resistances observed with deflocculated sludge can be attributed to small particles clogging, or blinding, the pores inside the cake, causing slower water flow and more significant cake compression due to liquid pressure.

4.4. *Effects on cake dry matter content*

The cake volume as well as the cake dry matter increases when the volumetric load and/or the suspended solid concentrations are increased. These observations might by merged according to Eq. (8). By expressing the mass of solids (M) as the volumetric load multiplied by the suspended solids concentration, and by substituting the solid volume fraction by cake dry matter content of the cake, the results of both experiments may be presented as shown in Fig. 8. The data are fitted by the rewritten version of Eq. (8), shown as Eq. (9).

$$DM_{cake} = DM_{cake,0} \cdot \left(1 + \frac{V_{load} \cdot SS}{A \cdot k}\right)^{\beta} \quad (9)$$

From Fig. 8 it can be seen that the cake solids are compressed as the dry matter load is increased. It may however also be noted that the solids compression has not reached a maximum (a plateau).

Cake compression was further studied by using analytical centrifugation. A cake was formed at low rotation speed;

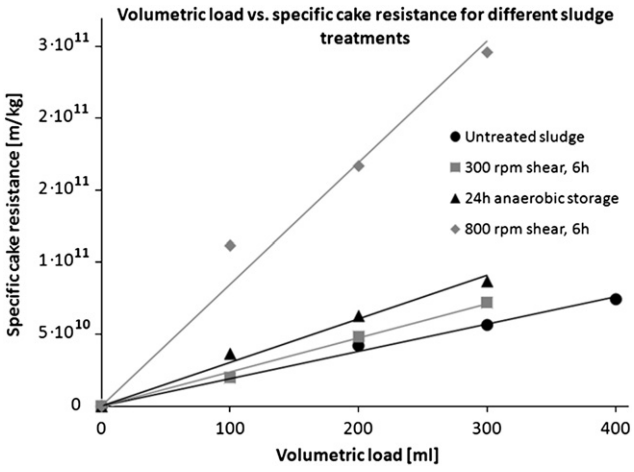

Fig. 7 – Results of the drainage experiments comparing the effect of 24 h anaerobic storage of activated sludge, shearing at 300 rpm and shearing at 800 rpm to the drainage properties of activated sludge from a single sample batch.

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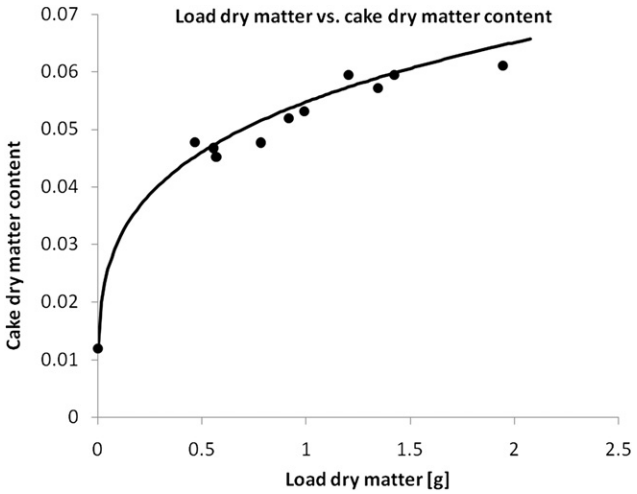

Fig. 8 – Relationship between the load dry matter content and cake dry matter content for combined data from experiments with varying loads and varying concentrations. Curve is fitted according to Eq. (9).

hence, the compression pressure through the cake was low. The rotation speed was then increased and it was observed that the cake collapses. By lowering the rotation speed after the collapse, it was possible to measure the degree of the cake reswelling. Fig. 9 shows data from the experiment. The cake height did not increase proportional with dry matter load because the cake was compressible cf. Fig. 8. Further, reswelling of the cake was below 20% of the compression and the assumption that cake compression was irreversible is found reasonable. Hence, if a sludge cake is compressed, it is not possible to reestablish the permeability of the cake by lowering the pressure.

5. Discussion

The present work demonstrates an experimental device for characterizing the drainage process by identifying the

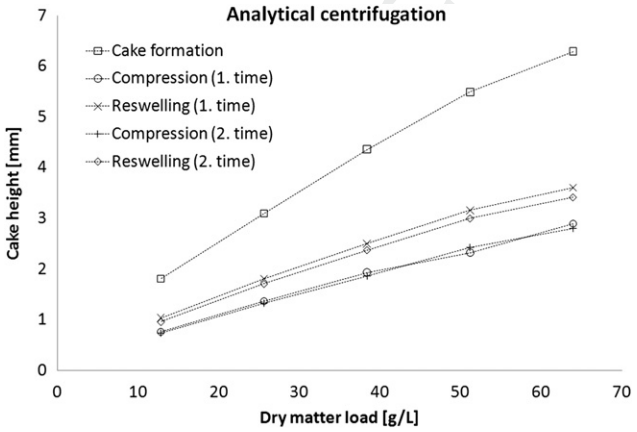

Fig. 9 – Results of analytical centrifugation experiment where the cake height is measured at different rotation speed (1000 rpm and 4000 rpm) and feed concentration.

sedimentation and filtration features in one experimental run. It was found the drainage process may by described by 3 stages: A – the initial sedimentation stage, B – the filtration step and C – the consolidation stage. The interpretation of the experimental data was made according to the model devised by Christensen et al. (2010).

It was found that the model, which originally was developed on MnO_2–dextran particles, adequately described the sludge data produced in this work (Fig. 2) An implication of this is that even at the low pressures encountered in drainage (0–2000 Pa), the compressibility of the sludge has to be included in the model. The compressible nature of sludge is reflected in the cake resistance. In the drainage experiments the specific cake resistance was found in the order of 10^{10} m/kg, whereas this value is in the order of 10^{12}–10^{13} m/kg in pressurized filtrations of unconditioned sludge. Indeed, the lower cake resistance makes drainage less costly than pressurized dewatering in terms of energy consumption, however at the expense of the cake dry matter, which for drainage is typically 4–6% compared to 13–17% for pressure dewatering.

When compressibility is a dominant feature, the dewatering is a non-uniform process rendering higher solid content in the bottom than in the top of the filter cake. This implies that a full description of the process requires a description of local properties such as flow, resistance, solid content. This in turn requires the use of numerical modeling of the process, which most often is a cumbersome affair. The authors set out to investigate how macroscopic process variables such as volumetric load, suspended solid content and sludge quality influenced the result of the drainage process expressed by time of drainage and cake dry matter.

It was found that the time of drainage depended on the volumetric load square ($t_D \propto V_{load}^2$). This experimental result was confirmed by the model of Christensen et al. (2010). As the load increases, the hydrostatic pressure on the formed cake increases, hence due to cake compression the cake resistance increases. This was confirmed by the linear increase in the specific cake resistance as a function of the volumetric load (Fig. 5b). When the sludge physical structure was deteriorated by e.g., intensive shearing or exposure to anaerobic conditions, the state of flocculation was altered. This implies an increased compressibility. The simple relations between drainage time and specific cake resistance versus volumetric load were however maintained, but the proportionality constants changed with the change of sludge structure: the more deflocculated the sludge, the higher the specific cake resistance and subsequently the longer the drainage time.

As the solids content is increased, maintaining the same volumetric load, the filtration properties expressed as the specific cake resistance are not altered, which results from the same hydrostatic pressure. However, a linear dependency of the drainage time on the solid content was found. The increase in solid content will increase the cake height, which again will increase the time of drainage. The formation of the cake is an increasingly slower process as the solid content is increased. This is seen on Fig. 6c, where v_s is depicted as function of solid content. As predicted by Vesilind, v_s decreases as solid content is increased. The impact of settling was discussed in Christensen et al. (2010). The cake dry matter (the solid volume fraction of the filter cake) was in Fig. 8 shown to depend on the

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dry matter load. As the dry matter load is proportional to the structure pressure in the cake, the fitting parameters obtained in Fig. 8 correspond to those obtained by the use of Eq. (7), namely: φ_0, p_a and β values at 0.012, 0.15 and 0.25, respectively. This corresponds very well to the findings of Curves et al. (2009), who found the parameters to be equal to 0.0095, 0.3 and 0.25, respectively. This means that the compression of activated sludge filtration cakes at low pressures can be accurately predicted by Eq. (7) and that this equation can be effectively used to describe data obtained with the experimental technique presented in this study, which in practice is easier to perform and requires much simpler equipment than the technique presented in Curves et al. (2009).

It is also important to note that in contrast to Sørensen and Sørensen (1997) the compression did not reach a steady state (plateau). They studied dead end filtration at low pressures, yet still pressures vastly exceeding those encountered in this study. This implies that the sludge is not completely collapsed in the gravity drainage and thus a filter skin, as known from pressure filtration, has not been formed. This explains why average values of resistances and cake dry matter are meaningful in this context, whereas they hardly are so in pressurized dewatering of compressible sludges.

6. Conclusions

- For gravity drainage, the specific resistance of the cake depends directly and linearly on the volumetric load of the sample, due to the compressible nature of activated sludge flocs which tend to produce more compact cakes when pressure of the liquid is higher.
- The final dry matter content of the cake is a function of both feed load and concentration (Eq. (9)). It is therefore of primary importance to choose the drainage conditions (feed volumetric load and concentration) carefully, so that the effect of the gravity drainage process is satisfactory, both in terms of the total drainage time and the final cake water content.
- The experimental technique is very simple, but very well capable of determining the settling velocity, average specific cake resistance and cake compressibility, and it provides a very good understanding of the process of gravitational drainage of activated sludge. Due to the compactness of the setup and the ease of operation, this technique could be applied to rapidly assess the quality of sludge in terms of drainability prior to its application to dewatering devices such as belt presses, or on sludge dewatering reed beds. Especially in the case of reed beds, the benefits would be immense, since the operational failures due to sludge overdosing could be avoided.

Nomenclature

List of symbols

A	cross-sectional area of the cylinder (m^2)
c	particle concentration in the feed (kg/m^3)
DM_{cake}	dry matter content in cake (–)
$DM_{cake,0}$	dry matter content in cake before compression (–)
g	gravitational acceleration (m/s^2)
h_0	initial level of the suspension (m)
h_c	height of the cake (m)
h_s	distance between cake surface and sample–water interface (m)
h_t	actual level of the suspension (m)
h_w	height of the clear water phase (m)
k	constant (m^2/kg)
M	mass of the sample (kg)
P	pressure (Pa)
p_a	fitting parameter in Eq. (7)
p_s	solids pressure (Pa)
R_m	media resistance (m^{-1})
SS	suspended solids (kg/m^3)
t_D	time of drainage (s)
V_{load}	volumetric load of the sample (m^3)

Greek symbols

α	specific resistance to filtration (m/kg)
β	fitting parameter in Eqs. (7) and (8)
ρ	density of the filtrate (kg/m^3)
ρ_s	density of the particles (kg/m^3)
τ	characteristic drainage time defined in Eq. (6) (s)
v_d	drainage rate (m/s)
v_s	settling velocity (m/s)
μ	filtrate viscosity (Pa s)
φ	solids volume fraction
φ_0	solids volume fraction for p_s equal to 0
ω	amount of deposited material per unit area of media (kg/m^2)

Parameter values

A	0.002827 m^2
p_a	0.15
β	0.25
μ	0.001 Pa s
g	9.81 m/s^2
ρ	987 kg/m^3

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Research paper 3

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Sludge quality aspects of full-scale reed bed drainage

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Abstract

Sludge drying reed beds can be a cost-effective and sustainable solution to surplus activated sludge dewatering and mineralization, especially for small wastewater treatment plants. However, the simplicity as well as low energy and monitoring requirements of this technology are often counterbalanced by frequent operational problems consisting of slow and insufficient dewatering, poor vegetation growth, odor, and overall poor mineralization of the sludge residues. The main reason is that the general rules for facility design and operation are based on empirical experience rather than on the actual and current sludge parameters. In this study a new method for the assessment of activated sludge drainage properties has been applied to determine the reasons behind operational problems faced by the operators of reed bed facility accepting surplus activated sludge from two wastewater treatment plants in Esbjerg, Denmark. The importance of sludge quality monitoring as well as the damaging effect of shear forces, oxygen depletion, and long-distance sludge transportation were demonstrated. Finally, more general guidelines for reed bed facility design and operation are given, based on experimental data from seven full-scale plants.

1. Introduction

Sludge drying reed beds can be a cost-effective and sustainable solution to surplus activated sludge dewatering and mineralization (Maeseneer, 1997). They have been widely applied in Denmark since 1988, where approx. 95 systems existed in 2002 (Nielsen and Willoughby, 2007). They have also become widespread in most of Europe (Haberl *et al*., 1995). Sludge volume reduction takes place due to both water drainage and plant-driven evapotranspiration, and mineralization of organic matter (Aagot *et al*., 2000). Simultaneous degradation of hazardous organic compounds and pathogen reduction occurs, which allows for the application of sludge residues in agriculture (Nielsen, 2005a).

However, the simplicity as well as low energy and monitoring requirements of this technology are often counterbalanced by frequent operational problems, consisting of slow and insufficient dewatering, poor vegetation growth, odor, and overall poor mineralization of the sludge residues (Nielsen, 2005b). Studies by Nielsen (Nielsen, 2002, 2003, 2005b) have led to the definition of certain guidelines for facility design and operation, all based on the capillary suction time (CST) as a measure of sludge dewatering characteristics at low pressures normally found on reed beds.

However, the operation of reed beds is generally only based on empirical experience and prescribed guidelines such as the dry matter loading limit of 60 kg dry matter/m^2/year, rather than on any sludge characteristics. For this reason, operational failures in reed bed facilities occur quite often and account for this technology's reputation of being unpredictable (Maeseneer, 1997). Although Nielsen's choice of the CST technique was correct from the viewpoint of low pressure compressibility of sludge, this method does not directly take into account the solids content of sludge, which has been shown to be one of the critical factors that decide the drainage rate and final cake water content (Dominiak *et al*., 2011). Furthermore, the measurement of cake compressibility with CST is impossible, so the actual hydraulic resistance cannot be calculated, and the drainage process cannot be correctly assessed or modeled.

In a recent study, we presented a novel technique for the determination of drainage properties of activated sludge during gravity dewatering (Dominiak *et al*., 2011), which we named the Specific Resistance to Drainage (SRD) method. This technique considers the settling velocity of sludge particles, the SRD value and compressibility at low pressure. It was found that the volumetric loading of sludge was most critical to the drainage rate as cake compressibility caused SRD to increase proportionally to increasing load (i.e. increasing pressure). It was also found that the compressibility depends on sludge properties and conditions and that treatments promoting sludge deflocculation, such as anaerobic storage and shear, worsen the drainage properties by increasing SRD under constant load. It is well known that anaerobic conditions, changes in microbial aerobic metabolism, and shear stresses all can cause deflocculation of activated sludge floc (Morgan-Sagastume *et al.*, 2003; Wilen *et al.*, 2000; Bruus *et al*., 1993) and eventually lead to changes in the normal high pressure dewatering (Bruus *et al*., 1992) These phenomena are expected to be even more pronounced in drainage of activated sludge, where both floc-settling velocity and drainability may be affected. Since the most common reason for poor dewatering in vertical flow reed beds is substrate clogging, believed to originate from the accumulation of suspended solids and their compaction (Platzer and Mauch, 1997; Langergraber *et al*., 2003), sludge handling prior to its application to reed beds appears to be critical to fast, efficient, and reliable operation of these facilities. Understanding the mechanisms behind reed bed operating problems and application of the SRD methodology presented in this paper should lead to improvements in the operation of sludge-drying reed bed facilities and, eventually, increase the reliability and competitiveness of this simple, sustainable, and cost-efficient technology.

In this study, a case story of two Danish wastewater treatment plants sharing a reed bed facility is presented. One treatment plant is located next to the reed beds, whereas the other is required to pump surplus sludge to the facility through a long pipeline. The initial observation that sludge from the distant treatment plant caused frequent dewatering problems in the reed beds, while sludge from the nearby plant did not, inspired the investigation of the reasons behind the dewatering problems and the factors of importance to effective and reliable operation of reed bed facilities.

The aims of this study were to investigate the reasons behind the operational problems faced by Esbjerg reed bed facility, and to test the hypothesis that the sludge gravity drainage characteristics depend on floc properties, and that deflocculation of activated sludge is responsible for the deterioration of these properties in both lab scale and full scale. Furthermore, by studying sludge quality variations in a number of full-scale activated sludge treatment plants, we wanted to find more general guidelines for reed bed facility design and operation.

2. Materials and methods

2.1. Site presentation

Two wastewater treatment plants located in Esbjerg in south-west Denmark by the North Sea were studied, Esbjerg East (design persons equivalents (PE) of 125,000) and Esbjerg West (PE of 290,000). Both plants perform nitrification and denitrification, as well as both biological and chemical phosphorus removal. The fraction of industrial wastewater in the influents of both plants is approx. 66% (by COD).

The reed bed facility, used for handling surplus sludge from the two treatment plants, is composed of 24 basins, each with an approximate area of 2,200 m^2 (Fig. 1). All basins are covered by vegetation of common reeds. Activated sludge from the aeration tank of each treatment plant (SS of 3 to 5 g/L) is pumped into a separate storage tank equipped with an aeration system and allowing calcium carbonate dosing. The distance between plant Esbjerg East and the reed bed facility is approx. 1,400 m, whereas plant Esbjerg West is located approx. 6,300 m away with a transportation time of about 6.5 hours. Table 1 presents the loading schemes for the reed bed basins. The lower loadings of sludge from Esbjerg West were implied by frequent operational failures consisting of sludge overflows and odor problems, but even with smaller portions, drainage takes much longer

than for sludge from Esbjerg East. Examination of reed bed residues in basins treating sludge from Esbjerg West revealed the presence of dark, dense, and sticky residue layers.

2.2. Samples of activated sludge

Samples of activated sludge for SRD determination were taken from aeration tanks from both Esbjerg wastewater treatment plants, the pipeline transporting sludge from Esbjerg West, and from the storage tank. Samples of mixed-liquor-activated sludge for SRD determination were also taken from aeration tanks of five other Danish wastewater treatment plants. The suspended solids (SS) and dry matter contents (DM) of activated sludge and filtration cakes were determined according to standard methods (APHA, 2005) by overnight weight loss at 104°C. Microscopic analysis of floc morphology and filament index (0-5 scale) was carried out with a light microscope and Eikelboom's manual for microscopic investigation of activated sludge (Eikelboom, 2000). SVI measurements were made by 30 min sludge settling in a 1L graded cylinder.

2.3. Gravity drainage experiments

Measurements of SRD and settling velocity were performed as previously described (Dominiak *et al*., 2011). A sample of activated sludge was introduced into a vertical transparent tube with a paper filter as a plug. The drainage process was recorded by a camera at a specified frame rate. The images were analyzed to determine the height of the clear liquid phase (h) that developed above the suspension during drainage. Initially, h increases due to particle settling and the settling velocity was determined from this increase i.e. $v_{sed} = dh/dt$. After the cake was fully developed at time t^*, h started to decline again. The following equation derived in Christensen *et al* (2010) was then fitted to experimental data and used to calculate SRD:

$$h = h^* e^{-\frac{(t-t^*)\rho g}{c h_0 \mu \cdot SRD}}$$

where ρ is the liquid density, g the gravity coefficient, c the dry matter content of the feed, h^* the initial height of the suspension, and μ is the viscosity. Time of drainage was determined as the point where 90% of the sample was drained. All the experiments were performed on site, immediately

after sampling, because it had been determined earlier that sludge transportation affects SRD negatively (data not shown). In each case, 200 ml of sludge were drained, and the dry matter content of filtration cakes was determined by measuring weight loss after drying at 104°C overnight.

2.4. Shear experiments

Shear experiments under aerobic, anoxic, and anaerobic conditions were performed on samples of activated sludge from Esbjerg East and West in order to simulate the effect of pumping and oxygen availability on the drainage properties of sludges. In each case, a 1-liter baffled reactor containing 600 ml of activated sludge was used, the shear rate was set to 300 rpm, and the experiment lasted for 6 hours (Klausen *et al*., 2004). Anaerobic and anoxic conditions during shear experiments were assured by seal-closed reactors and nitrogen gas bubbling, with addition of sodium nitrate (final concentration 15-20 mgN/L) and regular nitrate and nitrite monitoring with paper tests in case of anoxic trials.

3. Results and discussion

3.1. Esbjerg case study

3.1.1. Determination of SRD in Esbjerg treatment plants

The problems with sludge draining and mineralization were only noticed in reed bed basins handling sludge from Esbjerg West, but were not reported in basins handling sludge from Esbjerg East. In order to unveil the reason behind these differences, sludge quality in terms of drainage was measured by SRD on activated sludge from the aeration tanks of both treatment plants. The SRD of sludge from Esbjerg West was $1.4 \cdot 10^{10}$ m/kg, and was lower than in Esbjerg East with a value of $2.5 \cdot 10^{10}$ m/kg (Fig. 2). Lower SRD implies faster drainage, so the measured values for both plants contradicted the reports on operational failures.

SRD was also measured at the end of the transportation pipeline connecting Esbjerg West and the reed bed facility and in the storage tank with sludge from Esbjerg West after calcium carbonate

addition and prior to basin application (Fig. 2, black bars). Pumping of sludge over 6.3 km and lasting approx. 6.5 hours more than tripled the initial SRD value found in Esbjerg West. Addition of calcium carbonate (a common flocculant) to the sludge restored its drainability significantly, leaving the SRD value at approx. double the initial value found in the plant. The improvement caused by calcium carbonate indicated that the loss of drainability could originate from sludge deflocculation caused by shear due to pumping and extended anaerobic conditions. This assumption led to the hypothesis that sludge gravity drainage characteristics depend on floc properties in a similar way as does pressure dewaterability (Bruus *et al.*, 1992), and that deflocculation of activated sludge during transportation was responsible for the deterioration of the drainage properties.

3.1.2. Simulation of pumping and oxygen depletion

In order to test the hypothesis that the combination of shear and anaerobic conditions was responsible for sludge deflocculation and the resulting increase of SRD, drainage experiments were performed on sludge sample subjected to simulated pumping. In order to estimate the contribution of oxygen depletion to the overall loss of drainability, shear experiments under aerobic and anoxic conditions were performed at the same shear rate (Fig. 2, gray bars). In each case, shear caused a significant deterioration of sludge draining properties. Although the initial values of SRD of sludges from both plants were almost identical, sludge from Esbjerg East appeared to be much more susceptible to quality loss. In both cases, shear combined with anaerobic conditions caused the most damage to sludge drainability, whereas anoxic and aerobic conditions, respectively, limited the severity of SRD loss due to shear. It is interesting to note that the simulated pumping, which consisted of shearing under anaerobic conditions, raised the SRD value to almost that found at the end of the transportation pipeline (10% difference). This suggests that the set of conditions chosen for simulation of pumping reflected the actual situation quite accurately and that, most probably, shear and anaerobic conditions were responsible for sludge quality loss during its transportation in Esbjerg. Shear and anaerobic conditions had earlier been shown to worsen activated sludge quality, presumably through the lack of aerobic microbial activity, or by anaerobic respiration and reduction of trivalent iron (Bruus *et al.*, 1992; Wilen *et al.*, 2000). These experiments clearly show that such deflocculation has a substantial worsening effect on low-pressure drainage of activated sludge.

3.1.3. Remedies for sludge quality loss due to pumping

Several strategies for overcoming the difficulties with sludge dewatering on reed beds have been proposed to Esbjerg facility operators. The positive effect of calcium carbonate addition was verified, and this strategy is continuously applied. Furthermore, as in this study nitrate was shown to minimize the negative effects of anaerobic conditions, addition of nitrate to the pumped stream of surplus sludge from Esbjerg West was tested over a period of several months. Addition of approx. 15 mgN/L ensured that nitrate was still present after 6-7 hours pumping (5-7 mgN/L), and this combined treatment (calcium carbonate to pH 8 and nitrate addition) significantly improved the drainage situation by reducing the operational failures on reed beds, as indicated by the empirical experience of facility operators. These findings confirm the experimental evidence that shear and anoxic conditions cause less damage than the same shear imposed on sludge under anaerobic regime. A laboratory trial of anaerobic sludge deflocculation and subsequent aerobic reflocculation was performed on sludge samples from both Esbjerg plants (data not shown). The positive effect of extended aeration (6 hours) was only noted in connection with sludge from Esbjerg East, whereas the same treatment caused further drainability loss in sludge from Esbjerg West.

3.2. Survey of sludge drainage properties in Danish wastewater treatment plants

A number of SRD measurements on activated sludge from Aalborg East, Esbjerg East, and Esbjerg West wastewater treatment plants during a period of approx. two years showed a fairly constant level over time for each treatment plant (data not shown). In order to find out more about the variation in activated sludge drainage properties among different treatment plants, we performed a survey in seven Danish wastewater treatment plants representing different design types. The SRD value of samples in the aeration tanks analyzed on site turned out to be very different and ranged from $0.5 \cdot 10^{10}$ to $4.2 \cdot 10^{10}$ m/kg (Fig. 3). These differences are significant and clearly show that the drainage properties - thus the potential for using reed beds for dewatering – vary greatly among different wastewater treatment plants.

So far it is unknown why the drainage properties were so different among the 7 sludges investigated. Microscopic observation of each sludge sample revealed some potential factors (Table 2). The treatment plants presented in Table 2 are arranged according to increasing values of SRD, from left to right, i.e. sludge quality in terms of drainability decreases from left to right. It is easy to see that the SVI values, traditionally used to describe the quality of sludge in terms of its

settleability, also increase from left to right (with one exception in the case of Esbjerg West plant), following the SRD. High SVI values typically indicate many filamentous bacteria or deflocculated sludge with irregular floc structure and many small particles, which would naturally render filtering more difficult (Karr and Keinath, 1978; Barber and Veenstra, 1986; Mikkelsen *et al*., 1996). This is largely what was recorded during the microscopic investigation of sludge samples. The trend of increasing SRD of sludge is followed by a transition from large, compact, and regular flocs through medium-sized, slightly irregular ones, to small, irregular flocs of open structure, which resembles a decrease of floc strength and progression of deflocculation. Interestingly, sludges of good quality in terms of drainage tend to have a filament index of 1 to 2 (few to moderate filamentous bacteria), whereas those harder to drain exhibit values of 2-3.5. Filamentous bacteria could be part of the explanation, if one imagines that small particles could be entrapped by filaments protruding from flocs in the filtration cake, which would eventually lead to more resistance to water flow.

Usually a general correlation between number of filamentous bacteria and settling velocity exist in activated sludge (Eikelboom, 2000). Such connection also seemed to be present in the plants investigated. Flocs with the lowest filament index and with the most compact structure (Bramming South) settled most quickly, whereas those with the highest FI and with the most irregular structure (Bramming North) settled most slowly. The hydraulic drag posed by filaments and irregular floc structure as the floc settles may be responsible for higher flow resistance inside the cake, and thus for higher SRD of the entire cake.

All floc properties of importance to gravity drainage have not been revealed by this study, but it is clear that the morphology, size, and amount of filaments are important. Other factors known to be of importance for pressure dewatering may also be of interest, e.g. the amount and composition of extracellular polymers (and thus microbial composition producing these), cations, and the inorganic fraction (Frølund *et al*., 1996; Park and Novak, 2007). Future studies should investigate these factors better.

3.3. Recommendations for sludge handling and application to reed beds

The general guidelines for reed bed operation proposed by Nielsen (Nielsen, 2002, 2003, 2005b) distinguish between the maximum loading rates of 60 kg dry matter/m^2/year for surplus activated sludge and 50 kg dry matter/m^2/year for surplus sludge mixed with anaerobically digested sludge,

but do not include the actual sludge quality monitoring. The survey of seven Danish wastewater treatment plants revealed that the inherent quality of activated sludge in terms of gravity drainage can be very different for different treatment plants. Findings of this, and our previous study, indicate that regular sludge quality monitoring by means of SRD measurements are necessary and would make sludge treatment on reed beds much more predictable and efficient. The methodology is very simple and requires no sophisticated equipment. In the simplest approach, only a cylinder, a filter, scales, and a timer are needed. Plant performance optimization by 'trial and error' approach is not recommended, since the sludge residues, once formed, remain in the basin and determine its further hydraulic performance for the entire basin life cycle.

In our previous study describing gravity drainage of activated sludge, we presented some relationships between drainage and sludge loading (Dominiak *et al*., 2011), which can be used together with the results obtained in this study to establish some improved guidelines for sludge handling on reed beds. Fig. 4 presents the relationships between SRD and the time of drainage at different concentrations of suspended solids. The actual points, representing the values measured in the survey presented in this study, are mapped into the graph. The slope of SRD versus time of drainage increases with increasing SS concentration, which can be translated into longer drainage of the same sludge at constant load if it gets thicker. Fig. 5 illustrates the effect of sludge permeability, which depends on its condition and previous treatments. The harsher the treatment of sludge prior to its drainage, the higher the SRD at a given load, which translates to longer drainage time according to Fig. 4. A practical way of using these two graphs is to set a limit of time for drainage, which should not be too long if anaerobic conditions are to be avoided in the sludge layer on reed bed. Having decided about the time of drainage (e.g. 60 min), and knowing the SS content of sludge (e.g. 4 g/L), the desired SRD can be determined according to Fig. 4. It is only necessary to take one measurement of SRD of a certain activated sludge sample at one load value (one sample volume, e.g. 200 ml), and the slope of the relationship depicted in Fig. 5 can easily be determined, since it always transects point (0, 0). This reveals the permeability of sludge, which allows choosing a proper load in order to attain a desired SRD (read from Fig. 4) and, eventually, a desired time of drainage.

In the alternative case, when loading rate adjustments are impossible, the SRD should be measured for a given load, the relationship as shown in Fig. 5 should thus be determined, and the SRD corresponding to the present loading of the reed bed can be established. Fig. 4 would then help to

select the proper SS concentration (adjustable through dilution of sludge in the storage tank with effluent or thickening it by settling) in order to achieve sufficiently fast drainage. Generally, it might always be better to apply smaller portions of sludge more frequently than to overload the basins with large portions of sludge. Due to the compressible nature of activated sludge, loading is the most critical factor when deciding on the drainage rate (Dominiak *et al.*, 2010). If a large volume of sludge is applied to a bed, drainage will proceed very slowly or even stop in extreme cases, which can lead to the development of anaerobic layers in the sludge sediment. Since anaerobic conditions lead to reduced floc strength and deflocculation (Mikkelsen and Keiding, 1999; Wilen *et al.*, 2000), such anaerobic layers can turn into compacted, impenetrable skins creating a barrier to downward water flow. This can in the long run lead to a temporary or permanent loss of bed permeability. It is especially important not to overload the bed in the initial phase of its exploitation because a layer of high resistance present at the bottom of the bed would remain there for a long time and, in the worst case, the entire period of bed exploitation, which could be up to ten years. An alternative solution is to dilute the sludge with effluent, which would accelerate the drainage, but then the pressure would also be increased due to higher liquid levels, the risk of higher cake compaction also having to be taken into account. It is also necessary to evaluate all the possibilities of sludge quality improvement by flocculation through aeration or calcium carbonate addition so that the final effect is significant, but also economically acceptable.

Some general guidelines for the design of reed bed facilities can be formulated, based on the findings of this study. The most important operational parameter for a reed bed is the yearly average solids loading, hence the design process should start with the estimation of this value. Esbjerg East operates its basins at approx. 40 kg DM/m^2/year (Table 1) and reports consistently predictable operation with no significant problems. Whether this could be slightly increased is presently unknown. According to Fig. 3, the SRD of sludge in this plant is approx. $2.4 \cdot 10^{10}$ m/kg, which is an average value among the plants tested in this study. If a reed bed facility is to be designed, the first thing to do is to check the sludge- SRD as a measure of sludge quality in terms of drainability, taking into account the possible sludge transportation. If this value turns out to be high in the range presented in Fig. 3, it is worth running a series of tests, similar to those described in section 3.1.2., in order to check whether nitrate dosing, flocculation with calcium, or aeration can improve the drainage properties, and if so, to what extent. Having established the attainable value of SRD for a given sludge, it needs to be compared to that of Esbjerg East so that the annual average solids loading can be selected through comparison with the benchmark value of 40 kg DM/m^2/year

reported by that plant. The exact deviation from the benchmark value cannot, however, be given at the moment and requires more full-scale trials. Once the design yearly average solids load is known, the number of basins can be calculated based on the average sludge production for a given plant. Finally, Fig. 4 and 5 can be used to select the proper operational parameters for the facility (loading, sludge SS concentration). After the commencement of the facility operation, the sludge quality needs to be regularly monitored by means of the SRD technique so that the overall performance of the reed beds is consistent and high.

Experiments presented in this study and in our previous reports show the effect of sludge handling on its subsequent drainage properties and how these can be handled. The reed bed operators from Esbjerg introduced changes according to the recommendations presented in this paper. The SS concentration in the aeration tanks of both plants was lowered from 4-6 to 3.5-4 g/L. Nitrate was continuously dosed to the sludge transportation pipeline, and calcium carbonate was continuously used to flocculate sludge prior to its application to the basins. Finally, the sludge application program was changed for all basins, and sludge is now applied in smaller portions, but with higher frequency. It is now 2000 m^3/basin every 6 weeks, and this volume is divided into 5 batches on each basin. Each batch is pumped out during 1 hour with 25 hours to drain before the next batch is added. Thus, the problems with the operation of basins handling sludge from Esbjerg West were eliminated, and the overall performance of the reed bed facilities was significantly improved after 1 year.

4. Conclusions

The method for measuring the sludge specific resistance to drainage (SRD) allows quick assessment of sludge quality prior to its application to reed beds, and the guidelines given in this report help to select the proper load and concentration of sludge, so that efficient and predictable operation of reed beds is assured.

Drainage properties in two Esbjerg plants were followed over two years, showing significant differences in sludge drainability, even though the two plants are very similar in terms of design and inflowing wastewater composition. The long-distance transportation of sludge was revealed to be responsible for the poor performance of reed beds.

It is of utmost importance to keep the sludge aerobic and flocculated so that drainage proceeds fast and risk of flooding the beds is minimized. It is especially important to avoid operational failures in the initial phase of reed bed operation, since every failure leaves behind a compacted layer of sludge residue of high resistance, which negatively affects the bed performance for a long time and, in the worst case, throughout its entire operational period.

Seven full-scale wastewater treatment plants showed very significant differences in sludge-SRD values, which highlights the importance of direct and regular sludge quality measurements if a sustainable and high performance of reed beds is to be achieved.

The new approach to assess sludge quality opens the possibility of formulating new guidelines for reed bed designers and operators, based on direct measurements. This could lead to increased competitiveness of reed bed sludge handling by making this technique more efficient and reliable.

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Fig. 1

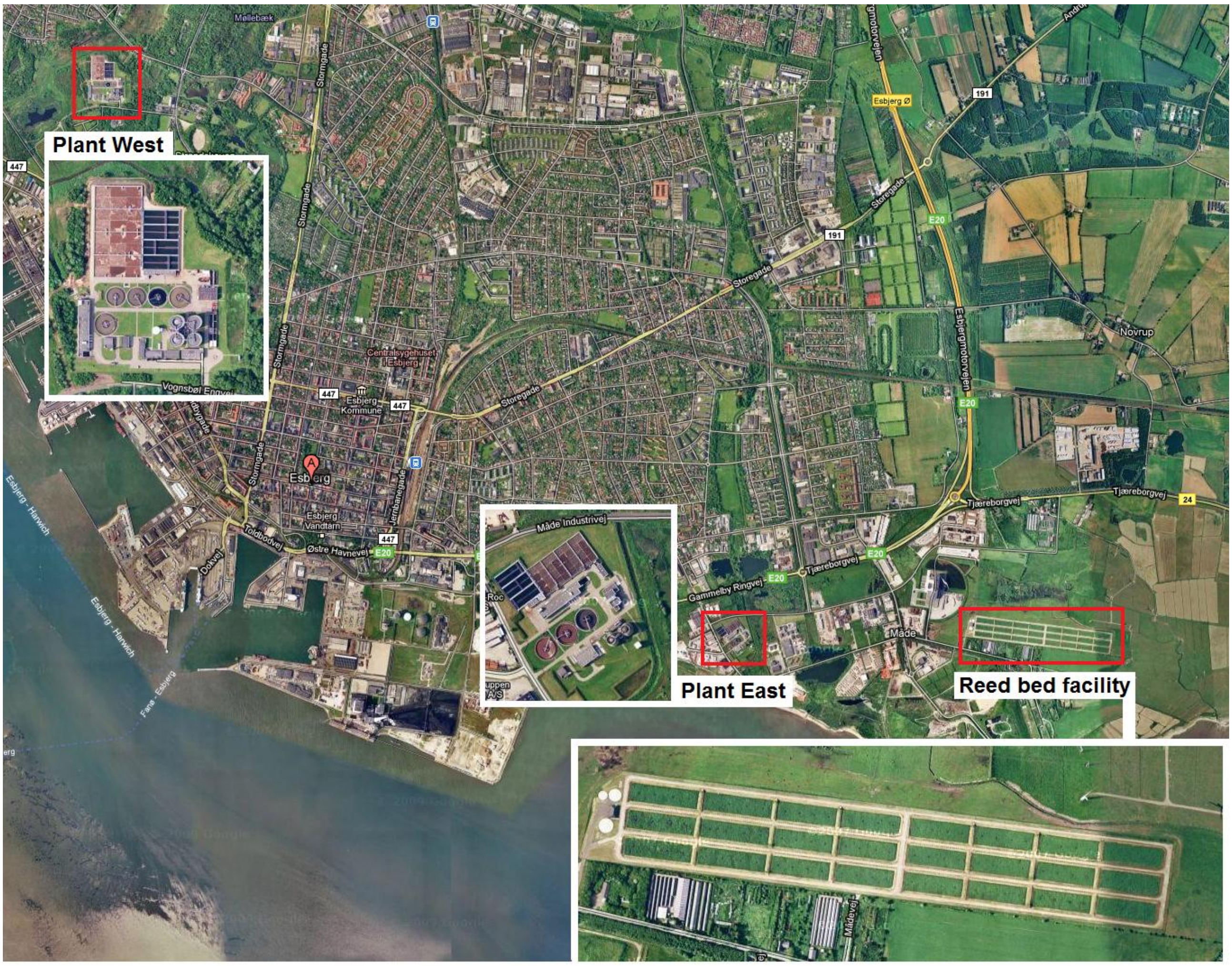
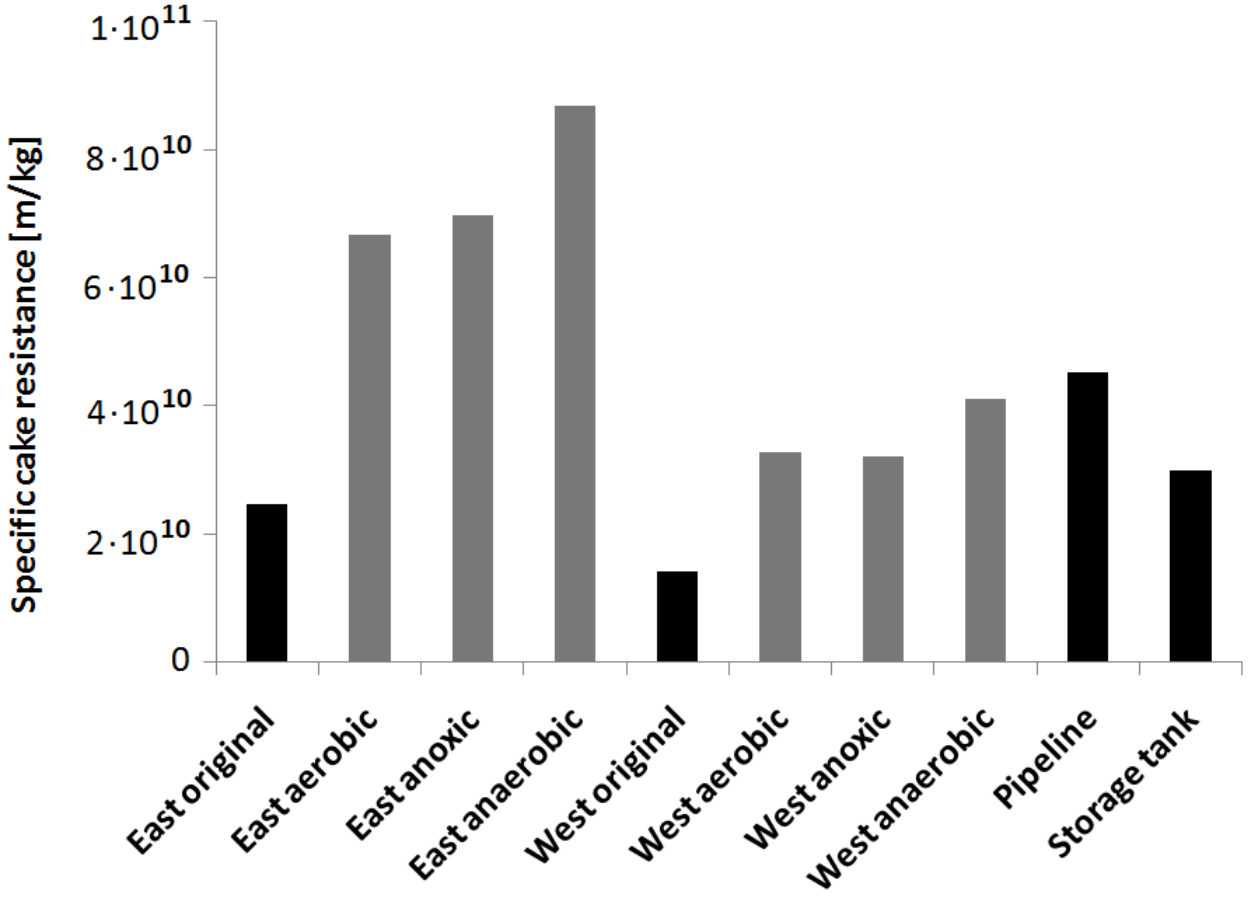

Plant West
Plant East
Reed bed facility
Esbjerg
Storegade
Stormgade
Novrup
Tjæreborgvej
Gammelby Ringvej
Måde Industrivej
Østre Havnevej
Toldbodvej
Esbjerg - Harwich
E20
447
191
24

Fig. 2


Specific cake resistance [m/kg]
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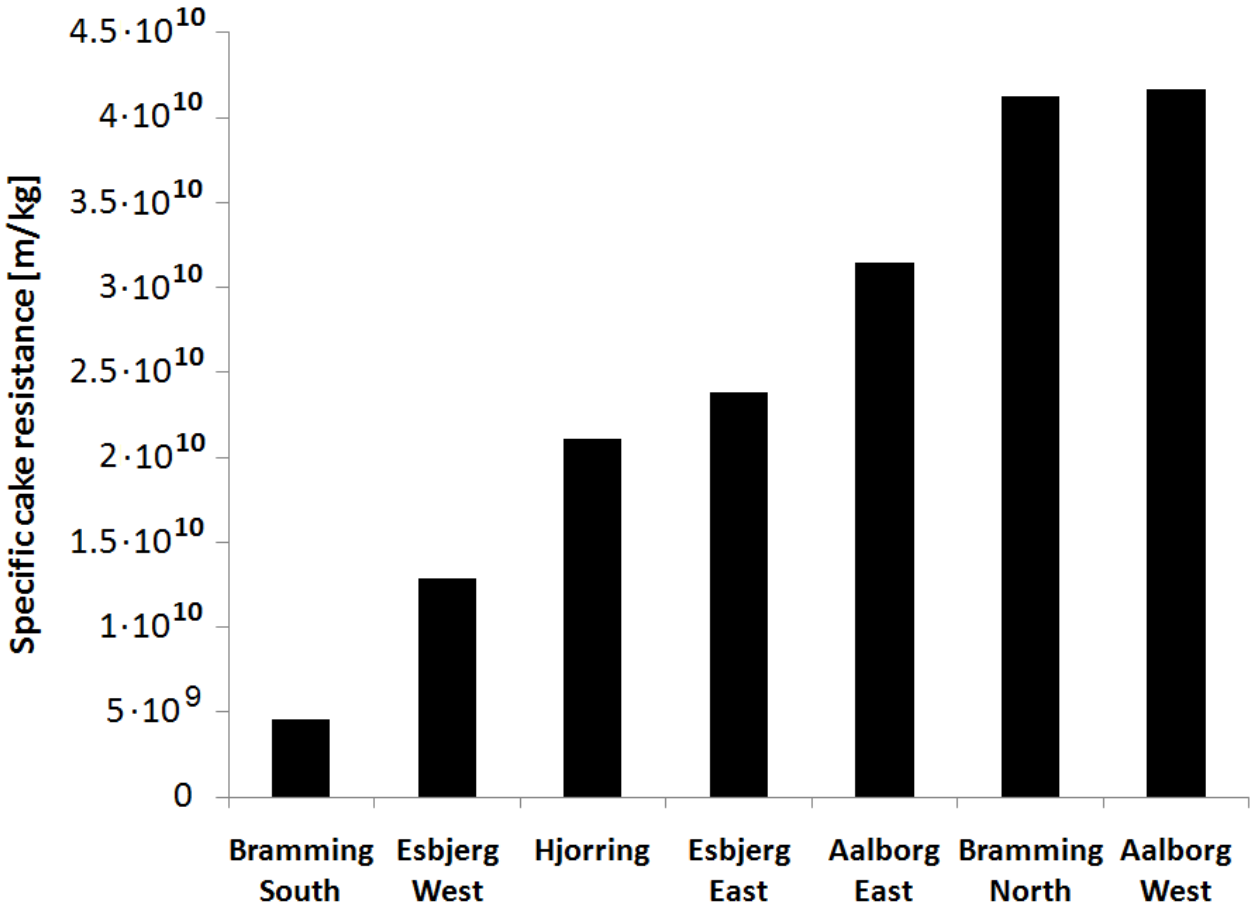
8·10^10
6·10^10
4·10^10
2·10^10
0
East original
East aerobic
East anoxic
East anaerobic
West original
West aerobic
West anoxic
West anaerobic
Pipeline
Storage tank

Fig. 3


Specific cake resistance [m/kg]
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4·10^10
3.5·10^10
3·10^10
2.5·10^10
2·10^10
1.5·10^10
1·10^10
5·10^9
0
Bramming South
Esbjerg West
Hjorring
Esbjerg East
Aalborg East
Bramming North
Aalborg West

Fig. 4

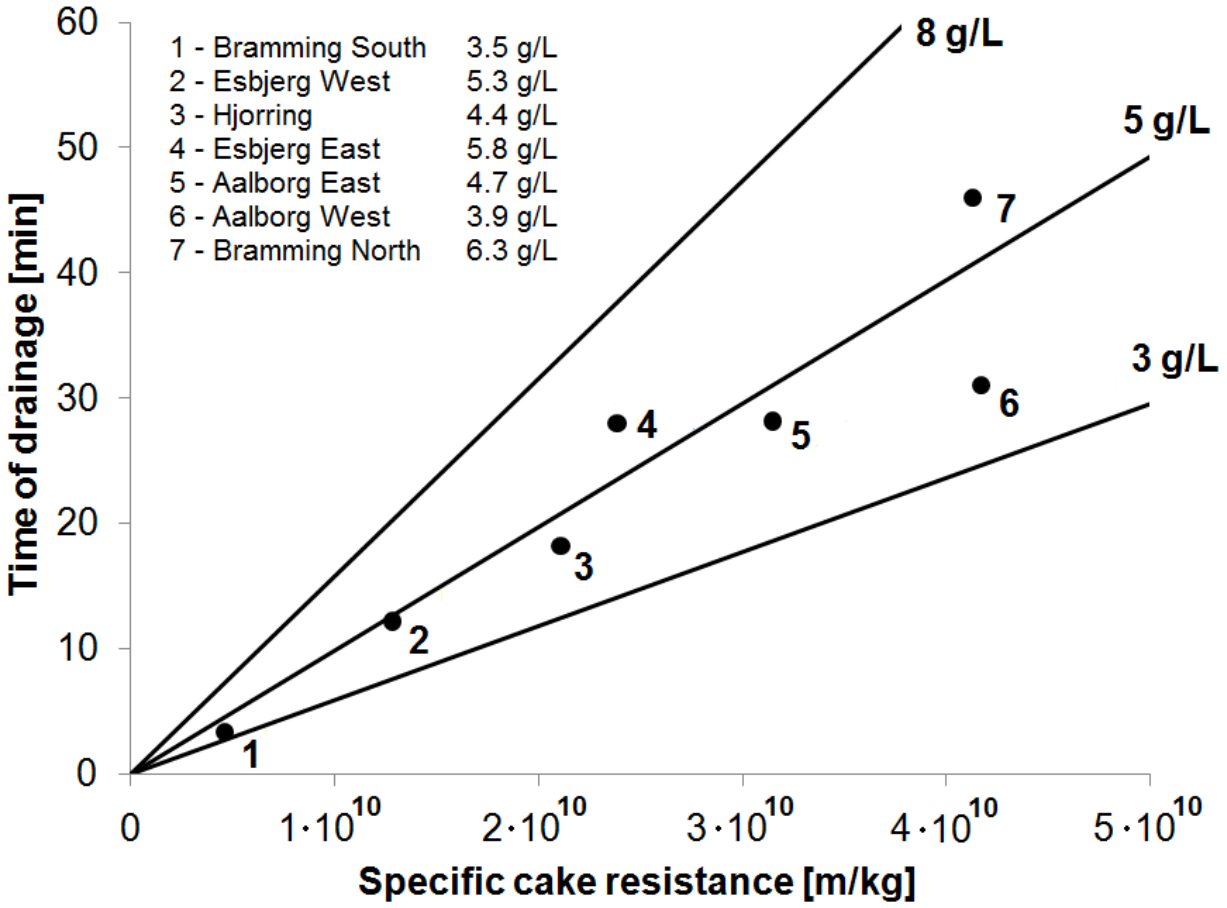
1 - Bramming South 3.5 g/L
2 - Esbjerg West 5.3 g/L
3 - Hjorring 4.4 g/L
4 - Esbjerg East 5.8 g/L
5 - Aalborg East 4.7 g/L
6 - Aalborg West 3.9 g/L
7 - Bramming North 6.3 g/L
8 g/L
5 g/L
3 g/L
Time of drainage [min]
Specific cake resistance [m/kg]
0
10
20
30
40
50
60
1·10^10
2·10^10
3·10^10
4·10^10
5·10^10

Fig. 5

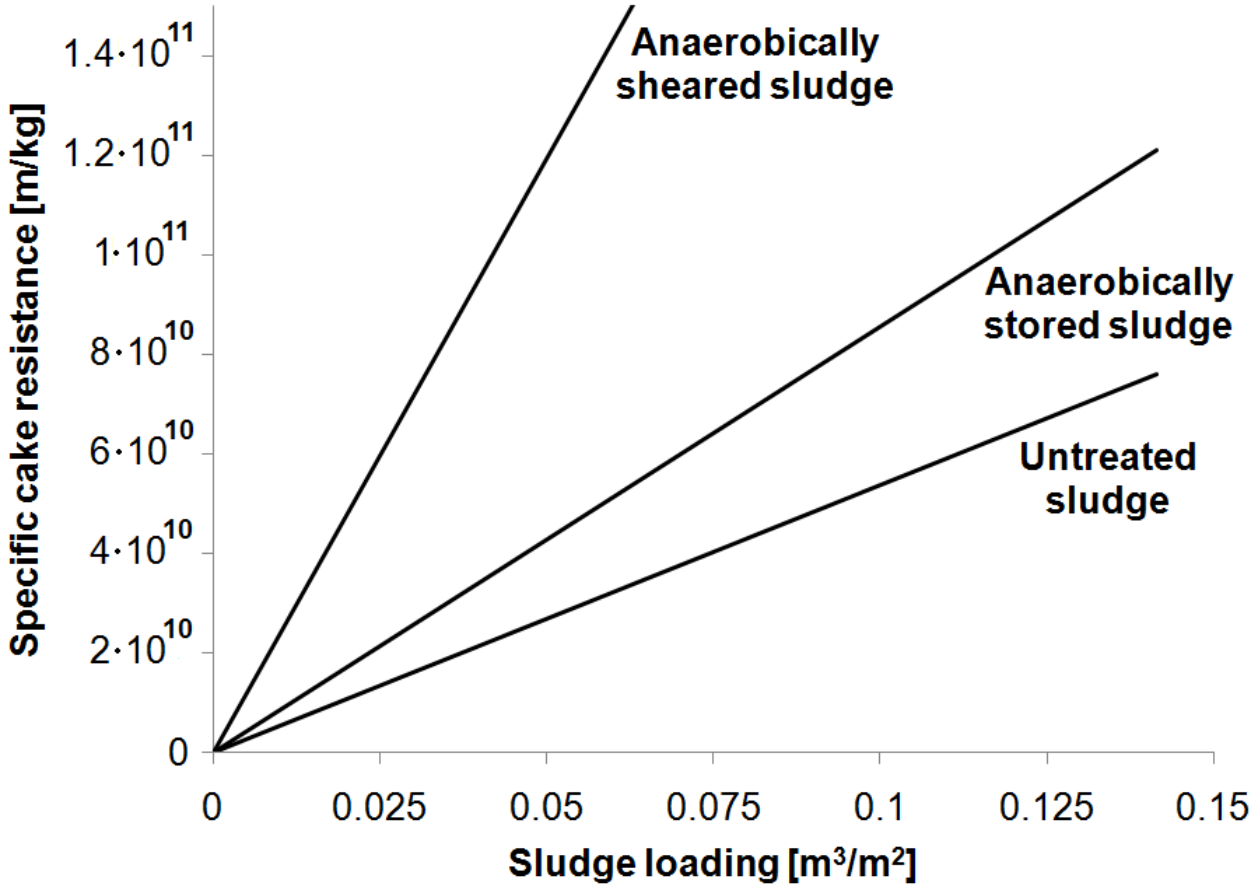

Table 1

	Plant Esbjerg East basins	**Plant Esbjerg West basins**
Design loading [kg DM/m^2/year]	55	55
Actual loading [kg DM/m^2/year]	38 – 42	27 – 32
Portion volume [m^3]	400 - 500	375 - 400
Drainage time [h]	20 – 25	30 – 36

Table 2

Wastewater treatment plant	Bramming South	Esbjerg West	Hjorring	Esbjerg East	Aalborg East	Bramming North	Aalborg West
Treatment plant information	PE 6000 C, N, DN, CP	PE 290000 C, N, DN, CP, BP	PE 160000 C, N, DN, CP, BP	PE 125000 C, N, DN, CP, BP	PE 125000 C, N, CP, BP	PE 6000 C, N, DN, CP	PE 330000 C, N, DN, CP, BP
Specific cake resistance [m/kg]	$0.5 \cdot 10^{10}$	$1.3 \cdot 10^{10}$	$2.1 \cdot 10^{10}$	$2.4 \cdot 10^{10}$	$3.2 \cdot 10^{10}$	$4.1 \cdot 10^{10}$	$4.2 \cdot 10^{10}$
Settling velocity [m/s]	$90 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$	$1.7 \cdot 10^{-5}$	$1.3 \cdot 10^{-5}$	$2.4 \cdot 10^{-5}$	$0.54 \cdot 10^{-5}$	$1.5 \cdot 10^{-5}$
Dry matter content of cake [%]	4.3	4.3	4.4	4.7	5.2	4.1	4.1
SVI [ml/g]	31	167	93	99	111	121	211
SS [g/l]	3.5	5.3	4.4	5.9	4.7	6.3	3.9
VS [g/l]	2.6	3.9	3.7	3.9	2.9	4.9	3.3
Microscopic floc observations	Large, compact, round, dark flocs	Large, regular, compact flocs	Medium-sized flocs, both round, regular and open, irregular	Open, irregular, medium-sized flocs, significant amount of inorganics	Medium sized flocs, both compact and open	Very small, irregular, disintegrated flocs, many branched filamentous bacteria	Small, irregular flocs of open structure
Filament index (0-5)	1	2	1	2.5	2	3.5	2
C – carbon removal; N – nitrification; DN – denitrification; CP – chemical phosphorus removal; BP – biological phosphorus removal							

Research paper 4

Dominiak, D.M., Nielsen, J.L., Nielsen, P.H. (2010) Extracellular DNA is abundant and important for microcolony strength of bacteria in mixed microbial biofilms. *Environmental Microbiology* (accepted).

environmental microbiology

Environmental Microbiology (2010) doi:10.1111/j.1462-2920.2010.02375.x

Extracellular DNA is abundant and important for microcolony strength in mixed microbial biofilms

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Summary

A new approach for quantification of extracellular DNA (eDNA) in mixed biofilms at microscale resolution was developed and combined with other staining techniques to assess the origin, abundance and role of eDNA in activated sludge biofilms. Most eDNA was found in close proximity to living cells in microcolonies, suggesting that most of it originated from an active secretion or alternatively, by lysis of a subpopulation of cells. When the staining was combined with fluorescence *in situ* hybridization for identification of the microorganisms, it was found that the eDNA content varied among the different probe-defined species. The highest amount of eDNA was found in and around the microcolonies of denitrifiers belonging to the genera *Curvibacter* and *Thauera*, the ammonium-oxidizing *Nitrosomonas* and the nitrite-oxidizing *Nitrospira*. Other floc-formers also produced eDNA, although in lower amounts. The total eDNA content in activated sludge varied from 4 to 52 mg per gram volatile suspended solids in different wastewater treatment plants. Very high local concentrations within some microcolonies were found with up to approximately 300 mg of eDNA per g of organic matter. DNase digestion of activated sludge led to general floc disintegration and disruption of the microcolonies with high eDNA content, implying that eDNA was an important structural component in activated sludge biofilms.

Introduction

The predominant lifestyle exhibited by bacteria in natural environments and engineered systems is growth on surfaces forming biofilms or bioaggregates. These microbial structures are held together by extracellular polymeric substances (EPS), which consist of a complex mixture of bacterial polymers forming a hydrated environment. The advantages of biofilm growth include protection against desiccation, mechanical shear and chemical toxins (e.g. Roberson and Firestone, 1992; Wolfaardt *et al*., 1994), and increased resistance to antibiotics (Mah and O'Toole, 2001). The composition of EPS is complex and depends greatly on the bacterial species and the growth conditions, but the compounds are often categorized to various polysaccharides, proteins and extracellular DNA (Watnick and Kolter, 2002).

Extracellular DNA (eDNA) has recently been shown to be an abundant component of many single- and multi-species cultured biofilms. Since the discovery of large quantities of eDNA in *Pseudomonas aeruginosa* (Hara *et al*., 1981), its mode of production, suspected roles and arrangement in *P. aeruginosa* biofilms in particular have been investigated. Also several other pure cultures have been studied, e.g. *Neisseria gonorrhoeae* (Dillard and Seifert, 2001), *Staphylococcus epidermidis* (Qin *et al*., 2007), *Shewanella* sp. (Pinchuk *et al*., 2008), *Acinetobacter calcoaceticus* and *Bacillus subtilis* (Lorenz *et al*., 1991). Extracellular DNA is also present in natural environments and engineered systems in considerable amounts. In marine sediments, the concentration can be 2 $\mu g\ g^{-1}$ dry soil (Niemeyer and Gessler, 2002), and eDNA can comprise more than 70% of the total DNA pool (Dell'Anno *et al*., 2002). In activated sludge from wastewater treatment plants eDNA has been reported up to 20 $mg\ g^{-1}$ organic matter (Palmgren and Nielsen, 1996).

The source of eDNA is yet to be established. Some authors have concluded that eDNA in *Staphylococcus* biofilms primarily originated from cell lysis, and was thus a natural and inevitable part of biofilm development (Qin *et al*., 2007). Recent experiments furthermore demonstrated that *cidA*-controlled cell lysis plays a significant role during development of *Staphylococcus aureus* biofilms and that released genomic DNA is an important structural component (Rice *et al*., 2007). However, the discovery of eDNA in young *Pseudomonas* biofilms (Whitchurch *et al*., 2002), where lysis is not a dominant process, suggests that lysis is most probably only part of the answer. In *P. aeruginosa* several biochemical pathways leading to eDNA production have been reported,

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such as the excretion of double-stranded DNA from living cells (Hara *et al*., 1981), release of vesicles containing DNA from living cells (Kadurugamuwa and Beveridge, 1996), and prophage-mediated lysis of a sub-population of cells (Webb *et al*., 2003). The production of eDNA in *P. aeruginosa* and other bacteria has also been linked to quorum sensing signals (Allesen-Holm *et al*., 2006; Spoering and Gilmore, 2006). The structure of this eDNA is reported to be double-stranded and largely similar to chromosomal DNA of the organism (Steinberger and Holden, 2005; Allesen-Holm *et al*., 2006; Böckelmann *et al*., 2006; Qin *et al*., 2007).

The role of eDNA in biofilms appears to be many. Studies of *P. aeruginosa* have documented the importance of eDNA for surface attachment and biofilm strengthening (Steinberger *et al*., 2002; Whitchurch *et al*., 2002). Similar discoveries have been made for other bacteria, e.g. *Staphylococcus epidermidis* (Qin *et al*., 2007), *Streptococcus* (Petersen *et al*., 2005), *Bacillus cereus* (Vilain *et al*., 2009) and marine photosynthetic bacterium *Rhodovulum* sp. (Watanabe *et al*., 1998). Extracellular DNA can also act as a nutrient source during starvation periods (Finkel and Kolter, 2001), indispensable link in phosphorus cycling in sea sediments (Dell'Anno and Corinaldesi, 2004; Dell'Anno and Danovaro, 2005) and in natural DNA transformation in single-species biofilms of *Acinetobacter calcoaceticus* and *Bacillus subtilis* (Lorenz *et al*., 1991). Extracellular DNA may also be a source of genes in the horizontal gene transfer (Molin and Tolker-Nielsen, 2003).

The abundance and importance of eDNA among uncultured bacteria in complex mixed biofilms is not well investigated, primarily due to lack of suitable methods for quantitative *in situ* studies. There are various methods for quantification of DNA, such as fluorescence staining, qPCR or spectrophotometric detection (Haque *et al*., 2003), but none of these methods has so far been used for assessment of distribution of eDNA in mixed microbial communities. A range of stains targets DNA, e.g. DAPI (4′,6-diamidino-2-phenylindole) or SYTO stains, but only certain stains such as DDAO (7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) and propidium iodide (PI) are capable of selectively targeting eDNA due to their molecular size, which does not allow the stains to penetrate intact cell membranes (Allesen-Holm *et al*., 2006). Both PI and DDAO were used in this study, but DDAO was chosen for quantitative analyses due to its better fluorescent properties and lack of interference with Cy3-labelled oligonucleotide probes.

The aim of this study was to develop an *in situ* technique for quantitative analysis of eDNA in mixed biofilms and to investigate the origin, and potential role of eDNA in complex biofilms, as exemplified by activated sludge flocs.

Results

Detection of eDNA in activated sludge flocs

DDAO was used to stain eDNA in the EPS matrix of activated sludge flocs and to reveal its abundance and distribution (Fig. 1A and B). Clear signals and a very uneven distribution were observed. To test the specificity of the DDAO stain for eDNA, the signal was compared before and after digestion with DNase I, which resulted in an almost complete removal of DDAO signal (Fig. 1C and D). SYTO 13 was simultaneously used to visualize intracellular DNA, and a distribution of both stains was observed. The incubation with DNase I digested eDNA, leaving intracellular DNA almost intact, thus indicating that DDAO only targeted DNA localized outside the cells (Fig. 1). This was also confirmed by simultaneous staining of eDNA with DDAO and PI, which both are unable to pass through intact cell membranes. DDAO-stained and PI-stained patches predominantly colocalized, as determined by image analysis (Fig. 2).

Origin of eDNA

A combination of SYTO 13, PI and DDAO staining was carried out in order to test the hypothesis that eDNA originates from the lysis of dead cells. Patches of eDNA, stained with DDAO, were checked for colocalization with membrane compromised cells, as evaluated with the SYTO 13 and PI stain combination (Fig. 2). The fraction of living cells (those with intact cell membranes – SYTO 13-positive and PI-negative) was approximately 85% of all cells, as indicated by direct counting. Most of the eDNA was found around living cells, and it was especially abundant in and around certain, but not all, bacterial microcolonies, which suggests that specific bacteria could be particularly involved in eDNA production. Only a small fraction of the eDNA was distinctly cell-shaped due to staining of intracellular DNA of cells with impaired cell membranes (as determined by visual microscopic investigation). Outside the microcolonies, eDNA was present as clouds in lower concentrations, and these covered most of the flocs' volume. Also, some protozoa appeared positive with DDAO stain, which seemed to have penetrated the protozoan cell membranes and targeted their intracellular DNA. Figure 2B shows some eDNA-rich microcolonies which were not detected by PI staining. In order to avoid such inconsistency, only DDAO was used as stain for visualization and quantification of eDNA in the rest of this study.

Abundance of eDNA in activated sludge flocs

Samples of activated sludge from aeration tanks of five Danish wastewater treatment plants were taken and

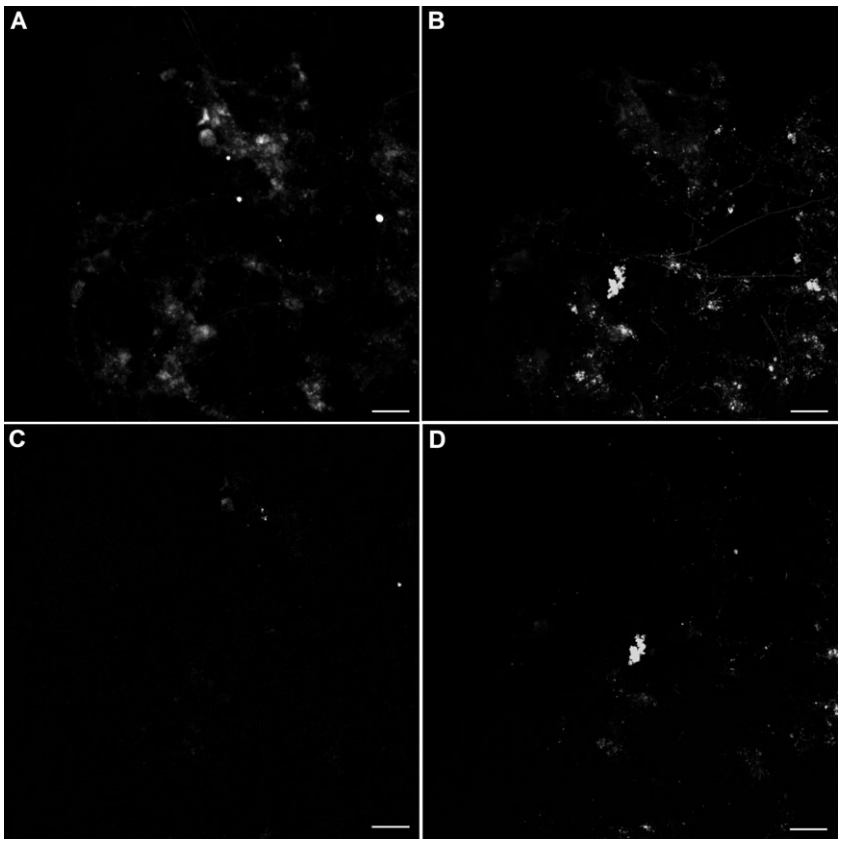

Fig. 1. Extracellular DNA in activated sludge floc before (A and B) and after (C and D) a 60 min incubation with DNase I. Extracellular DNA was stained with DDAO stain (A and C). Intracellular DNA was stained with SYTO 13 (B and D). Both images show the same microscopic field of view, relocated after DNase digestion. Some inevitable loss of SYTO 13 signal took place during DNase digestion. Scale bars correspond to 20 μm.

assayed for the overall average concentration of eDNA (Table 1). Extracellular DNA was found in all plants and the concentrations ranged from 4 to 52 mg eDNA g^{-1} VSS, which corresponds to a range from approximately 21–134 mg eDNA l^{-1}.

Short-term variations of eDNA concentration were investigated for Aalborg East treatment plant, where sampling took place every hour for 7 h in the aerobic nitrification tank and the anaerobic tank for biological P removal/release. No differences in the tanks were observed although some fluctuations of the average eDNA concentration were observed (approximately 30% deviations from the mean value). The content in the treatment plant remained at a relatively constant level of 10–30 mg g^{-1} VSS in both tanks over a time scale of 1 year (data not shown).

Table 1. The amount of extracellular DNA in activated sludge samples from five Danish wastewater treatment plants.

Wastewater treatment plant	Concentration of eDNA (mg eDNA g^{-1} VSS ± SE; $n = 3$)
Aalborg East	16.6 (0.8)
Aalborg West	52.2 (2.5)
Hjørring	42.9 (2.2)
Aabybro	6.5 (0.3)
Aars	4.2 (0.2)

Parentheses give standard errors.

The eDNA appeared to be clustered around microcolony-forming cells. Local concentrations of eDNA in particularly eDNA-positive microcolonies were approximately 10 times higher than the average concentration found for a given sample, ranging from 200 to 770 mg eDNA l^{-1}. The background concentration in the cloud-like eDNA patches could be as low as approximately 5% of the average concentration in a sample, ranging from approximately 1 to 7 mg eDNA l^{-1}. Around 95% of eDNA was localized into recognizable structures (primarily microcolonies) fluorescing with an intensity higher than the average background intensity in the floc EPS. An example of eDNA distribution inside activated sludge flocs is given in Fig. 3. Figure 3A and B shows the distribution of eDNA in flocs with average amount of 15 mg eDNA g^{-1} VSS. They show that most eDNA was found in close proximity to its presumed origin (microcolonies), but a detectable concentration could be found in almost any part of each floc. The area with eDNA concentrations below or equal to the average background concentration occupied approximately 95% of the floc area (Fig. 3C).

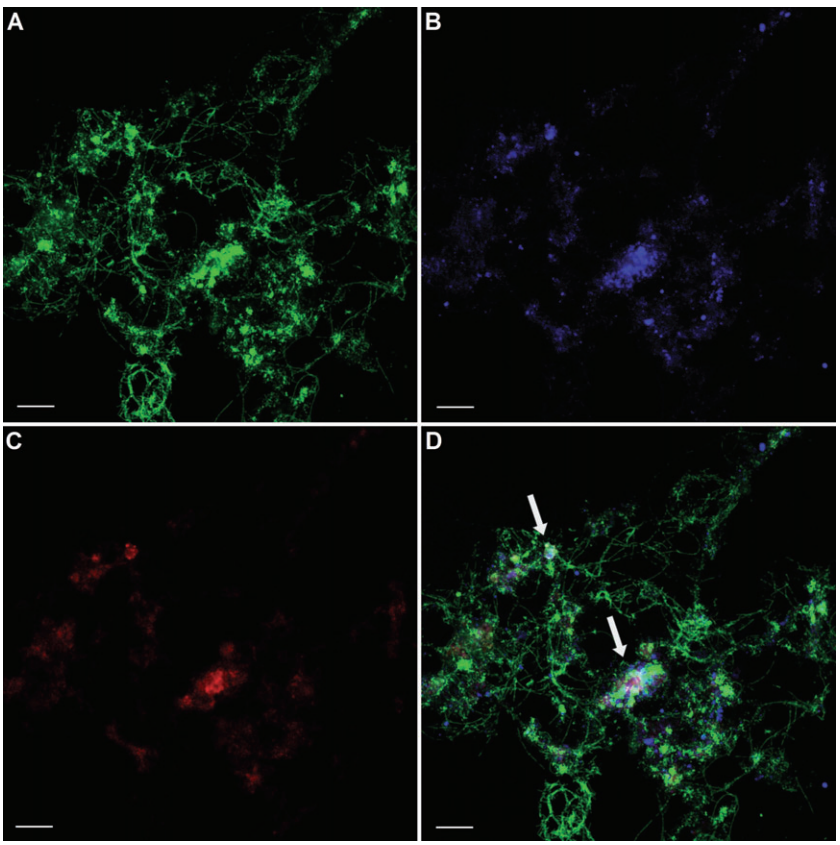

Fig. 2. Combination of SYTO 13 (A, green), DDAO (B, blue) and propidium iodide (C, red) staining. Image D is a merge of the three channels. Arrows in (D) show places in the floc which are especially rich in eDNA, as indicated by both DDAO and propidium iodide. Scale bars correspond to 20 μm.

The total eDNA pool consisted primarily of microcolonies containing 17–50 mg eDNA g^{-1} VSS.

Identity of bacteria with high levels of eDNA

The identity of bacteria with high amounts of eDNA was investigated by combining DDAO staining and FISH (Fig. 4) in samples from three wastewater treatment plants (Table 2). All probe-defined bacteria were present in an abundance of 2–8% of the total number of bacteria as measured by EUBmix. Most floc-forming bacteria tested were surrounded by eDNA. Some probe-defined species were surrounded by particularly high amounts of eDNA (up to 300 mg g^{-1} VSS) in and around the microcolonies. These were denitrifiers from the genera *Curvibacter* and *Thauera* and the nitrite oxidizer *Nitrospira*. Also *Accumulibacter* and *Competibacter*, two groups involved in enhanced biological phosphorus removal, and the ammonium oxidizer *N. oligotropha* were also consistently surrounded by eDNA, but at a lower concentration (60–140 mg g^{-1} VSS). Filamentous bacteria did not show any detectable eDNA production. The common denitrifier *Azoarcus* exhibited some eDNA production in one treatment plant, but none in another plant.

Table 2. Probe-defined major microbial producers of extracellular DNA in activated sludge samples from three Danish wastewater treatment plants.

	eDNA production		
Identity	Aalborg East	Aalborg West	Aars
Nitrifiers			
Nitrosomonas oligotropha	+	+	
Nitrospira	++	+	
Denitrifiers			
Thauera	+	++	
Azoarcus	–	+	
Curvibacter	++	++	
Polyphosphate Accumulating Organisms			
Accumulibacter	+	+	
Tetrasphaera	–	–	
Glycogen Accumulating Organisms			
Competibacter	+	+	
Filamentous bacteria (Gram –)			
Saprospiraceae	–	–	
Chloroflexi	–	–	
Some *Flavobacteria*	–	–	
Filamentous bacteria (Gram +)			
Candidate division TM7	–	–	
Microthrix parvicella	–	–	–
Skermania			–
Gordonia			–

++, most positive; +, some positive; –, all negative.

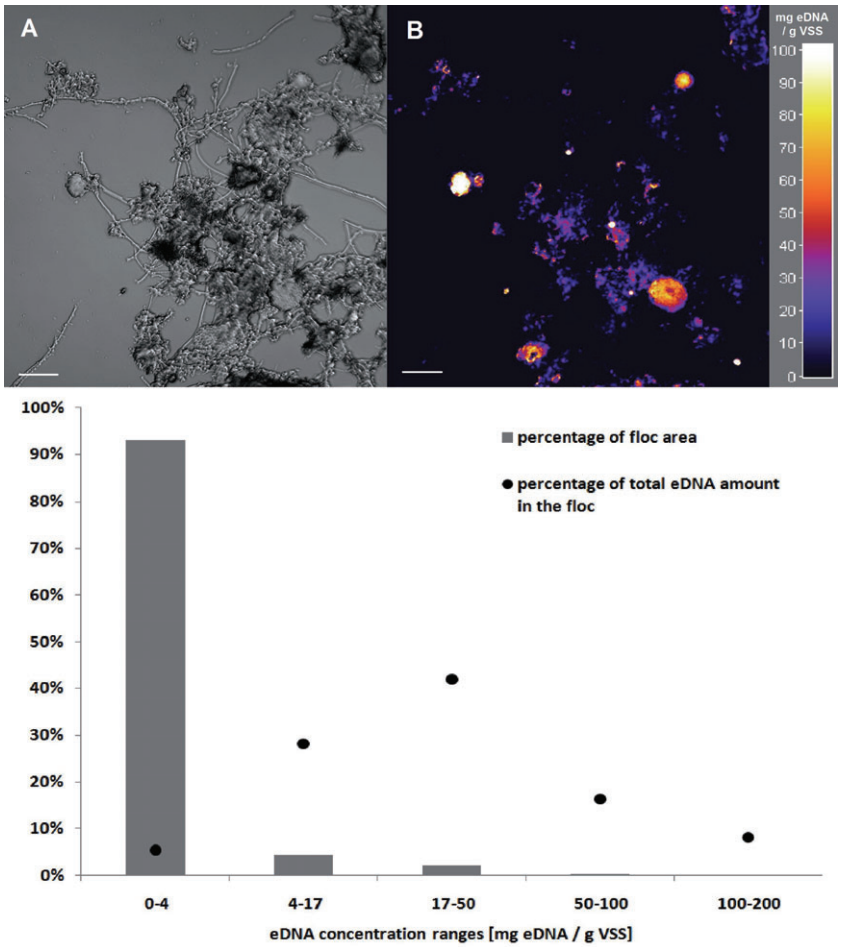

Fig. 3. Example of a distribution of eDNA inside an activated sludge floc. Nomarski microscopy (A) and DDAO staining (B). Certain microcolonies contained up to 100 mg g^{-1} VSS of eDNA, whereas the average concentration of eDNA in this floc was around 15 mg g^{-1} VSS. The colour code at right illustrates the eDNA content. The graph presents the distribution of eDNA given as area of the floc (grey bars) and as percentage contribution to the total eDNA amount in the floc (black dots). Scale bar corresponds to 20 μm.

Importance of eDNA for floc and microcolony strength

The importance of eDNA for floc strength was assessed in a series of deflocculation experiments with DNase I treatment of activated sludge from Aalborg East WWTP and Aalborg West WWTP. Addition of DNase I (100 U in each case) caused a significant deflocculation effect in both treatment plants. Figure 5 shows that activated sludge flocs deflocculated due to shear forces (stirring) and that DNase treatment promoted faster and larger deflocculation. The concentration of eDNA was approximately three times greater in activated sludge from Aalborg West than from Aalborg East, which may explain the difference in deflocculation patterns caused by the DNase treatments.

The general effect of deflocculation of activated sludge flocs, observed by turbidity measurements during the shear experiment, was confirmed by size distribution analysis before and after the shear treatment on a sample from Aalborg East WWTP, both with and without DNase I addition (Fig. 6). The largest fraction (in terms of bioarea) of particles in an untreated sludge sample were the large flocs and floc assemblies with diameters

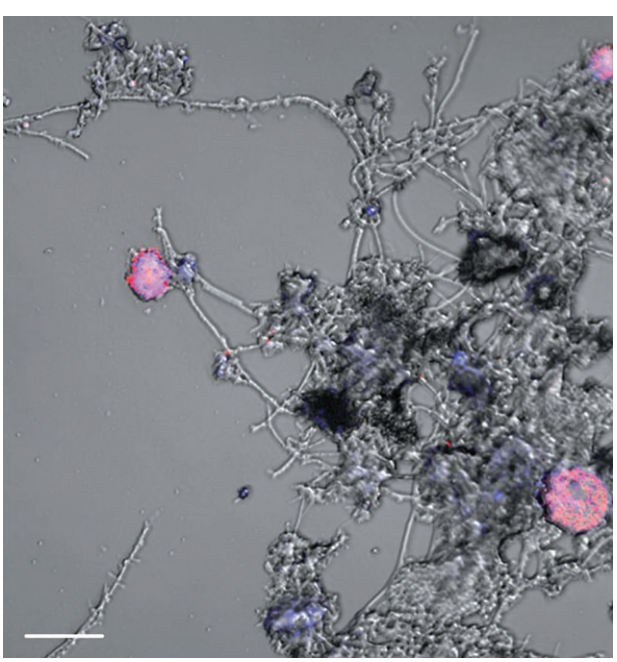

Fig. 4. FISH-defined *Curvibacter* microcolonies in activated sludge (red), contained high concentrations of eDNA (blue). They appear pink in the figure. Scale bar corresponds to 20 μm.

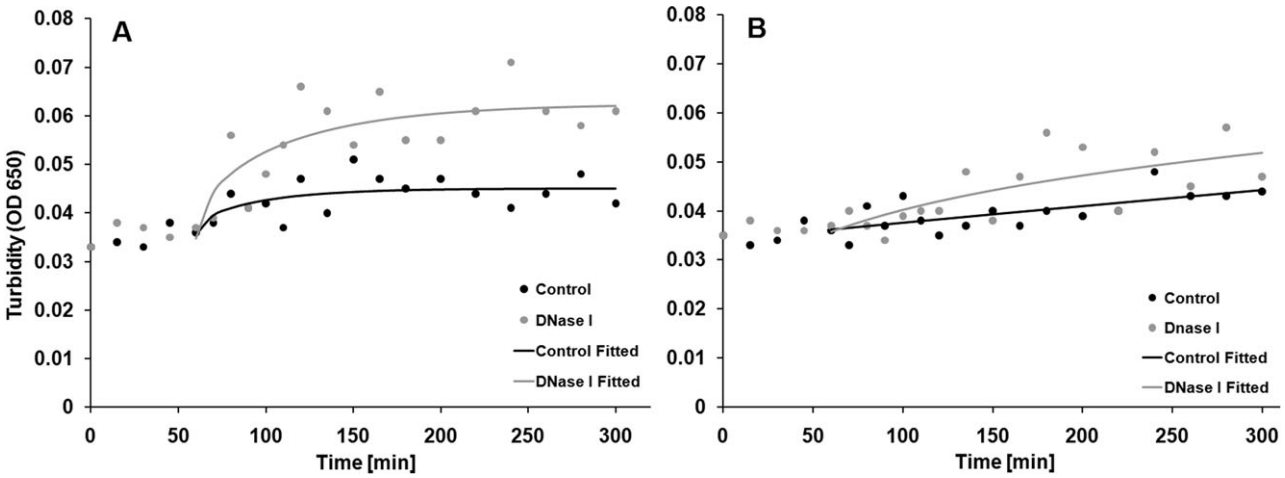

Fig. 5. Deflocculation curves obtained in shear experiments with and without addition of DNase I to activated sludge from Aalborg East WWTP (A) and Aalborg West WWTP (B).

above 200 μm, the rest were flocs with diameters of 42–200 μm with a very small share of particles with lower diameters. Shear alone produced sludge with an increased fraction of smaller particles. DNase I addition caused an almost complete dissociation of the large flocs and floc assemblies (Fig. 6A), resulting in a production of smaller cell aggregates (from 6 to 24 μm) and especially single cells, which were found to constitute almost 80% of the particles.

Since many microcolonies contained large amounts of eDNA, the size distribution was also analysed for three of these bacterial groups, *Curvibacter* (Fig. 6B), *N. oligotropha* cluster 6A (Fig. 6C) and *Nitrospira* (data not shown). Most *Curvibacter* cells in the untreated sludge sample were aggregated into microcolonies ranging from 30 to 100 μm in diameter with a small share of low-diameter cell assemblies (Fig. 6B). Shear alone caused a pronounced fragmentation of the largest fractions (36–100 μm) into pieces of sizes of 18–24 μm in particular. A combination of shear and DNase I digestion almost completely dissociated the particles larger than 18 μm and produced small fragments of microcolonies ranging from 6 to 18 μm in diameter. Erosion of single cells from the surface of microcolonies hardly took place.

The size distribution of *N. oligotropha* microcolonies in the untreated sample of activated sludge was more diverse than in the case of *Curvibacter* (Fig. 6C). Forty-four per cent of the aggregates ranged in diameter of 12–18 μm but larger aggregates were also abundant (over 35%), and single cells accounted for less than 1.5%. Shear alone caused a fragmentation effect, disrupting the larger microcolonies into smaller pieces, ranging from 6 to 24 μm. Digestion with DNase I combined with shear forces had a clear effect of reducing the larger fragments into very small aggregates of up to 12 μm (52%) and single cells or very small cell assemblies of up to 6 μm (48%). This reduction was most probably a combination of microcolony fragmentation and surface cell erosion. All the larger aggregates present in the untreated sample were replaced by single cells or very small fragments of microcolonies, with an almost equal share of particles smaller than 6 μm and particles ranging from 6 to 12 μm in diameter. The change in size distribution after DNase treatment for *Nitrospira* very much resembled that of *N. oligotropha* (data not shown).

Discussion

Abundance of eDNA in activated sludge

Only few studies have tried to quantify the amount of eDNA in complex biofilms such as activated sludge. Based on a cation-based extraction procedure, levels of 10–20 mg eDNA g^{-1} VSS have been found in a number of WWTPs (Frølund *et al.*, 1996; Palmgren and Nielsen, 1996). It was estimated to be significantly more than present inside the cells (up to 10 times). The level was in the same range as measured in this study, proving the importance of DNA as a constituent of the EPS matrix in mixed biofilms, although the actual content differed significantly between different treatment plants. The eDNA concentration at a certain time point in a treatment plant is the net result of inflow with wastewater and microbial production and removal. However, variations in eDNA concentration during one day in one plant were small, and the levels turned out to be constant in both aerobic and anaerobic tanks over long time periods. Therefore, the different contents of eDNA in activated sludge of different treatment plants are most probably due to different microbial populations and operational conditions affecting their activity.

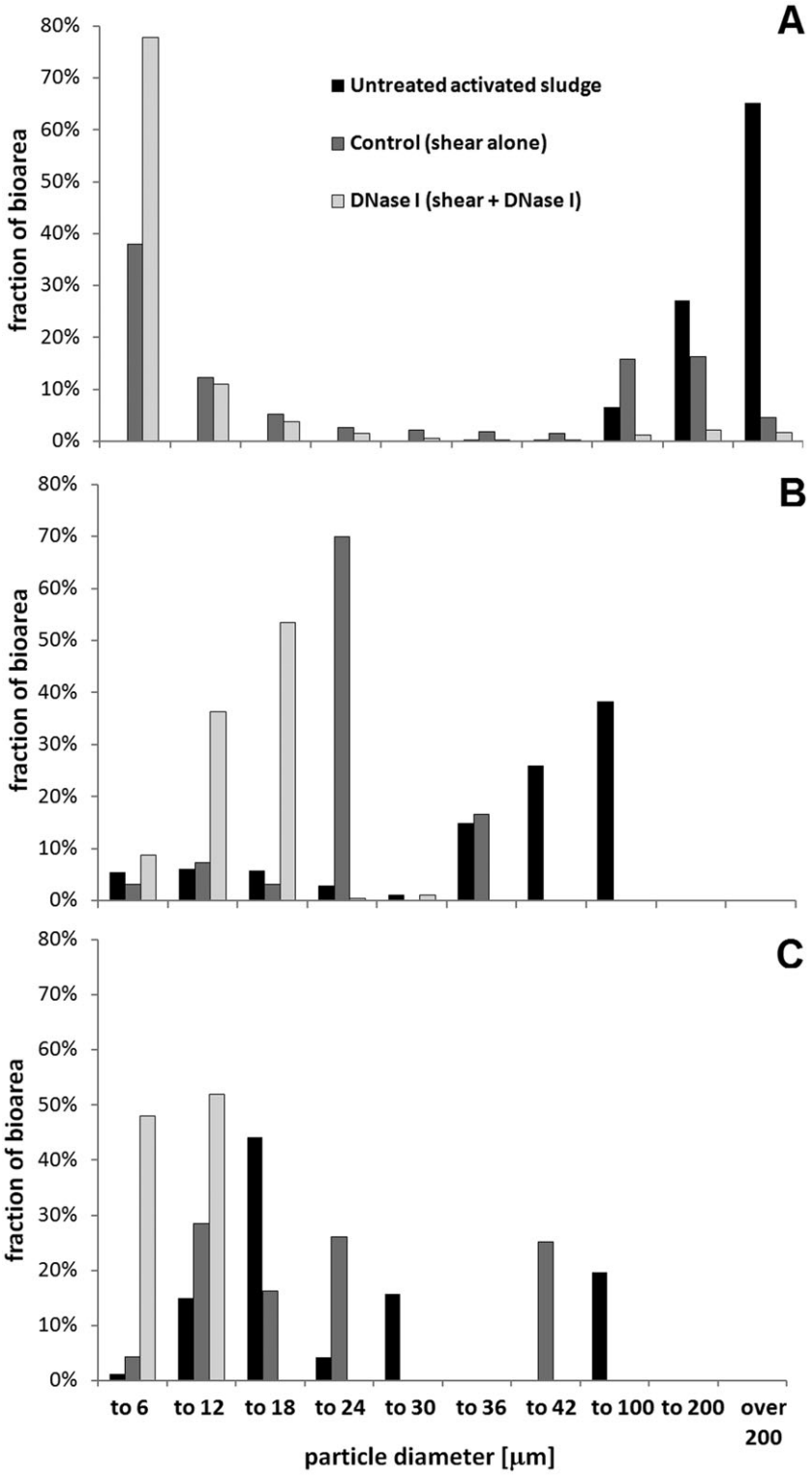

Fig. 6. Size distributions in terms of bioarea fractions measured for all bacteria in activated sludge (A), *Curvibacter* microcolonies (B) and microcolonies of *Nitrosomonas oligotropha* cluster 6a (C), before and after shear treatment both with and without the addition of DNase I.

The DDAO staining has been used for qualitative visualization of eDNA in pure culture studies (Allesen-Holm *et al*., 2006; Qin *et al*., 2007), and this was also a fast and reliable method for visualization of eDNA in activated sludge. Colocalization of signal with the other well-known DNA stain PI and the disappearance of signal after DNase treatment demonstrated the specificity of this stain towards the double-stranded DNA

located outside living bacterial cells. Furthermore, the development of this method as a quantitative measurement of eDNA using internal standards will be useful for future studies of eDNA, particularly in mixed microbial environments.

Producers of eDNA and importance for structural integrity

Most eDNA was found around living single cells and microcolonies, as determined by SYTO 13 and PI staining, suggesting active production of this polymer. However, we cannot exclude the possibility of lysis from a subpopulation of bacteria in the microcolonies (by autolysis or phages attack) not detected by the PI staining. Some membrane-compromised cells were also positive with DDAO stain, probably on the way to becoming extracellular material. Diffusion of eDNA into the EPS matrix could be an explanation for the presence of low concentration of this substance all around the flocs.

The FISH identification revealed that many microcolonies and particularly those of *Curvibacter*, *Thauera* and *Nitrospira* almost always had very high levels of eDNA. Concentrations within these microcolonies were found to be up to 300 mg g^{-1} organic matter. It is a very high amount which must strongly affect or define the microenvironment. Interestingly, these bacteria are also reported to form very strong microcolonies that are extremely difficult to shear apart (Larsen *et al*., 2008), indicating that a possible major function of eDNA is related to structural integrity. Such function has also been suggested by several other authors with regard to biofilm formation and stability in pure culture studies (Steinberger *et al*., 2002; Whitchurch *et al*., 2002; Petersen *et al*., 2004; Böckelmann *et al*., 2006). The deflocculation studies by adding DNase I support a structural role for eDNA, although some differences between the probe-defined species investigated were found. The deflocculation pattern for *Curvibacter* suggested fragmentation of microcolonies into larger pieces, while the pattern for *N. oligotropha* rather suggested erosion of single cells and small aggregates. Furthermore, DNase I also had a substantial deflocculating effect on the entire flocs, strongly indicating that even the low levels of DNA in the overall EPS matrix distant from the cells may be of importance to the floc strength.

Interestingly, not all microorganisms in activated sludge were surrounded by eDNA, and there were some variations for the same probe-defined species from plant to plant implying that the eDNA level was dynamic and the content may depend on both the species composition and the actual growth conditions. High abundance of certain potential eDNA producers could lead to higher eDNA contents in the overall EPS, but possibly also larger variations in the eDNA concentrations due to changing growth conditions.

The structural role of eDNA may affect the overall treatment plant performance. Various EPS components affect floc formation and floc strength influencing the settling properties as well as drainage and dewatering properties (Mikkelsen and Keiding, 2002a; Novak *et al*., 2003; Park and Novak, 2007; 2009; Park *et al*., 2008). The structural role of eDNA in activated sludge flocs has not been demonstrated so far, but as shown in this study eDNA seems to be an important component involved in forming strong flocs, resistant to shear forces present in wastewater treatment plants. Stronger flocs mean better settleability and better performance in pressure dewatering and gravity drainage (Christensen *et al*., 2010). As also shown in this study, the highly variable content of eDNA in different bacterial species means that the species composition in the wastewater treatment plants must affect the floc properties and thus also drainage and dewatering. The same is most likely the case in biofilms in any natural or engineered environment, where the species composition and their EPS production (and among these the eDNA) will affect biofilm properties.

Experimental procedures

Activated sludge samples

Samples of activated sludge were collected from the aeration tank in five wastewater treatment plants (WWTPs) in Denmark. Three plants have biological N removal (nitrification and denitrification) and enhanced biological phosphorus removal (Aalborg East, Aalborg West and Hjørring), whereas Aabybro and Aars only have biological N removal. All experiments were initiated within 1 h after sampling. Suspended solids (SS) contents for all plants were between 3 and 5 gSS l^{-1}, and volatile suspended solids (VSS) were 55–70% of SS. Values were determined according to Standard Methods (APHA, AWWA and WEF, 2005).

Staining techniques and microscopy

Extracellular DNA was stained with the fluorescent dye 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one (DDAO) (Invitrogen). It was chosen for its affinity to double-stranded DNA, good fluorescence properties, molecular size preventing it from penetrating intact cell membranes, and therefore targeting only eDNA (Allesen-Holm *et al*., 2006), and also the fact that its emission wavelength does not interfere with Cy3-labelled oligonucleotide probes. Verification of DDAO target was performed by simultaneously staining with PI (Invitrogen) having similar properties and also by observation of signal disappearance after digestion with DNase I (Sigma-Aldrich). Intracellular DNA was stained with SYTO 13 stain (Invitrogen), capable of penetrating intact cell membranes. Distinction between living and dead cells was made with a Live/Dead stain combination (Invitrogen). The working

concentrations of all stains were determined by preparation of saturation curves and by choosing the concentrations that were sufficient to saturate the target.

Staining was carried out on glass slides. Biomass was distributed on the glass surface and dried at 46°C. It was covered with a drop of working solution (10 μM in case of each stain, see below) and incubated at room temperature in the dark for 30 min. Slides were then washed by submersion in cold, sterile-filtered tap water and dried, before investigation with fluorescence microscopy. In some experiments, the eDNA was removed from the sample by digestion with DNase I (0.5 U μg^{-1} VSS) on slide-attached biomass for 60 min at 37°C.

A confocal laser scanning microscope (LSM 510 META, Zeiss) was used. Oligonucleotide probes labelled with Cy3 and PI stain were excited with a HeNe laser (543 nm), probes labelled with FLUOS and SYTO stains were excited with an Ar laser (488 nm), and DDAO stain was excited with a HeNe laser (633 nm). Digital image analysis was performed with ImageJ software (http://rsbweb.nih.gov/ij/) using self-written macros. In case of FISH combination, DDAO-stained sample was investigated microscopically with a 40 × objective, positions of interest were recorded with use of an automatic stage controller, FISH procedure was conducted, and the positions were relocated for final investigation.

FISH analysis

The FISH oligonucleotide probing was performed according to Amann (1995). The identity of bacteria in biofilms was investigated with the following oligonucleotide probes: PAOmix (mixture of PAO462, PAO651 and PAO846) for *Accumulibacter* (Crocetti *et al*., 2000); GAOmix (mixture of GAOQ989 and GB_G2) for *Competibacter* (Crocetti *et al*., 2002; Kong *et al*., 2002); Aqs997 + competitor for *Curvibacter* (Thomsen *et al*., 2004); Cluster6a-192 for *Nitrosomonas oligotropha* (Adamczyk *et al*., 2003); NTSPA662 + competitor for genus *Nitrospira* (Daims *et al*., 2000); Thau-646 for *Thauera* (Lajoie *et al*., 2000); Azo644 for most members of *Azoarcus* cluster (Hess *et al*., 1997); Nso190 for betaproteobacterial ammonia-oxidizing bacteria (Mobarry *et al*., 1996); SAP-309 for *Saprospiraceae* (Schauer and Hahn, 2005); CFXmix (mixture of GNSB941 and CFX1223) for *Chloroflexi* (Gich *et al*., 2001; Bjornsson *et al*., 2002); TM7-905 for candidate division TM7 (Hugenholtz *et al*., 2001), Actino-221 and Actino-658 for *Tetrasphaera* (Kong *et al*., 2005); MPA_all_1410 for *Microthrix parvicella* (Levantesi *et al*., 2006); Spin1449 for *Skermania* (Eales *et al*., 2006); and GOR-596 for *Gordonia* (de los Reyes *et al*., *1997*). The gene probes were labelled with 5(6)-carboxyfluorescein-*N*-hydroxy-succinimide ester (FLUOS) or with sulfoindocyanine dye (Cy3). Hybridization conditions and probe details can be found at probeBase (Loy *et al*., 2007).

Quantification of eDNA

Quantification of eDNA was carried out by the application of internal standards. Increasing amounts of pure DNA (Salmon sperm DNA, 10 mg ml^{-1}, Invitrogen) were added to gently homogenized activated sludge samples. A sample in which eDNA was removed by digestion with DNase I served as control. An even distribution of salmon sperm DNA and its proper adhesion to activated sludge flocs were assured by gentle shaking of sludge/DNA mixture for 30 min at 4°C, and good adherence of DNA to flocs was confirmed by DNA concentrations of < 1 ng μl^{-1} left in the liquid phase, determined spectrophotometrically (NanoDrop Technologies). Extracellular DNA was stained with DDAO according to the protocol described above. Flocs were visualized with Normarski microscope without any fluorescent markers, because numerous trials aiming at obtaining a linear standard curve with fluorescent dyes targeting intracellular DNA were unsuccessful, possibly due to stain competition with DDAO over the DNA target. Finally, the average ratio of DDAO fluorescence intensity to cells biovolume (factor referred to as the 'relative intensity') was measured for each sample, and a standard curve was prepared describing the relationship between the content of eDNA in the sample and DDAO relative intensity. The linear nature of this relationship (Fig. S1) allowed for the estimation of eDNA concentration in the original sample (where no external DNA was added) and finally, estimation of eDNA concentration in any activated sludge sample, based on the intensity of DDAO signal, provided that the same settings of the microscope were used every time. Determination of eDNA concentration around single cells and microcolonies was possible by comparing the intensity of DDAO fluorescence around a particular target with the average DDAO intensity for a given specimen. The range of eDNA concentrations covered by the standard curve was chosen so as to include the highest concentrations found in some microcolonies.

Shear experiments

The impact of DNase I (Sigma Aldrich) treatment on the microcolony strength was determined with shear tests in 1 l baffled shear reactors with 600 ml of activated sludge under defined shear conditions as described elsewhere (Klausen *et al*., 2004). Each experiment was divided into two stages and 30 U of enzyme were added at the very beginning of each trial. The first stage consisted in gentle mixing of activated sludge at 60 r.p.m. for 60 min, in order to assure thorough mixing and allow its action. In the second stage the stirring rate was increased to 800 r.p.m. Samples were taken from both reactors at certain time intervals, centrifuged for 3 min at 426 *g* and the deflocculation evaluated by optical density of the resulting supernatant measured at 650 nm. The experiment was continued until the optical density measurements reached a plateau. Numerical fitting of the deflocculation curves was performed according to Mikkelsen and Keiding (2002b). The effect of DNase I treatment on the overall floc strength was assessed in 300 ml baffled reactors with 100 ml of activated sludge, and the concentration of DNase I was increased to 100 U. This concentration was determined experimentally as the threshold DNase I concentration above which no more DNase-based deflocculation took place (data not shown).

Microcolony size distribution upon DNase treatment was measured in paraformaldehyde fixed samples after quantitative FISH. Another set of slides was used for SYTO 13

staining, which stained all the cells in the sample. Finally, images were recorded with a confocal laser scanning microscope, and the particle size distributions (referred to as bioarea) were manually acquired with ImageJ software for at least 100 microcolonies, cell aggregates and single cells of *Curvibacter*, *Nitrosomonas oligotropha* cluster 6A, and *Nitrospira*.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Standard curve for quantification of eDNA concentration (mg g^{-1} VSS) in activated sludge. Relative intensity, describing the average ratio of DDAO fluorescence intensity to the biovolume of cells in a batch of at least 30 images, can be translated to the concentration of extracellular DNA in a given sample. The first point without eDNA concentration was obtained by digestion of eDNA with DNase I. The curve presented is an average of four consecutive experiments which provided very repetitive data. Error bars represent the standard error of the mean.

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