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Properties of Activated Sludge Flocs

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CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden 1999

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT

The quality of the effluent from activated sludge treatment plants is highly dependent on the efficiency of the solid-liquid separation process. Small flocs and free bacteria, which cannot be separated by means of settling, contribute to the turbidity of the effluent. The suspended solids responsible for the turbidity contain most of the organic material, phosphorus and, sometimes, nitrogen that leave activated sludge treatment plants. Small particles also cause deteriorated dewaterability of the sludge. The sludge must have good settling and thickening properties to avoid accumulating in the settlers, which may cause discharge of sludge or other operational problems.

The aim of this thesis was to study (i) the effects of dissolved oxygen (DO) concentration on effluent turbidity and flocculation, as well as on the settling properties of activated sludge, for both short term and long term scales, and (ii) the influence of aerobic microbial activity on the stability of activated sludge flocs.

Relatively short periods of oxygen limitation (a few hours) caused increased turbidities of the effluent from both a pilot plant and a full scale plant. In addition, the adsorption of colloidal material onto the sludge flocs decreased under anaerobic conditions. The variations in activated sludge floc structure, size and size distribution were studied for DO concentrations of 0.5 to 5 mg/l, and sludge ages of 1.25 to 5 days, in pilot scale, completely mixed reactors. There was a trend towards larger flocs at higher DO concentrations. The size distribution by volume of flocs within the range of 10 - 1100 μm fitted well to log-normal distribution functions, while the size distribution by number of the small flocs (1 - 100 μm) in the supernatant after settling fitted best to power functions. Lower DO concentrations produced sludge with poorer settling properties and higher turbidities of the effluent than higher DO concentrations.

Activated sludge deflocculated when it was subjected to anaerobic conditions and shear. The extent of deflocculation varied with the length of the anaerobic period. Activated sludge of a low sludge age deflocculated more than older sludge. To restore the deflocculated sludge, oxygen was added; thereafter, in less than an hour, most of the deflocculated matter was reflocculated. However, part of the deflocculated sludge did not reflocculate. The deflocculated material was composed mainly of bacteria and extracellular polymeric substances (EPS) attached to cells or floc fragments. Particles deflocculated under anaerobic conditions included more bacteria and protein than there were the composition of the total sludge. Greater floc strength was obtained when the aerobic microbial activity was stimulated, while deflocculation increased when the aerobic microbial activity was inhibited. Not only inhibition of the aerobic microbial activity increased the deflocculation, but the extent of deflocculation under anaerobic conditions could also be increased by stimulating the anaerobic microbial activity, e.g. Fe(III)-reduction. Field experiments showed seasonal variation in floc strength with the largest proportion of weak flocs present in the winter: there was a temperature effect on the degree of deflocculation. The degree of reflocculation could be increased by stimulating the aerobic microbial activity.

Key words: activated sludge, dissolved oxygen concentration, floc size, settling, filamentous microorganisms, deflocculation, reflocculation, shear forces, aerobic microbial activity, floc strength.

SAMMANFATTNING

Kvaliteten på utgående vatten från ett avloppsreningsverk beror till stor del på hur effektiv separeringen av slam från det renade vattnet är. Små flockar som inte kan avskiljas vid sedimenteringen bidrar till turbiditeten på utgående vatten. Flockarna innehåller organiskt material. Dessutom är fosforn till stor del partikelbunden. Slammet måste, förutom ha goda flockuleringsegenskaper, också ha goda sedimenterings- och förtjockningsegenskaper för att undvika slamflykt från sedimenteringsbassängerna. Målet med den här studien var att studera (1) syrehaltens inverkan på turbiditeten samt flockulerings- och sedimenteringsegenskaperna hos aktivt slam och (2) hur bakteriernas aeroba mikrobiella aktivitet påverkar slamflockarnas styrka.

Korta perioder av syrebrist (några få timmar) orsakade en ökad turbiditet i utgående vatten från både en pilotanläggning och en fullskaleanläggning. Slammets förmåga att adsorbera partiklar och kolloider från avloppsvattnet minskade dessutom under anaeroba förhållanden. Variationen i struktur, storlek och storleksfördelning hos slamflockarna, studerades för olika syrehalter (0.5 till 5 mg/l) samt slamåldrar (1,25 till 5 dagar) i en pilotanläggning. Flockarna ökade något i storlek med ökad syrehalt. Storleksfördelningen för flockarna i storleksintervallet 100–1100 µm kunde anpassas till log-normalfördelningar. Storleksfördelningen för de små flockarna som fanns i supernatanten efter sedimentering (1–100 µm) kunde anpassas till exponentiella fördelningar. Låga syrehalter producerade flockar med sämre sedimenteringsegenskaper och högre turbiditet på utgående vatten, jämfört med vid högre syrehalter.

Aktivslamflockarna deflockulerade när de utsattes för anaeroba förhållanden och turbulens. Graden av deflockulering ökade med längen på den anaeroba perioden. Slam av en låg slamålder deflockulerade mer än slam av en hög slamålder. Flockarna kunde delvis fås att återflockulera genom luftning. En fraktion av de deflockulerade flockarna kunde dock ej fås att återflockulera. De deflockulerade slampartiklarna bestod av bakterier och extracellulära polymerer som var bundna till slampartiklar. Partiklar som deflockulerats under anaeroba förhållanden, innehöll mer bakterier och protein jämfört med den totala mängden i slam. Slamflockarnas styrka ökade då den aeroba mikrobiella aktiviteten stimulerades. Däremot, då den aeroba mikrobiella aktiviteten reducerades, minskade flockstyrkan. En sänkt flockstyrka observerades under anaeroba förhållanden på grund av en anaerob mikrobiell aktivitet, tex mikrobiell reduktion av järn. Graden av återflockulering vid luftning kunde fås att öka genom att stimulera den aeroba mikrobiella aktiviteten, tex genom att tillsätta substrat. Vid en reducerad mikrobiell aktivitet minskade återflockuleringsgraden avsevärt.

Det fanns en årstidsvariation i flockstyrkan; flockarna var svagast under den kalla årstiden. Deflockuleringsexperiment visade att det också fanns ett temperaturberoende hos flockstyrkan; låga temperaturer reducerade flockstyrkan.

LIST OF PAPERS

This thesis is based on the work contained in the following papers which are referred to by their Roman numerals in the text.

I. **Short term effects of dissolved oxygen concentration on the turbidity of the supernatant of activated sludge**
Britt-Marie Wilén and Peter Balmér (1998), *Water, Science and Technology*, **38** (3), pp. 25 - 33.

II. **The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs**
Britt-Marie Wilén and Peter Balmér (1999), *Water Research,* 33(2), pp. 391 - 400.

III. **Anaerobic deflocculation and aerobic reflocculation of activated sludge**
Britt-Marie Wilén, Kristian Keiding and Per H. Nielsen, *Submitted to Water Research.*

IV. **Influence of microbial activity on the stability of activated sludge flocs**
Britt-Marie Wilén, Nielsen, J. L., Kristian Keiding and Per H. Nielsen, *Submitted to Colloids and Surfaces B: Biointerfaces.*

V. **Reflocculation of activated sludge by stimulation of the biological activity**
Britt-Marie Wilén, Kristian Keiding and Per H. Nielsen, *To be submitted to Applied and Environmental Microbiology.*

VI. **Effect of temperature and cold-storage on activated sludge floc strength**
Britt-Marie Wilén, *Working paper.*

ABBREVIATIONS

BOD	Biological Oxygen Demand
DSVI	Diluted Sludge Volume Index
DLVO	Deryagin, Landau, Verwey and Overbeek theory
DNA	Deoxyribonucleic Acid
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substances
F/M	Food to Microorganisms Ratio
HRT	Hydraulic Retention Time
MLSS	Mixed liquid suspended solids concentration
RNA	Ribonucleic Acid
SRT	Solids Retention Time
SS	Suspended Solids
SSVI	Stirred Specific Volume Index
SV	Sludge Volume
SVI	Sludge Volume Index
SQI	Sludge Quality Index
TS	Total Solids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

CONTENTS

1 INTRODUCTION

1.1 Background

Stricter regulations issued by authorities to limit the maximum concentrations of pollutants allowed, mainly organic matter and nutrients such as nitrogen and phosphorus, in the effluent from wastewater treatment plants, have made it necessary to optimize treatment processes. Usually, wastewater is treated biologically by means of the activated sludge process (Tchobanoglous and Burton, 1991). In this process, the microorganisms, which degrade the pollutants, grow as suspended flocs. Before the effluent can be discharged to the receiving waters, the activated sludge has to be separated from the treated water.

The separation takes place in the secondary settler, which is often the most critical process in an activated sludge treatment plant. For successful separation, the microorganisms must form flocs which settle and compact well without leaving a high concentration of suspended solids in the supernatant. The result of poor separation properties is often discharge of suspended solids into the receiving water, and can cause several operational problems in the wastewater treatment plant. When the sludge compacts poorly, enough sludge cannot be recycled to the aeration tank; this will cause the settlers to overflow. It also decreases the retention time of solids, thereby reducing treatment efficiency. The formation of flocs with poor flocculation properties, furthermore, reduces dewaterability which is particularly affected by the amount of small particles.

Organic matter discharged from activated sludge plants is to a large extent particulate (Neis and Tiehm, 1997). Apart from generating an oxygen demand in receiving waters, particles in the effluent may contain particulate bound phosphorus (and a minor amount of nitrogen). Discharge of the nutrients phosphorus and nitrogen may lead to eutrophication.

There is a clear relationship between the hydraulic loading of the secondary settlers and the concentration of suspended solids in the effluent from activated sludge treatment plants (Bergh and Olsson, 1996). In particular, if the wastewater treatment plant is connected to a combined sewer system, the fluctuations in hydraulic loading can be very large due to storm water entering the sewage system. Perturbations propagate fast through the treatment plant and the processes taking place in the aeration tank, as well as in the settlers, are affected. Larger hydraulic loads generally lead to greater concentrations of suspended solids in the effluent; although this is caused mainly by a rise in sludge blanket level, it is also due to erosion of small and light flocs from the sludge blanket. Thus, the capacity of the treatment plant to handle changes in hydraulic load, without producing higher concentrations of suspended solids in the effluent, is directly linked to the floc properties. Even small changes in floc structure can cause large deviations in the effluent quality.

Basically, there are two main types of settling problems: (i) bulking sludge due to the proliferation of filamentous bacteria and (ii) poor flocculation properties of the microorganisms. Much effort has been made to solve the problem of bulking sludge and reasonably good control strategies have been developed (e.g. Wanner, 1993). In spite of taking careful precautions, the growth of filamentous bacteria can be very difficult to control, since there are a vast number of different types of filaments with different optimal growth conditions. The problem of deflocculation of sludge flocs has been less well investigated. Although the parameters influencing the flocculation of activated sludge are to some extent known, their relative significance is still largely unknown. The flocculation of activated sludge is an active process and depends on physical, chemical and biological factors. The major problems are the great complexity of the activated sludge floc composition, coupled with the various parameters that interact in the flocculation process. Basically, any change in the treatment plant affects the sludge floc structure (e.g. surface properties, strength, morphology) and, thereby, also the solid-liquid separation properties.

To run wastewater treatment plants efficiently without adding large quantities of flocculation agents, which is both expensive and incompatible with the desire to use as little resources as possible, more research is needed to increase the understanding of which parameters influence the floc formation. The introduction of new process solutions may alter the floc properties considerably. This is something which has often been ignored in the development of new process solutions for the removal of nitrogen and phosphorus. The most sophisticated treatment plant can malfunction if the flocculation properties are poor, since a process is never stronger than its weakest link.

1.2 Aim

The purpose of this thesis was to increase the understanding of which parameters affect the flocculation and settling properties of activated sludge. The work includes studies of the effects of dissolved oxygen concentration on the separation properties of activated sludge flocs, on both long term and short term scales. Furthermore, the importance of microbial mechanisms in the flocculation of activated sludge flocs was studied.

1.3 Scope of the Thesis

The research was basically divided into two main projects. In the first one, the effects of anaerobic conditions or oxygen limitation on the flocculation and settling properties were studied (***Papers I and II***). The study was arranged as follows.

- The first part deals with the short term effects of dissolved oxygen (DO) concentration on the turbidity of the supernatant of activated sludge. The effect of DO concentration on the adsorption of particulate and colloidal material from wastewater onto the sludge flocs was studied. In pilot plant studies, the effect of short periods (a few hours) of oxygen limitation on the effluent turbidity was investigated. In full scale studies made to verify the results from previous studies, the DO concentration was changed along the aeration tank to see how this would affect the effluent turbidity at a conventional activated sludge treatment plant.

- The second part of the research concerned the long term effects of DO concentration on the structure, size and size distribution of activated sludge flocs. The work was carried out in an activated sludge pilot plant with continuous completely mixed reactors. The experiment was conducted in stages: the pilot plant was run at DO concentrations of 0.5 to 5 mg/l and at sludge ages of 1.25 to 5 days.

In the second project, controlled experiments were carried out to study the effects of different parameters on the floc strength (***Papers III and VI***). The first experiment was a quantitative study of *deflocculation* of activated sludge under anaerobic conditions and the extent to which the sludge can *reflocculate* again when it is subjected to aerobic conditions. The second experiment examined how the microbial activity affects the floc stability, during both deflocculation and reflocculation.

This thesis is based on six papers linked together. Chapter 2 contains a comprehensive literature review which includes the latest advances in the various aspects of floc formation and the separation properties of activated sludge. In Chapter 3 the experimental approach and set-ups used in the research are described briefly, while in Chapter 4 the results are summarized and discussed in relation to the literature. Chapter 5 gives the conclusions and ideas for future work.

2 LITERATURE REVIEW

2.1 Wastewater Treatment

Wastewater is derived from domestic sewage and in many cases also from industrial effluents and storm water. Ultimately, collected and treated wastewater must be returned to receiving waters or to land. For reasons of public health, environmental protection and aesthetics, untreated wastewater cannot be discharged to receiving waters. Wastewater can vary widely in composition, due mainly to variations in water consumption, infiltration of groundwater, and dilution with storm water. Wastewater contains both organic and inorganic components. The discharge of organic matter causes oxygen depletion in receiving waters; also, nutrients such as nitrogen and phosphorus may cause an excess growth of algae (eutrophication). These algae can produce toxins and unpleasant odours, which makes the recipient water unsuitable for bathing and fishing. In addition, wastewater usually contains numerous pathogenic or disease-causing microorganisms which can constitute a public health hazard. Sometimes, wastewater can also contain toxic substances.

Wastewater comprises a very complex mixture of compounds, both dissolved and solid. In the treatment plant, the contaminants must be reduced to acceptably low concentrations. The basic principle of a wastewater treatment plant is to convert dissolved compounds into solids that can be removed from the treated water. The type of process used depends mainly on the kind and amount of wastewater as well as on economic and environmental considerations. For domestic wastewater, a combination of mechanical, biological and chemical treatment is normally used. Wastewater is generally treated in a series of steps or unit processes (Tchobanoglous and Burton, 1991).

Primary treatment is a physical separation: sewage entering the treatment plant is passed through a bar screen which remove gross solids, gravels and floating solids. Thereafter the suspended solids in the wastewater settle for some hours to remove larger solids (primary settling).

Secondary treatment is the stage in which biological and/or chemical processes are used to remove organic matter. This treatment can be designed to remove the nutrients nitrogen and phosphorus biologically or by means of chemical precipitation. The biological stage is carried out either in a suspension of bacteria aggregated into flocs, known as activated sludge, or in biofilm systems (Henze *et al.,* 1995). Sometimes, activated sludge and biofilm systems are combined. There are three main types of biological processes: aerobic (DO present), anoxic (DO absent, nitrate present) and anaerobic (DO and nitrate absent).

Tertiary treatment is a complementary handling of the effluent from the secondary stage. This is a physico-chemical process, which can involve precipitation, filtration, flotation and chlorination, to reduce the levels of inorganic nutrients, especially phosphorus and nitrogen, from the final effluent. Tertiary treatment is, however, uncommon.

Sludge treatment, the handling and disposal of sludge, is probably the most complex and difficult part of the whole process. Sludge is produced in the primary stage (settleable solids), in the secondary treatment (biomass and chemical solids when chemical precipitation is employed) and in the tertiary one (chemical solids and biomass). The principal methods used are thickening to reduce sludge volume and dewatering to reduce the moisture content. Other unit processes may be included, such as stabilization to reduce mass and content of pathogens as well as to reduce offensive odors and the potential for putrefaction, and conditioning to improve the dewaterability and drying. Ideally, the organic material and nutrients in the sludge should be returned to the farmland. This is, not always possible, however, due to demanding requirements for the quality of the sludge.

The dewatering of sludge is very costly for two main reasons: when the sludge does not have good dewatering properties, which is almost always true, conditioning agents have to be added to improve the solid-liquid separation; when the dry matter content in the filter cake is low, the cost of transportation and disposal of sludge increases dramatically.

There are many technical solutions used in the different treatment stages. Much research has been carried out to optimize the various stages to meet the increasingly stringent effluent standards. Nowadays, there is a growing demand to analyse and evaluate the treatment of wastewater from a more holistic point of view. It is desirable that the use of energy as well as precipitation and conditioning chemicals be minimized, and much attention has accordingly been given to biological treatment processes. However, biological processes are complex, due to the large variety of microorganisms present, and that many of the microbiological processes involved are still poorly understood. Hence, to optimize the biological treatment step further, more research is needed.

2.1.1 The activated sludge process

The most widespread biological wastewater treatment method is the activated sludge process which was developed in England by Arden and Lockett in 1914. This process has been subjected to many improvements throughout the years (Tchobanoglous and Burton, 1991). Its popularity is due primarily to its simplicity and robustness. Basically, the conventional activated sludge process (Figure 1a) consists of an aerated suspension of a mixed bacterial culture which carries out the biological conversion of the contaminants in the wastewater. The mixed bacterial culture forms flocs spontaneously. The suspension, the activated sludge, is supplied with oxygen by means of compressed air, pure oxygen or by mechanical aeration. The aeration serves two functions: to supply the microorganisms with oxygen and to keep the activated sludge in suspension. After an initial contact time between wastewater and micro-organisms, the suspension enters a settler where the microorganisms are separated from the treated wastewater to produce a clear effluent. One part of the settled biomass is recycled back to the inlet of the aeration tank and the other part is disposed of as excess sludge. The amount of withdrawn sludge is the same as that of the sludge produced to avoid accumulation of biomass in the system. Some sludge also leaves the system with the effluent; however, when the solid-liquid separation works well, its contribution to the withdrawn sludge is small. Sludge production is influenced by the type of process used, the operation of the wastewater plant, and the wastewater characteristics. The recycling ensures a continuous sludge inocula-tion and extends the sludge residence time to give the microorganisms a chance to adapt to the new environment. It also enables the adsorbed organic material to be oxidized. In addition, recirculation also ensures a high sludge concentration in the aeration tank.

The efficiency of the activated sludge process is strongly linked to the ability of the sludge flocs to settle and compact. The sludge must be thickened enough so that the amount of it necessary to maintain a desired concentration of suspended solids in the aeration tank, can be recycled. Also, a clear effluent must be produced and the excess sludge withdrawn should be easy to dewater. To avoid the loss of sludge from the wastewater treatment plant to the effluent, the load of suspended solid reaching the settler must not exceed its capacity, either for thickening or clarification, which is a varying function of the sludge characteristics (Lumley, 1985).

There are various configurations for the activated sludge process, such as different shapes of the aeration tank (plug-flow or completely mixed stirred tanks), combinations of several tanks, differing feed patterns of the wastewater, dissimilar aeration systems etc. (Henze *et al.*, 1995). Biological nitrogen and phosphorus removal can be achieved by expanding a conventional activated sludge treatment plant with anoxic and anaerobic tanks in various configurations (Figure 1b). Nitrogen removal is a two-step process: nitrification and denitrification. Nitrification requires aerobic conditions under which the nitrogen in the wastewater is transformed from ammonium to nitrate. Denitrification requires anoxic conditions by which nitrate is transformed into nitrogen gas. Biological phosphorus removal is carried out by a specific group of phosphorus accumulating bacteria (Bio-P bacteria) which require both aerobic and anaerobic conditions (Henze *et al.*, 1995). Phosphorus can also be removed by chemical precipitation with metal salts (iron or aluminium) or lime.

Activated sludge is a complex mixture of diverse microorganisms (mainly mixed types of bacteria), dead cells, extracellular polymeric substances (EPS) and particulate organic and inorganic material (Eikelboom and van Buijsen, 1983). The population of microorganisms present in the activated sludge depends on factors such as the composition of the wastewater, growth rate, electron acceptor (oxygen or nitrate), pH and temperature. Settling and flocculation characteristics also determine the microbial population, since microorganisms that do not settle are washed out from the system. Bacteria are the most important ones for the degradation of organic material in wastewater; they are predominant in terms of biomass and biochemical activity. Bacteria can grow in three basic modes: dispersed, floc-forming and filamentous. Other types of microorganisms are fungi, algae, protozoa, rotifers and nematodes. Higher microorganisms such as rotifera and protozoa consume small biological flocs and dispersed bacteria, thereby keeping the effluent clear. Algae and fungi have a minimal influence on the degradation of organic material. The activated sludge process is very adaptive to changing compositions of the wastewater, a characteristic that makes the process highly efficient.

A special property of the activated sludge is its capacity for high adsorption of suspended and colloidal material. According to Boller (1993), more than 50% of the contaminants in waste-water are larger than 0.001 μm and cannot be taken up directly by the bacteria. The initial step in removing material from the wastewater is its attachment to the flocs. This process depends primarily on the adsorptive capacities of the flocs, which are influenced by the floc composition and structure. Knowledge of the mechanisms involved in these adsorption processes is still limited. The subsequent step in the degradation of organic material is the oxidation of adsorbed particles, by which enzymes break down the larger particles into smaller constituents, that can pass through the cell wall.

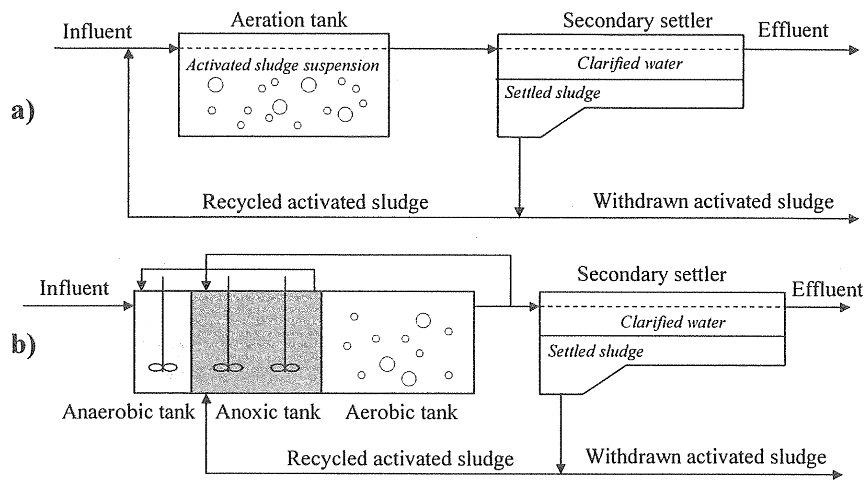


Figure 1 *Schematic drawing of two activated sludge treatment plants: (a) conventional, and (b) biological N and P removal.*

2.1.2 Factors affecting the suspended solid concentration in effluent

An activated sludge treatment plant is a very complex system: most of the processes involved affect the function of the secondary settlers. First, the wastewater coming into the plant varies, both in composition and amount, virtually minute-by-minute. This affects the biological and chemical processes taking place in the aeration tank, as well as the hydraulics in the whole wastewater treatment plant. Second, the design and operation of the plant influences the characteristics of the activated sludge flocs (Section 2.3) and, consequently, the separation efficiency in the settlers. In other words, when looking into the solid-liquid separation processes taking place in the settlers, it is fundamental to keep in mind that hydraulic effects and biological-chemical effects are interrelated.

Wastewater characteristics

The composition of the wastewater depends not only on the source but also on transformations taking place in the sewer system (Nielsen *et al.*, 1992). The composition of wastewater is not uniform: it changes with time (hourly, daily, monthly and yearly). Infiltration of ground water to the sewerage system often has the greatest impact on the variation of the composition over the year. Intermittent discharges from industries connected to the sewerage system increase the variations in composition still more. Often the systems are combined and sometimes storm water also enters the pipe network. During storm water events, large quantities of water enter the system and the wastewater becomes very diluted; furthermore, the temperature of the wastewater can decrease considerably (Mattson, 1997).

Domestic wastewater consists mainly of organic compounds, microorganisms which provide a continuous mixed-culture inoculum for the treatment process, nutrients such as nitrogen and phosphorus, metals, inorganic materials (e.g. hydrogen sulphide), inorganic ions and sometimes toxic compounds (Henze *et al.*, 1995). The composition of drinking water also influences the composition of the wastewater, mainly as regards the amount and types of ions present. The organic material can be divided into three major categories according to the rate of biodegradation (Wanner, 1994). *Readily biodegradable compounds* have low molecular weight and can be metabolized immediately by the bacterial cells (methanol, ethanol, volatile fatty acids, monosaccharides and lower amino acids). *Slowly biodegradable compounds* have to be hydrolyzed by means of extracellular enzymes before they can be transported into the cells (soluble or suspended). *Unbiodegradable compounds* are those which pass through the biological treatment and remain in the effluent, dissolved or adsorbed to flocs, or which are metabolic by-products.

The organic contaminants in wastewater are a mixture of particulate and soluble compounds of different sizes, see Figure 2 (Levine *et al.*, 1985). In biological wastewater treatment, contaminants of widely differing sizes can be treated. Processes that take place in the treatment plant (settling, mass transfer, adsorption, diffusion and biochemical reaction rates) are determined by the size distribution of the various contaminants. Small organic compounds can be taken up directly by the bacteria, while larger molecules must first be broken down into smaller constituents by means of enzymes outside the bacteria. The organic fraction of a settled domestic wastewater can be roughly fractionated into 45 - 55% dissolved components (< 0.001 μm), 20 - 35% colloidal particulates (0.001 - 1 μm) and 25 - 35% supra-colloidal particulates (1 - 100 μm) (Boller, 1993).

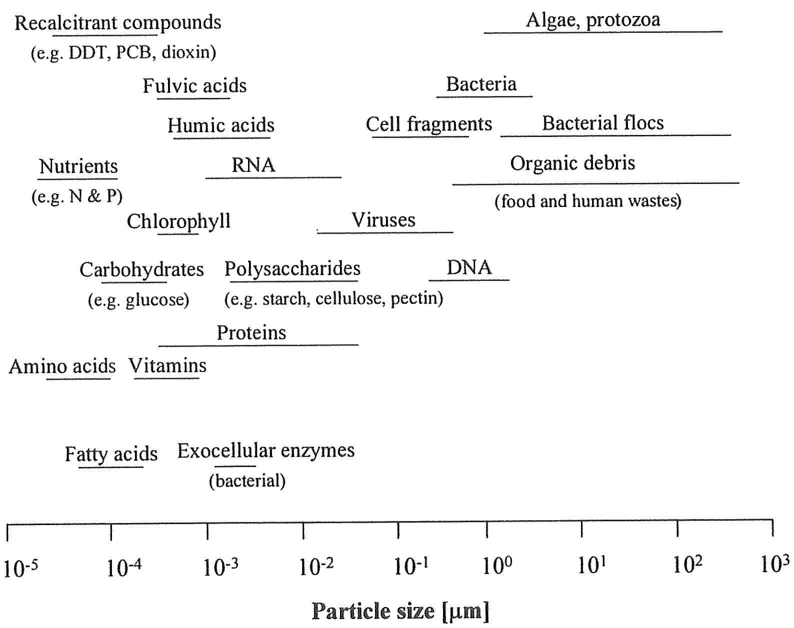


Figure 2 *Organic constituents and their approximate size ranges in settled municipal wastewater (adapted from Levine et al., 1985).*

The composition of the organic fraction in the wastewater affects the micro-flora, a process that takes place on a longer time scale (days). On the other hand, bacterial surface chemistry and the production of metabolic products such as EPS can probably change fast due to changes in the composition of organic compounds in the wastewater (Section 2.2). The types of bacteria present determine to a large extent the structure of the activated sludge flocs and, thereby, the separation properties (Section 2.3). Components such as toxic compounds, sulphides, detergents and metals can disturb the biological processes, sometimes enough to produce instantaneous floc break-up, which deteriorates the quality of the effluent and causes poor settling and compaction properties. Other factors such as ionic strength, water hardness, pH, and temperature may also affect the flocculation properties of the activated sludge (Section 2.2.4).

Hydraulics

Large changes in the hydraulic loading of a wastewater treatment plant cause major disturbances in the process, especially in the clarification. Often, the result of increased hydraulic loadings is an increase in the concentration of suspended solids in the effluent. During high hydraulic loadings, the sludge blanket in the settlers rises since sludge is pumped to the settler faster than it settles. Often, the return sludge pumping is increased to remove some of the accumulated sludge, something that may fuel the hydraulic disturbances even more. As the water entering the settler and the clarified water have different densities, a density current is formed which flows close to the sludge blanket (Lumley and Balmér, 1991). Consequently, the increased water flow through the settler may scour the sludge blanket and suspended solids are lost to the outlet of the settler. Overloaded settlers therefore exhibit large variations in effluent suspended solids concentration. According to Bergh and Olsson (1996), there is a relationship between the flow into the treatment plant and the concentration of suspended solids in the effluent; the time constant of this relationship is strongly dependent on the floc properties. Small changes in floc structure can have large consequences for the performance of the settlers. Other factors such as transportation in channels, pumping, aeration etc. may affect the sludge flocs, as is described in Section 2.3.4.

During high hydraulic loading, the composition of the wastewater is changed as well (e.g. lower temperature, lower ionic strength, lower concentrations of organic material than for normal inflows), which can affect the floc stability very fast and, thereby, also the suspended solids concentration in the effluent (Sections 2.2 and 2.3). To minimize this type of problem, the flow into the wastewater treatment plant can be equalized by means of buffer tanks.

2.1.3 Concluding remarks

The introduction of new treatment processes for wastewater, for example biological N and P removal, has made the activated sludge systems even more complicated. New process designs can alter the microbial composition of the activated sludge quite radically. This makes it very important to understand the microbiological processes taking place in the degradation of organic material, nutrient removal, and the processes that affect the floc properties. Besides, the increasing demand for more energy and resource efficient systems has made it necessary to run treatment plants in such a way that sludge with good flocculation properties is produced without adding flocculation agents (e.g. poly-electrolytes). Tertiary treatment of the effluent with, for example, filtration is efficient, but rather costly.

Therefore, to run treatment plants in an efficient way, the connections between wastewater composition, treatment plant configuration and operation, and microbiological processes have to be elucidated.

2.2 Flocculation and Adhesion of Bacteria in Activated Sludge

Microorganisms often occur as flocs or biofilms in natural aquatic systems (Marshall, 1981). For example, in freshwater rivers, particles are often transported as large flocs (Droppo and Ongley, 1994). Although various chemico-physical parameters influence the flocculation in fresh water, such as ionic strength, pH and amount of organic compounds, the amount of bacteria present is decisive. Bacteria, which colonize the particles, produce extracellular polymers that contribute to the formation of dense aggregates. These freshwater flocs seem to have much in common with activated sludge flocs. Microorganisms attached to surfaces or aggregated in flocs or biofilms in an aquatic environment probably have advantages in their enhanced nutrient supplies from material adsorbed to the surface, as well as their protection from shear forces (Fowler 1988). Microorganisms in flocs and biofilms can also establish close interactions with each other, which helps to protect them from desiccation. Usually, flocs and biofilms contain extracellular polymeric substances (EPS) which act as ion exchange resins, attracting ions and molecules that contribute to the formation of dense flocs or biofilms. Similar mechanisms are probably involved in the aggregation of many different types of cells (Atkinson and Daoud, 1976), which is why knowledge of the flocculation of other types of cells can contribute to the understanding of the mechanisms involved in the flocculation of bacteria in activated sludge. However, the flocculation of activated sludge cannot be explained by a single mechanism: many factors interact. In contrast to pure cultures, the bacteria in activated sludge flocs constitute only a minor fraction of the organic material and the interaction between other floc components is also of great significance.

2.2.1 Composition of activated sludge flocs

Activated sludge flocs are a complex mixture of different microorganisms (mainly diverse types of bacteria, of which only a small fraction has been identified), dead cells, particulate organic and inorganic material, and extracellular polymeric substances (EPS). The EPS bind the floc components together and contribute to the formation of dense colonies of bacteria within the floc (Snidaro *et al.*, 1997). The structure of the flocs is very heterogeneous (Li and Ganczarczyk, 1990; Zartarian *et al.*, 1994) and the size range can be as wide as from a few microns up to 1000 μm (Parker *et al.*, 1971; Li and Ganczarczyk, 1991). The sludge flocs contain large quantities of water (Li and Ganczarczyk, 1990).

The flocs contain approximately 30 - 40% inorganic matter and 60 - 70% organic matter. The organic fraction is composed of 50 - 60% EPS, 10 - 30% other organic matter and only 2 - 20% cell biomass (Frølund *et al.*, 1996). The chemical composition of the organic fraction of the activated sludge flocs changes with wastewater composition and treatment plant design and operation. Approximately, the organic fraction of the sludge contains 50% protein, 20 - 30% humic substances and 10 - 30% carbohydrate (Nielsen *et al.*, 1996; Frølund *et al.*, 1994). Since organic compounds such as humic substances are absorbed in the EPS matrix, the concentration in the sludge increases at higher sludge ages (Keiding and Nielsen, 1997). The rest of the organic matter can be compounds such as uronic acid and nucleic acids (Urbain *et al.*, 1993; Nielsen *et al.*, 1996).

The EPS constitute the main components of the organic fraction of the activated sludge flocs. Since they are believed to play an important role in the floc formation, their composition and structure are described in more detail in the next section.

2.2.2 Extracellular polymeric substances (EPS)

Origin and function of EPS

The EPS can have two possible origins: the metabolism or lysis of microorganisms (proteins, DNA, polysaccharides and lipids) or the compounds in the wastewater, which are absorbed in the EPS matrix, e.g. cellulose and humic acids (Urbain *et al.*, 1993). The EPS excreted by the bacteria exist in two forms: as slimes or as capsular polymers (Madigan *et al.*, 1997). The capsule can be found close to the cell surface, while the slime layer is more loosely attached to the cell (Decho, 1990). The EPS have many functions in bacteria, such as retaining exo-enzymes near the cell surface (Frølund *et al.*, 1995; Thörn, 1997), and the binding of organic matter; they are also important for the attachment of cells to surfaces (Fowler, 1988). The DNA accumulates in the EPS matrix of activated sludge (Palmgren and Nielsen, 1996).

Chemical composition of EPS

Very heterogeneous data on the chemical composition of EPS can be found in the literature. The probable reason is the diverse polymer extraction and analysis methods used; this makes a comparison of studies all but impossible. To analyse the EPS, they have to be extracted from the microorganisms. The problem has been to find a method that extracts the EPS efficiently without causing lysis of the cells, which would contaminate the sample with intra-cellular polymers. The various extraction methods include: (1) heat extraction (Forster, 1971, 1985; Brown and Lester, 1980; Goodwin *et al.*, 1985; Morgan *et al.*, 1990); (2) ultracentrifu-gation (Brown and Lester, 1980; Novak and Haugan, 1981; Pavoni *et al.*, 1972; Eriksson *et al.*, 1992; Sanin and Vesilind, 1994; Magara and Nambu, 1976); (3) sonication (Jorand *et al.*, 1995); (4) chemical extraction (Brown and Lester, 1980; Horan and Eccles, 1986; Eriksson *et al.*, 1992); and (5) cation exchange resins (Frølund *et al.*, 1996). Ultracentrifugation is most likely too weak a method (Novak and Haugan, 1981), while heat extraction and chemical extraction are probably too harsh, thus causing cell lysis. In more recent studies it has been reported that extraction by means of a cation exchange procedure is efficient and it causes little or no cell lysis (Frølund *et al.*, 1996). The chemical composition of EPS from six studies is summarized in Table 1. It should be noted that comparing these studies is difficult since both the activated sludge compositions and the extraction methods used differed. In addition, the components in the EPS are measured as standard equivalents (in units of glucose, BSA, humic acid etc.) which do not say anything about the molecular structure of the various components.

Protein is the dominating compound, which contradicts older studies where it was often claimed that polysaccharides were the dominating compounds (e.g. Forster, 1971; Horan and Eccles, 1986). The EPS contain approximately 2 - 4 times more protein than the rest of the sludge (Nielsen *et al.*, 1996). Higgins and Novak (1997b) studied the composition of the protein fraction of the EPS and their amino acid analysis showed that the proteins were lectin-like. They suggested that lectins, which are non-enzymatic proteins that bind sugar residues, are strongly involved in the bioflocculation. Dignac *et al.* (1998) analysed the EPS and found that the dominating compounds were negatively charged and hydrophobic amino acids, which would promote flocculation. The polysaccharides in the EPS consist mainly of monomers of glucose, mannose, galactose, glucuronic acid and uronic acid (Steiner *et al.*, 1976; Horan and Eccles, 1986).

Table 1 *Chemical composition of extracted extracellular polymeric substances (EPS).*

	Extraction procedure	EPS [mg/gVS*]				
		Protein	Humus	Carbohydrate	DNA	Uronic acid
Urbain *et al.* (1993)	Sonication	8 - 204	–	8 - 31	12 - 37	–
Frølund *et al.* (1994)	Cation exchange resin	49 - 54	–	6	–	1.6 - 2.0
Jorand *et al.* (1995)	Sonication	120	–	30	30	–
Frølund *et al.* (1996)	Cation exchange resin	243 ±7	126 ±1	48 ±1	–	6.1 ±0.2
	Sodium hydroxide	96 ±4	–	22 ±2	–	3.1 ±0.3
	Heat	121 ±3	–	8 ±2	–	2.2 ±0.2
Nielsen *et al.* (1996)	Cation exchange resin	212 ±1	101 ±2	40 ±3	16 ±0.5	3.9 ±0.2
Dignac *et al.* (1998)	Sonication+exchange resin	242 ±24	–	11.4 ±2	–	–

**VS = volatile solids (i.e. the organic fraction of the activated sludge)*

Physical properties of EPS

Most of the EPS contain differently charged functional groups, such as hydroxyl and carboxyl groups, with a high binding capacity of metals, particles and microorganisms (Christensen, 1989). They also contain hydrophobic sections. Urbain *et al.* (1993) suggested that hydrophobic bonds between EPS molecules are involved in the flocculation. Due to the heterogeneous functional groups, the EPS can probably bind by hydrophobic interaction, protein-polysaccharide interaction, hydrogen bonding and ionic interaction (Higgins and Novak, 1997b). The EPS are long chain molecules that can also bind the distinct floc components together by physical entanglement. The physical characteristics of the EPS are influenced by ionic strength, temperature and chemical composition of the polymers (Sutherland, 1988).

Production of EPS

The production of EPS may be a feature of a specific growth phase of the bacteria; different types of bacteria synthesize EPS of differing compositions (Sutherland, 1988; Whitfield, 1988). In several studies it has been found that the production of EPS is affected by their growth conditions. The types and concentrations of substrates have been found to affect EPS production, both in activated sludge (Horan and Shanmugan, 1986; Jorand *et al.,* 1994) and in pure cultures (Allison and Sutherland, 1987; Knox *et al.*, 1985; McEldowney and Fletcher, 1986). It has also been found that starvation leads to increased EPS production (Bengtsson, 1991; Brown *et al.*, 1977). In another study, Jorand *et al.* (1994) found that when a dose of glucose was added to activated sludge, the production of exopolysaccharides increased. Palmgren (1996) has made an extensive summary of the various environmental factors that affect EPS production in pure culture. As the composition of activated sludge flocs is complex, small changes in EPS composition and concentration during short term changes of the environment surrounding the flocs are difficult to determine; however, this is possible in pure cultures. Palmgren and Nielsen (1998) showed that the EPS production of the model organism *Pseudomonas putida* decreased when oxygen was limited.

Few studies can be found in the literature that compare the amount of EPS in different types of sludges. Frølund *et al.* (1994) compared EPS from an advanced biological nitrogen and phosphorus removal treatment plant and a traditional one. The sludge from the conventional plant contained younger flocs, which had more polysaccharides than those in the advanced plant. The polymers in the advanced plant were more hydrophobic than those in the conventional one, which could explain why sludge from plants performing biological P removal often have very good flocculation properties (Andreasen and Sigvardsen, 1996). Urbain *et al.* (1993) investigated the chemical composition of the EPS for 16 different sludges and found that the ratio between protein, DNA and polysaccharide was almost constant for all of them.

The role of EPS in the floc formation and settleability

The exact role of EPS in the floc formation of activated sludge is not completely understood: various contradictory data can be found in the literature. Busch and Stumm (1968) found that EPS extracted from well-flocculated activated sludge improved the performance of poorly-flocculated sludge. Pavoni *et al.* (1972) found that there was a positive correlation between amount of EPS and flocculation properties and that the removal of the EPS caused floc dispersion. The extracted EPS could flocculate inorganic dispersions of kaolinite, silica and alumina. Wahlberg *et al.* (1992) and Mikkelsen and Keiding (1999b) found a reduction in floc strength at decreased concentrations of EPS. Mikkelsen and Keiding (1999b) also observed that when the amount of EPS fell below a certain level, the flocs became smaller. In various studies it has been found that the settling properties, measured as sludge volume index (SVI), deteriorate as the amount of EPS increases (Forster, 1968; Magara and Numbu, 1976; Forster, 1985; Urbain *et al.*, 1993). Mikkelsen and Keiding (1999b) showed that increasing the amount of EPS in the sludge could be directly related to the negative charge of the flocs. The increase in SVI could therefore be due to a stronger electrostatic repulsion between floc components as described by the DLVO theory (Section 2.2.4). Many of the earlier studies of EPS concentration in sludge in relation to the settling properties are questionable due to poor extraction and analysis techniques; it is also quite probable that other parameters in the sludge were changed during handling. The relationship between the relative amounts of the diverse constituents in the EPS and the flocculation properties is not yet fully elucidated.

2.2.3 Cations

In several studies, it has been reported that the removal of the divalent cation Ca^{2+} causes removal of biofilms (Turakhia *et al.*, 1983) and deflocculation of activated sludge (Eriksson and Axberg, 1981; Kakii *et al.*, 1990; Eriksson and Alm, 1991; Eriksson *et al.*, 1992; Bruus *et al.*, 1992; Urbain *et al.*, 1993; Zita and Hermansson, 1994; Higgins and Novak, 1997a; Keiding and Nielsen, 1997). The EPS, cell surfaces and inorganic particles (e.g. calcium phosphate, iron oxides) in the sludge have negatively charged groups which have a high affinity for cations (Forster, 1968; Steiner *et al.*, 1976; Goodwin and Forster, 1985; Urbain *et al.*, 1993). Bruus *et al.* (1992) suggested that divalent cations bridge negatively charged groups on alginate-like polysaccharides within the flocs. Higgins and Novak, (1997b) suggested, on the other hand, that divalent cations bind mainly to the proteins in the EPS. Besides calcium, other cations such as copper and cobalt were found to have a higher affinity for the EPS than for the sludge as a whole; adsorption isotherms are not the same for different types of metal ions (Steiner *et al.*, 1976; Forster, 1985). Bruus *et al.* (1992) observed that Ca^{2+}-ions were replaced by Cu^{2+}-ions, which resulted in a more stable floc structure. There are also some indications that Mg^{2+} is a poorer flocculant than Ca^{2+} (Higgins and Novak, 1997a; Urbain *et al.*, 1993), due possibly to the higher hydration of Mg^{2+}. Zita and

Hermansson (1994) suggested that cations contribute to the flocculation because they contribute to ionic strength, according to the DLVO theory.

Fe-ions, especially Fe^{3+}, are strongly bound to the sludge flocs (Nielsen and Keiding, 1998); their removal causes a much stronger deflocculation than the removal of, for instance, Ca^{2+}. Humic substances, which are adsorbed in the EPS matrix, also exhibit a high affinity for metal ions, especially Fe^{3+} (Stevenson, 1982). Cations that have a higher valency and are less hydrated seem to bind better to the sludge flocs, thereby forming flocs of a higher stability.

High concentrations of monovalent cations can cause deflocculation, probably by means of ion exchange process in which the monovalent ions, with much poorer flocculation properties than divalent cations, replace the divalent cations (Bruus *et al.*, 1992; Higgins and Novak, 1997a). Increased turbidities of effluents from wastewater treatment plants have been observed during the snow-melting period (Eriksson *et al.*, 1992). This could be due to an ion exchange process involving high concentrations of Na^+ with Ca^{2+}. Cations such as NH_4^+ may have the same effect on floc stability, as for example Na^+ (Murthy *et al.*, 1998). Nitrification (conversion of NH_4^+ to NO_3^-) could thus have a positive effect on the floc properties.

2.2.4 Conceptual floc models

The complexity of the flocculation of activated sludge is due to the large variety of bacteria and other components present. In the literature only few studies can be found that deal with flocculation properties of bacteria isolated from activated sludge (Jorand *et al.*, 1994; Kurki *et al.*, 1989; Singh and Vincent, 1987; Zita and Hermansson, 1997a; Zita and Hermansson, 1997b). The studies show that various types of interactions are involved. Mechanisms responsible for pure culture flocculation vary for different species and may not be the same as the mechanisms involved in flocculation of activated sludge. Several conceptual models for the mechanisms of flocculation have been put forward.

The polymer bridging model

In the polymer bridging model (Busch and Stumm, 1968; Pavoni *et al.*, 1972; Eriksson *et al.*, 1992), it is suggested the bacteria excrete EPS which join the cells together by means of di-valent cations such as Ca^{2+} and Mg^{2+}. The EPS are, as mentioned before, high-molecular-weight compounds that extend outward from the cell surface and can form bridges between several cells (steric interaction). Furthermore, EPS contain functional groups that are primary anionic and nonionic in the neutral pH range. Divalent cations bind electrostatically to the negatively charged functional groups to generate strong bridges between floc components.

The filamentous backbone model

In the filamentous backbone model (Sezgin *et al.*, 1978; Parker *et al.*, 1971), it is suggested that filamentous bacteria form a backbone onto which EPS producing bacteria can attach themselves. It has also been proposed that the filaments give rise to stronger flocs (Parker *et al.*, 1971, 1972). However, the filaments are not believed to affect the attachment of other floc components to the flocs. Nowadays it is recognized that filamentous bacteria are not a pre-requisite for flocculation.

The colloidal interaction model

The colloidal interaction model is based on the idea that the interactions between the floc components can be described by the DLVO theory for colloidal stability (Zita and Hermansson, 1994), developed independently by Deryagin and Landau (1941) and Verwey and Overbeek (1948). The DLVO theory has also been shown to describe the interactions involved in bacterial attachment to surfaces (e.g. Fowler, 1988; Loosdrecht *et al*,. 1990; Loosdrecht *et al.*, 1987a; Mozes *et al.*, 1989; Rijnaarts *et al.*, 1995). Not only single bacteria but also larger floc constituents can be described as colloids. Colloidal systems include particles in the size interval 1nm - 1 µm (Shaw, 1992). These particles are affected by Brownian movements: they are not settleable. Larger particles are influenced more by gravity in the particle interaction. In practice, however, particles up to 1000 µm can be described as colloids (Gregory, 1989).

In this model for colloidal stability, the degree of interaction, depends on the surface potential and on the thickness of the electrical double layers. The principles for the DLVO theory are briefly outlined. Most natural surfaces, including activated sludge flocs, are negatively charged (Gregory, 1989). In aquatic environments the negative surfaces are surrounded by oppositely charged ions, of which some are bound to the surface and others are distributed in a diffuse layer. This leads to repulsive electrostatic interactions between approaching surfaces. The most common interactions between colloids are the *van der Waals attraction* and *electrical repulsion.*

In the DLVO theory, the van der Waals attraction and electrical double layer repulsion are assumed to be additive and combined to give the total Gibbs interaction energy between particles as a function of the separation distance (Figure 3). When the repulsion outweighs the attraction, there is a potential energy barrier, which hinders contact between particles. For two particles to aggregate, this energy barrier must be overcome. Once the barrier has been overcome, the particles are strongly held in the deep primary minimum. If the particles are interacting with each other in the secondary minimum, which is much shallower, the bonding is weaker. The van der Waals attraction forces are determined basically by the separation distance between two particles, the geometry of the system, and the Hamaker's constant (Shaw, 1992). The Hamaker's constant depends on the hydrophobicity of the interacting compounds (Loosdrecht *et al.*, 1990). The electrical repulsion is influenced by the thickness of the double layer, which is inversely proportional to the square root of the ionic strength. This means that, if the electrolyte concentration is high or if there are polyvalent counterions present, the electrostatic repulsion is reduced (decreased energy barrier) and bacteria and other floc constituents can more easily adhere to each other. Divalent and trivalent cations affect the interactions between colloids in two ways: they both reduce the particle surface potential, since they adsorb in the Stern-layer, and they increase the ionic strength (Shaw, 1992).

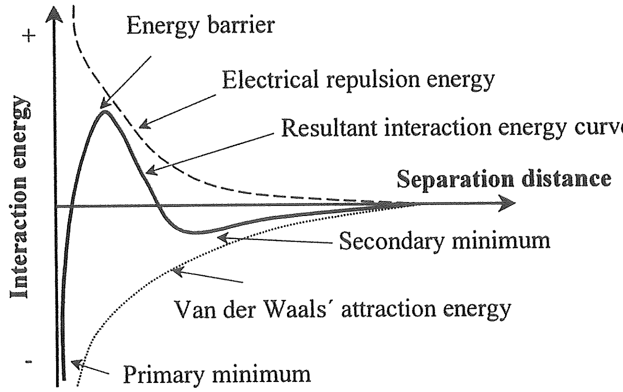


Figure 3 *Principal curve for the interaction energy between two particles as a function of separation distance according to the DLVO theory.*

The DLVO theory seems to be qualitatively valid for complex systems such as activated sludge flocs (Zita and Hermansson, 1994), even though other forces, e.g. hydrophobic interaction, hydration interactions and steric hinderance, are not included in this model.

Modified floc models

The three floc models described have been modified in a few ways to give a better description of an activated sludge floc. Eriksson *et al.* (1992) suggested a general model for activated sludge properties according to sludge ages or to variations in organic loading of the sludge flocs (Figure 4). It is based on the hypothesis that with high organic loading (or with a low sludge age), the EPS production is low and the cell growth is high (Busch and Stumm, 1968; Pavoni *et al.*, 1972; Li and Ganczarczyk, 1990). Therefore, the outer surface of flocs, which has younger cells, contains less EPS than the interior of flocs; the bacteria are bound together by a few polymer bridges. This would explain why the outer parts of the floc have a chain-like structure where the floc components are bound with weak forces due to the limited amount of EPS. It also explains why the outer parts of the sludge flocs are sensitive to high shear (Parker *et al.*, 1971). In the central part of the flocs, the cells are embedded as agglomerates in a strong EPS matrix. When a sludge floc is exposed to periods of higher organic loading, the outer surface of the flocs take on the properties of a "young" floc. This could explain why higher turbidities is often observed at high F/M ratios.

Jorand *et al.* (1995) suggested a modified floc model similar to the one proposed by Eriksson *et al.* (1992). The model has three structural levels of microflocs: primary particles (2.5 μm) and secondary particles which are microbial colonies (13 μm) that are linked together by exopolymers to form tertiary structures having a mean diameter of approximately (125 μm). Keiding and Nielsen (1997) modified the model further by describing the floc as being a cloud of organic macromolecules and single bacteria attached by very weak forces to a more rigid backbone consisting of fibers, filamentous bacteria and bacterial colonies embedded in a pool of EPS. It was found that the removal of Ca^{2+} from the bulk water by an ion exchange resin led to a release of Ca^{2+} ions from the flocs and a desorption of macromolecules such as humic substances and single bacteria. The removal of Ca^{2+} with an ion exchange resin yielded a higher negative surface charge of the flocs without significantly changing the ionic strength. Therefore, it was suggested that the observed deflocculation was due to an increase in the negative surface charge rather than to a decrease in ionic strength as suggested by Zita and Hermansson (1994).

The quality and properties of the EPS (i.e. molecular weight, charge density, and hydrophobic/hydrophilic properties) and of the charge neutralising ions (valency, polarizabilities and hydration) seem to decide the floc stability.

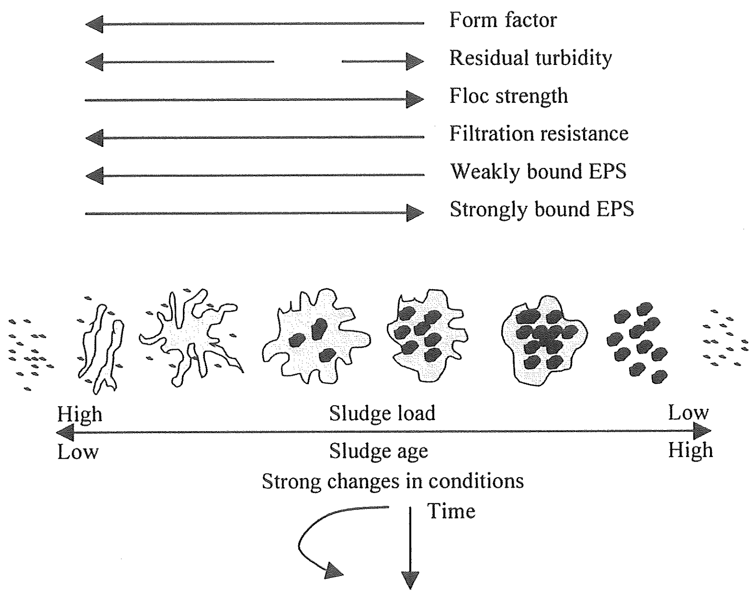


Figure 4 *A general model for activated sludge properties according to sludge ages or to variations in organic loading (adapted from Eriksson et al., 1992).*

Higgins and Novak (1997b) studied the relationship between the amount of exocellular polymer concentration and cation concentration in sludge flocs. They found that, as the concentration of divalent cations ($Ca^{2+}$and Mg^{2+}) was increased in the feed to an activated sludge pilot plant, the amount of extracellular protein rose in the sludge at the same time as the concentration of suspended solids in the effluent fell. When a monovalent cation (Na^{+}) was added, the amount of bound protein decreased and the concentration of suspended solids in the effluent increased simultaneously. Also, when an enzyme that degraded exocellular proteins (pronase) was added, a deflocculation was observed. When a polysaccharide-degrading enzyme was added, no deflocculation could be seen. Based on these results a new floc model was suggested. In the new model, the proteins in the EPS (which were found to be lectin-like) bind polysaccharides that are cross-linked to adjacent proteins, and divalent cations bridge the negatively charged sites on the EPS to stabilize the network structure. These results indicate that the amount of EPS in the sludge flocs is determined by both the production rate and the amount of divalent cations present which can bind the polymers.

In a study by Mikkelsen and Keiding (1999b), it was found that, as the concentration of EPS in the sludge become higher, the sludge flocs became less sensitive to shear. The stabilising role of the EPS on the floc structure was not in accordance with the DLVO theory, since the zeta-potential rose at high EPS concentration due to increased EPS charge content. It was therefore suggested that the physical entanglement of the EPS is more important than DLVO-type interactions for the floc structure, which is contrary to their earlier findings (Mikkelsen *et al.*, 1996). The results indicate that it is necessary to distinguish between long term and short term changes in floc structure. When various sludges are compared, the flocs with the higher content of EPS should be less shear sensitive. However, for a given sludge, sudden changes in the chemical environment could lead to greater shear sensitivity due to increased electrostatic repulsion or due to reduced hydrophobic interaction.

Hydrophobic interactions

Besides polymer bridging, steric interaction and DLVO-type interactions, other interactions such as hydrophobic interactions are involved in the flocculation. A colloid that is charged or polar is hydrophilic (strongly soluble in water) while a nonpolar colloid is hydrophobic (not soluble in water). In aqueous solutions, molecules with hydrophobic segments (e.g. long hydrocarbon chains) can be associated in such a way that contact between water and the hydrophobic regions is minimized. This is known as hydrophobic interaction (Gregory, 1989). Several studies have shown that the degree of hydrophobicity of bacteria influences the cell adhesion (Tenney and Stumm, 1965; Loosdrecht *et al.*, 1987b; Loosdrecht *et al.*, 1990; Mozes *et al.*, 1989; Rosenberg and Kjelleberg, 1986). Diverse factors have been found to affect the cell surface hydrophobicity: (1) growth conditions (Eisen and Reid, 1989; Grotenhuis *et al.*, 1992; Hazen *et al.*, 1986; Knox *et al.*, 1985; Loosdrecht *et al.*, 1987b; McEldowney and Fletcher, 1986); (2) starvation (Kjelleberg and Hermansson, 1984); (3) growth phase (Hazen *et al.*, 1986); and (4) temperature (Blanco *et al.*, 1997; Hazen *et al.*, 1986). Oxygen limitation can reduce the hydrophobicity of bacteria from activated sludge (Palmgren *et al.*, 1998).

Relatively little is known about the role of hydrophobicity in the flocculation of activated sludge. Valin and Sutherland (1982) and Singh and Vincent (1987) observed that there was a positive correlation between the hydrophobicity of activated sludge flocs, or bacteria isolated from activated sludge, and their flocculation properties. Jorand *et al.* (1994) found that hydrophobic and hydrophilic bacteria coexist in the activated sludge flocs and that the degree of hydrophobicity could change in the course of growth. Zita and Hermansson (1997a) measured the hydrophobicity of single bacteria between the activated sludge flocs and found that most of them were highly hydrophilic. This indicates that the cell surface hydrophobicity is of significance for bacterial adhesion to flocs. In another study, Zita and Hermansson (1997b) found that the cell surface hydrophobicity of both well-characterized *Escherichia coli* strains and isolates from wastewater correlated well with increased adhesion to sludge flocs. Olofsson *et al.* (1998) studied the attachment of hydrophobic and hydrophilic bacteria to activated sludge flocs. High degrees of cell surface hydrophobicity correlated well with adhesion to flocs. Hydrophobic cells attached not only on the surface but also within the flocs, whereas hydrophilic cells did not. Urbain *et al.* (1993) proposed that hydrophobic bonding inside the flocs, between EPS molecules, is important for the flocculation process.

The cell surface hydrophobicity of bacteria from activated sludge has been measured by several methods such as bacterial adhesion to hydrocarbons (Zita and Hermansson, 1997a; Jorand *et al.*, 1995; Palmgren *et al.*, 1998); hydrophobic interaction chromatography (Zita and Hermansson, 1997b); salting out techniques (Urbain *et al.*, 1993); and microsphere adhesion to cells (Olofsson *et al.*, 1998; Zita and Hermansson, 1997a). The biggest problem is to interpret the results from such a heterogeneous system as activated sludge.

Based on the literature review, a schematic description of an activated sludge floc is suggested (Figure 5).

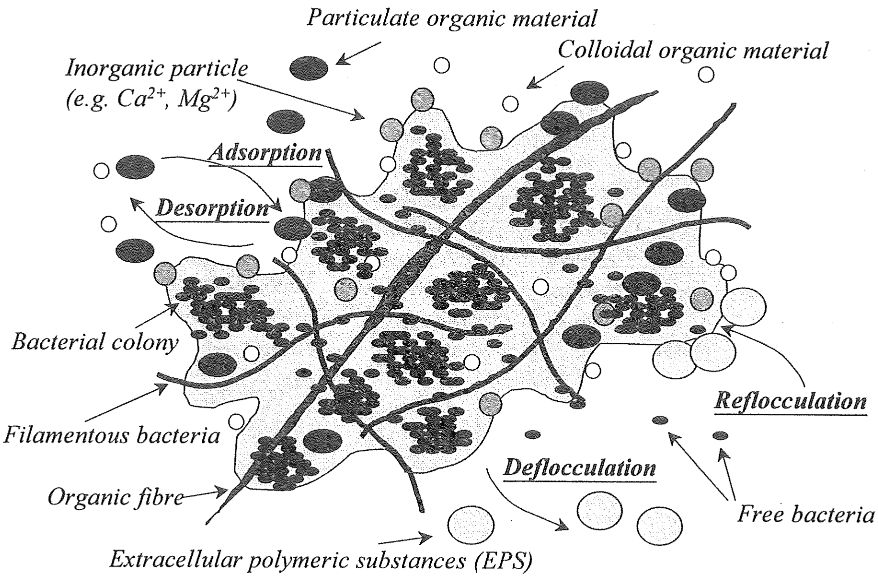


Figure 5 *Schematic drawing of an activated sludge floc.*

2.2.5 Conclusions

It can be concluded in general that activated sludge flocs have a very complicated structure and several mechanisms are involved in the floc formation. Furthermore, it is not only physico-chemical aspects that must be taken into account, since the composition of the flocs is constantly changing due to microbial degradation and the formation of new products (e.g EPS). Some properties of the sludge flocs change on a long term scale (e.g. structure, bacterial population, floc size), while other properties change fast (e.g. surface chemistry).

In activated sludge flocs, bacterial colonies, single bacteria, and organic and inorganic particles are embedded in a matrix of extracellular polymeric substances. The bacteria constitute only a small fraction of the organic part of the flocs, while the EPS is the major organic fraction. This makes it quite clear, that floc stability rests largerly on both the amount and the properties of the EPS.

It is still not known what components in the EPS are most important for the flocculation of sludge. The EPS consist mainly of protein, carbohydrate and humic substances. Proteins are the dominating compounds and, in recent studies, it has been suggested that the proteins are particularly important for the flocculation. The concentration of humic substances depends primarily on the amount adsorbed to the sludge matrix from the wastewater. However, since the humic substances appear to be loosely bound to the flocs, they may be desorbed easily if the sludge flocs are subjected to changed environmental conditions.

The importance of divalent cations for good flocculation is indisputable. However, the exact mechanisms involved are still being debated. Three mechanisms have been suggested: (i) the flocs are glued together by negatively charged EPS that are joined electrostatically by divalent cations such as Ca^{2+}; (ii) the divalent cations determine the charge density of the EPS which glue the floc components together by means of electrostatic interactions, van der Waals interaction and hydrophobic interactions; and (iii) cations decrease the electrical double layer thickness (which is inversely proportional to the square root of the ionic strength) according to the DLVO theory, thereby promoting flocculation. Most probably, a combination of the suggested mechanism is responsible for the importance of cations (mainly divalent) in floc formation.

The amount and structure of EPS decide the physico-chemical properties of sludge flocs, provided enough divalent cations are present to flocculate the EPS components. The EPS components at the outer parts of the flocs, in particular, are bound with weak forces and are sensitive to small changes in cation concentration. The interior is more firmly bound in a dense matrix of EPS.

Little is known about the variation in EPS production and cell surface hydrophobicity. Research on pure cultures shows, however, that the EPS production as well as hydophobicity is affected by the growth conditions and it is probable that the same applies for bacteria present in activated sludge.

2.3 Settling and Separation Properties of Activated Sludge

Many of the operational problems at a wastewater treatment plant are directly caused by poor floc quality. The three separation processes taking place in a treatment plant are favoured by different properties of the sludge flocs.

The *clarification* process is favoured by irregularly shaped and large flocs that sweep smaller flocs with them during settling. The amount of single bacteria and small flocs between the larger flocs must be low in the bulk phase. The flocs should also be strong to avoid break-up, which leads to increased concentrations of suspended solids in the effluent.

The *settling and thickening* process is promoted by large, regularly shaped and compact flocs, with few filamentous bacteria which settle fast and compact readily.

The *dewatering* process is favoured by large and strong flocs of a uniform size. Small flocs increase the filtration resistance in particular (e.g. Karr and Keinath, 1978).

This shows that it is not easy to define an "ideal" floc which is appropriate for all solid-liquid separation processes. There are various types of separation problems. Sometimes, the problems are related to the structure and size of the flocs and in other cases the problems are due to substances in the raw wastewater or compounds produced during the metabolism. To take appropriate measures, the reasons for the poor separation properties must be known. Hence, several investigations have been made to correlate the morphology of the flocs to the process parameters by means of microscopy (Eikelboom and van Buijsen, 1983; Jenkins *et al.*, 1993). Flocs with good separation properties are generally thought to be made up of floc-forming bacteria and a moderate number of filamentous bacteria that form a backbone onto which the floc-forming bacteria can attach (Sezgin *et al.,* 1978). In Figure 6, examples of activated sludge flocs with different settling, compaction and clarification properties are schematically illustrated. The five most common separation problems are outlined.

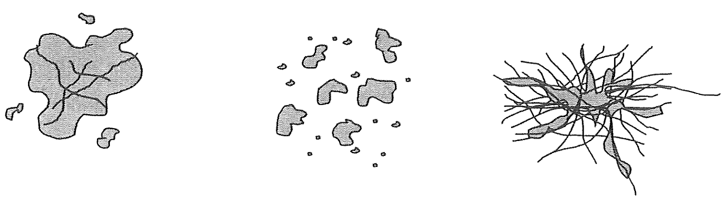
Pin-point flocs are small flocs and dispersed bacteria which do not settle by gravity. They cause a poor clarification with high turbidity of the effluent (Pipes, 1979) and decreased dewaterability (Karr and Keinath, 1978; Barber and Veenstra, 1986; Novak *et al.,* 1988; Bruus *et al.*, 1992; Rasmussen *et al.,* 1994; Sørensen *et al.,* 1995; Mikkelsen *et al.,* 1996).

Filamentous bacteria are a problem when excessive growth (Eikelboom and van Buijsen, 1983): this produces a sludge with less efficient compaction and settling ability (bulking sludge).

Zoogloeal bulking is caused by zoogloeal microorganisms forming finger-like colonies that excrete EPS, which gives the flocs a very voluminous and slimy character. In excessive amounts it can cause foaming and scumming and a sludge that settles and compacts poorly, often called non-filamentous bulking (Novák *et al.*, 1993, 1994).

Rising sludge originates from biological denitrification, by which nitrogen gas is formed. The nitrogen gas is poorly soluble and bubbles can adhere to activated sludge flocs and flotate them to the surface of the settlers (Henze *et al.,* 1993).

Scumming describes another common problem arising from the formation of foam caused by non-degradable surfactants or by the presence of the bacteria *Nocardia sp., Actinomycetes* or *Microthrix parvicella.* The sludge is then transported to the surface of the settlers as a thick foam (Kappeler and Gujer, 1994; Blackall, 1994).



a) Floc with good settling properties: large and compact with few filamentous bacteria

b) Pin-point flocs: small, porous and weak flocs

c) Floc with poor settling properties: excessive numbers of filamentous bacteria and with an irregular shape

Figure 6 *Schematic illustration of activated sludge flocs with different clarification, settling and compaction characteristics.*

2.3.1 Characterization of settling and separation properties

Sludge volume index

The most common way to evaluate how well a sludge settles and compacts is to measure the sludge volume index (SVI) which is the volume occupied by 1 g of sludge after 30 minutes of settling in a cylinder. This is not a scientific parameter and it does not say anything about the composition of the sludge. However, it is a valuable operational parameter that is well correlated to the function of the settlers. A low SVI generally indicates a well flocculated sludge with good settling properties. Values of SVI up to 100 ml/g are considered satisfactory. When the SVI > 150 ml/g, the sludge is considered be bulking, i.e. the sludge has very poor settling and thickening properties. The SVI is very non-specific and it is, apart from the structure of the flocs, affected by parameters such as suspended solids concentration, cylinder diameter and height, temperature and stirring (Dick and Vesilind, 1969). There are several modifications of the traditional SVI, to minimize the influence of suspended solids concentration and wall effects, such as stirred specific volume index (SSVI), diluted sludge volume index (DSVI) and sludge quality index (SQI) (Eriksson and Härdin, 1984; Hultman *et al.*, 1991). The use of DSVI is often recommended. It is important to note that the settling characteristics in a settling cylinder are not the same as in a full-scale plant; hence, it is meaningless to compare SVIs for different plants. Even though the SVI has been criticized for its inconsistency, it is measured regularly at most treatment plants. Its main advantages are that it is easy to measure and that by following it at a specific wastewater treatment plant, changes in the floc structure can be detected.

The floc characteristics which have been found to affect the SVI are: (1) number and type of filamentous bacteria and/or zoogloeal bacteria (e.g. Eikelboom and van Buijsen, 1983; Eriksson and Härdin, 1984); (2) median floc size and density for non-filamentous sludge (Andreadakis, 1993; Barber and Veenstra, 1986; Sadalgekar *et al.*, 1988); and (3) amount of EPS in the sludge (Urbain *et al.*, 1993).

Initial settling velocity

The initial settling velocity is a key parameter, since it determines how much the secondary settlers can be loaded. When the initial settling velocity is measured at different mixed liquid suspended solids (MLSS) concentrations, it describes the settling properties according to the solids flux theory; this makes it possible to model mathematically the performance of the settlers. When a concentrated suspension of activated sludge settles in a cylinder, various types of settling take place as illustrated in Figure 7. In the hindered settling zone, a relatively sharp interface forms between the sludge and the water; the downwards movement of this interface with time is defined as the initial settling velocity (or zone settling velocity). This is often measured routinely at wastewater treatment plants. The initial settling velocity depends on the diameter of the settling cylinder, the concentration of mixed liquid suspended solids, the sludge volume, the temperature in the water as well as on the floc structure (e.g. density, shape, filamentous bacteria), and size (Daigger and Roper, 1985; Göhle and Björlenius, 1996). By looking at the interface between sludge and water, some hints about the floc structure can be obtained. When filamentous bacteria are present, the interface is often very sharp but when lots of small flocs and dispersed bacteria are present, the interface can be very blurred. Various attempts have been made to correlate the SVI and/or suspended solids concentration to the initial settling velocity (e.g. Pittman, 1984; Daigger and Roper, 1985; Cho *et al.*, 1993) with reasonable success.

By measuring the initial settling velocity regularly at a wastewater treatment plant, changes in the floc structure can be detected. However, if filamentous bacteria are present and when the

SVI is high, the values can deviate strangely. Better results are generally obtained when the sludge is diluted to a sludge volume (SV) lower than about 250 ml/l. The initial settling velocity is more valuable in designing secondary settlers or in modelling their performance than for investigating the structure of the flocs.

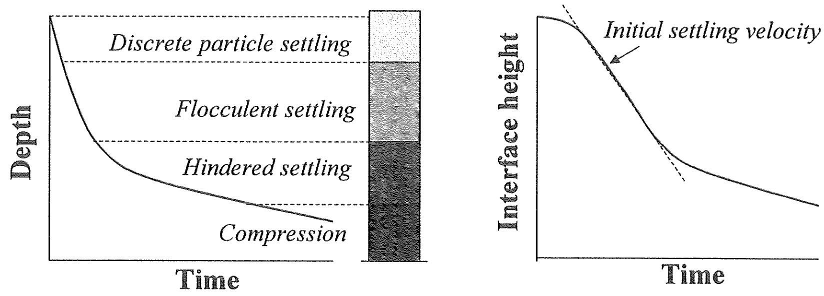


Figure 7 *Settling of activated sludge in a cylinder.*

Non-settleable solids
To determine the number of small flocs and free bacteria that cannot settle, the amount of suspended solids remaining in the supernatant after settling is measured. Often, turbidity is measured instead of suspended solids concentration because of its simplicity and sensitivity. This turbidity is often called the residual tubidity after settling. Reasonably good correlations between suspended solids concentration and turbidity have been found (Wahlberg, 1992; Wilén, 1997). The turbidity has been found to correlate well with the number of free cells in the supernatant and with the sludge filterability (Rasmussen *et al.*, 1994; Sørensen *et al.*, 1995). The results obtained when the sludge settles in a cylinder are not necessarily the same as the ones obtained in a full scale plant, since the hydraulic conditions are different. When the sludge flocs settle, small flocs that cannot settle by themselves are trapped in the floc matrix; thus the residual turbidity can be affected by the floc morphology.

2.3.2 Bulking sludge

Since the field of bulking sludge is very large and complicated and an enormous amount of research has been carried out, this summary is selective. High SVIs are usually a result of excessive growth of filamentous microorganisms. Generally, a sludge with SVI > 150 ml/g is considered be being bulking. In severe cases, the activated sludge hardly settles at all and the sludge flocs escape the secondary settlers into the effluent. If too much sludge is lost from the system, the sludge age gradually decreases and the treatment efficiency deteriorates. Filamentous bacteria exist in most activated sludge treatment plants and they can contribute to stronger flocs and a clearer effluent (Parker *et al.*, 1971). However, if the filaments grow too much, the problems arise. The basis for the study of filamentous bacteria has until recently been the identification scheme of Eikelboom and van Buijsen (1983), in which the identification was based on morphological characteristics and staining techniques. Recently, molecular methods have been introduced to improve identification of the filaments (Amann *et* *al.*, 1990; 1995), since this can be very difficult when done by micrcoscopic investigation. The filamentous bacteria that cause bulking are a wide and heterogeneous group (Eikelboom and van Buijsen, 1983; Jenkins, 1993). The various filamentous microorganisms can grow in dissimilar forms such as rigid, straight or coiled. Some filamentous bacteria grow mainly inside the flocs, while others grow predominantly between them. Since the forms of filamentous bacteria vary, it can be expected that differing types of filaments affect the settle-ability in different ways. The colloidal properties of the flocs also affect the settling properties. For instance, if the flocs are very compact, they can have good settling properties even though they contain high numbers of filamentous bacteria.

The factors which influence the formation of bulking sludge can be divided into general factors and specific ones (Jenkins, 1992). The general factors are sludge age, aeration basin configuration, reactor design and settler design. Exemples of specific factors are dissolved oxygen (DO) concentration, concentration of nutrients such as nitrogen and phosphorus, pH, sulphide concentration, nature and biodegradability of the organic substances in the waste-water, and temperature. The problem with bulking sludge is complicated by the great variety of filamentous microorganisms, many of which can thrive in different environments. However, it is often possible to minimize the growth of filamentous bacteria after identifying them and changing some of the factors mentioned above. The specific factors are often easiest to change.

The most usual way to change the general factors is to install selectors in the treatment plant (Rensink and Donker, 1991; Jenkins, 1993; Wanner, 1994; Chudoba and Pujol, 1994). The selectors promote the growth of floc-forming bacteria and suppress the growth of filamentous bacteria (Chudoba *et al.,* 1973a,b, 1974, 1994; Rensink, 1974; Wanner, 1994). In a selector, the food-to-microorganisms (F/M) ratio is high or the conditions are anoxic, which repress the growth of several types of filamentous bacteria. It has been suggested that at low substrate concentrations the filamentous bacteria grow faster than floc forming bacteria due to their higher surface-to-volume ratio, while the floc forming bacteria have higher growth rates at high substrate concentrations (Chudoba *et al.*, 1973a,b). Hence, the bacteria with lower specific growth rates and half saturation constants (which is typical for filamentous bacteria) have a competitive advantage at low substrate concentrations. This principle is known as the kinetic selection theory. Some filaments are unable to use nitrate as an electron acceptor or to take up substrate during anaerobic conditions; hence, anoxic or anaerobic selectors can be used to promote the growth of floc forming bacteria. This is known as metabolic selection. These types of filaments cannot grow in biological nitrogen and phosphorus removal plants. When installing selectors there is, however, a risk that solving one problem can cause others. The main reason is that knowledge of the microbiology of filamentous bacteria, as well as other bacteria, in the activated sludge is still poor.

It has been suggested that the ability to accumulate substrate is a key factor in the kinetic selection of bacteria in an activated sludge plant (Chudoba, 1985). The microorganisms which take up most substrate, in the selector or in the inlet part of the aeration tank, will dominate. Floc formers seem to have a greater ability to store substrates than filamentous micro-organisms, i.e. the floc formers have a competitive advantage under conditions of unbalanced growth (Majone *et al.*, 1999). Information about maximum growth rates and half-saturation constants for various substrates and oxygen can be used to predict which bacteria have an advantage over others (Lau *et al.*, 1984; Slijkhuis, 1983). The problem is that it is very difficult to determine the limiting factor. Wanner (1993) suggested that for mixed cultures cultivated with real wastewater, a multiple limitation is quite probable. This makes it very difficult to know what the limiting factor is.

Chiesa and Irvine (1985) suggested that there exist two main groups of filamentous bacteria: (i) fast-growing, starvation susceptible filaments, and (ii) slow-growing, starvation resistant filaments. Continually low substrate concentrations, as in extended aeration systems, was thought to favour the growth of slow growing filaments which have a high substrate affinity and a maximal resistance to starvation. On the other hand, a high substrate concentration and low dissolved oxygen concentration would favour the growth of fast growing filaments which have a high affinity for dissolved oxygen. Some filamentous bacteria can grow in a wide range of sludge ages while others grow in a narrower range.

In plants operated with very long sludge ages, typical of nitrifying-denitrifying activated sludge systems, a certain type of filaments has been found: low F/M filamentous bacteria (Gabb *et al.*, 1991). In many treatment plants built for biological nitrogen and phosphorus removal serious problems with bulking sludge and foaming have been experienced, due mainly to the filamentous bacteria *Microthrix parvicella* (e.g. Eikelboom and Andreasen, 1995; Andreasen and Sigvardsen, 1996; Knoop and Kunst, 1998, Wanner *et al.*, 1998). Because of the way they grow, *Microthrix parvicella* causes particularly severe bulking and foaming problems (due to their hydrophobic surfaces). It is not known exactly what factors govern its growth in nutrient removal plants. It is hard to isolate in the laboratory and, thus difficult to study (Slijkhuis, 1983; Blackall *et al.*, 1994, 1996). Sludge from biological phosphorus removal plants has been found to have good settling properties, even though filamentous bacteria often grow in this type of systems; this is due to the formation of more dense flocs (Andreasen and Sigvardsen, 1996).

Five factors that have been found to promote filamentous growth include: (1) readily degradable compounds such as saccharides, alcohols, low fatty acids, long chain fatty acids and amino acids (Wanner, 1993; Jenkins, 1992); (2) inorganic sulphur compounds (Echeverría *et al.*, 1992); (3) low dissolved oxygen concentrations (Palm *et al.*, 1980; Jenkins *et al.*, 1993); nutrient depletion (Horan and Shanmugan, 1986; Ericsson and Eriksson, 1988; Echeverrią *et al.*, 1993); and (5) low temperature (Knoop and Kunst, 1998).

To evaluate the impact of the filaments on the settling characteristics, the number of filaments has to be estimated. There are several methods, of which the easiest is to determine the filament index (Eikelboom and van Buijsen, 1983; Jenkins *et al.*, 1993). Although this index is based on personal judgement, it is easy to carry out. It is also possible to measure the length of the filaments protruding from the floc surface (Sezgin *et al.*, 1978).

2.3.3 Floc strength

The number of small particles present in the bulk water when sludge is exposed to turbulent shear is a measure of the floc strength. These small particles have been eroded from larger flocs , which is known as deflocculation (Parker *et al.*, 1971; 1972). The particles in activated sludge are divided into flocs approximately 25 - 300 μm and primary particles approximately 0.5 - 5 μm which are basically single bacteria and other floc components (Li and Ganczarczyk, 1990; Jorand *et al.*, 1995; Snidaro *et al.*, 1997). The number of primary particles at a given shear is a function of the flocculation properties of the sludge flocs. The primary particles are very small compared with the flocs; they are generally regarded as colloids with physical properties that differ from those of flocs.

Filamentous bacteria form a backbone in the floc and, due to their tensile strength, it has been suggested that they produce stronger flocs (Parker *et al.*, 1971). It is, however, not clear whether they also affect the erosion of small particles. In other words, floc strength is a decisive parameter, not only for the clarification process but also for the dewatering process, since both of these processes require low numbers of primary particles for a good function.

Erosion takes place when the shear forces exceed the strength of the bond between the primary particles and the floc. Consequently, the size of flocs subjected to shear is a function of the floc strength. According to Parker *et al.* (1971), small-scale eddies, on the same scale as the flocs, cause the greatest surface shear. Furthermore, the surface shear increases with floc diameter; thus there should be a maximum stable floc size at which the shear stress equals the shear strength. Parker *et al.* (1971) also introduced the concepts of fragmentation, which is the break-up of flocs into smaller ones, and aggregation, which is the formation of larger flocs when smaller ones adhere to each other. Fragmentation has a greater effect on the floc size than erosion; the number of primary particles can increase significantly without affecting the floc size much. Consequently, it is important to distinguish between these two processes which are very dissimilar in nature. Presumably, erosion is more common in wastewater treatment plants than fragmentation (Mikkelsen, 1999). The probable reason for this is that the EPS are very strongly crosslinked within the flocs so that strong forces are necessary to break the flocs.

Sometimes, particles collide: the probability of collision increases with turbulence. The turbulence also gives the particles kinetic energy. If the kinetic energy is high enough, the probability of flocculation increases (i.e. primary particles adhere to the existing flocs) in the primary minimum, according to the DLVO theory (Figure 3, Section 2.2.4.). Here the particles are held together very firmly in an irreversible manner. If the potential energy of the particles is too low to pass the energy barrier between them, a flocculation in the secondary minimum may occur (reversible flocculation). Consequently, when a suspension is stirred, flocculation and deflocculation (or erosion) occur simultaneously.

Flocculation of activated sludge has been explained by flocculation in the secondary minimum (Valin and Sutherland, 1982). This can be seen as deflocculation under turbulent conditions and flocculation at low shear levels. Hence, the flocculation is reversible. Sludge never deflocculates completely. Sometimes the bacterial colonies in the sludge are irreversibly flocculated in the primary minimum, while the colonies themselves are more weakly flocculated to each other in the secondary minimum.

Parker *et al.* (1971), and later Wahlberg *et al.* (1994), adopted a model, originally developed by Argaman and Kaufman (1968), which describes mathematically the simultaneous deflocculation and flocculation of activated sludge. Their purpose was to study the potential of activated sludge for improving the suspended solids removal by mechanical flocculation to incorporate the small particles into the larger flocs. This is very critical from a practical point of view since small particles can have a large impact on the effluent quality. They suggested that the change in number of primary particles (measured as the turbidity or suspended solids concentration after settling) in a stirred activated sludge suspension can be written as

$$\frac{dn}{dt} = k_B X G^2 - k_A X n G .$$

Integration of this equation gives

$$n_t = \frac{k_B G}{k_A} + \left(n_o - \frac{k_B G}{k_A} \right) e^{-k_A X G t}$$

where n_t is the number of primary particles at time t [number/l]; n_0 is the number of primary particles at time 0 [number/l]; t is time [s]; X is the concentration of suspended solids [g/l]; G is the velocity gradient [s^{-1}]; k_A is the floc aggregation rate coefficient [l/g]; and k_B is the break-up rate coefficient [number·s/g]. G is defined as (Camp and Stein, 1943)

$$G = \sqrt{\frac{P}{\eta_0 \cdot V}}$$

where P is the power input to the reactor [W]; V is the reactor volume [m^3]; and η_0 is the viscosity of the suspension [kg/m·s]. The break-up rate coefficient, k_B, is inversely proportional to the floc strength, while the aggregation rate coefficient, k_A, is dependent on the flocculation equipment. The flocculation-deflocculation model is illustrated in Figure 8. At low G values, the number of primary particles, or turbidity, decreases (flocculation) while at higher G values, the number of primary particles increases with time (deflocculation). Wahlberg *et al.* (1994) showed that activated sludge possesses varying flocculation properties and that the supernatant could remain turbid also after flocculation. The experiments by Wahlberg *et al.* (1994) were carried out at low shear levels, since the main purpose was to flocculate the sludge (to reduce the number of primary particles with time); consequently, the fit of data from experiments carried out at higher G values are more uncertain. Besides, the variation in parameters other than shear was not investigated.

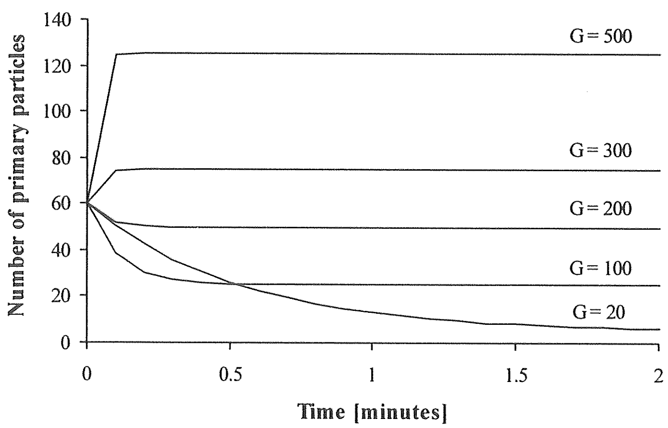


Figure 8 *Illustration of the flocculation-deflocculation model according to Wahlberg et al. (1994): $n_0 = 60$; $k_A = 40 \cdot 10^{-5}$; $k_B = 10 \cdot 10^{-5}$; and $X = 4$.*

Mikkelsen *et al.* (1996) defined the concept of shear sensitivity which is the ratio k_B/k_A; this k_B/k_A describes the interaction energy measured in terms of the degree of dispersion relative to the shear intensity. The parameters k_B and k_A are decreasing and increasing functions of the secondary minimum potential energy, respectively; thus, the combination k_B/k_A is also a decreasing function of this energy. Mikkelsen *et al.* (1996) changed the colloidal stability of the activated sludge by chemically manipulating it. The shear sensitivity was well correlated to the residual turbidity after settling, as well as to the specific resistance to filtration.

The study of the balance between flocculation and deflocculation has until recently been very empirical. Mikkelsen and Keiding (1999a) made a more rigorous analysis of the equations adopted by Wahlberg *et al.* (1994) to include desorption of primary particles from activated sludge in response to different suspended solids concentrations and turbulent shear (G values between 500 and 1700 s^{-1}). It was found that the turbidity increased more than linearly at higher suspended solids concentrations, at a given turbulent shear, and that the equation adopted by Wahlberg *et al.* (1994) therefore seemed to be somewhat erroneous, since it assumed that the desorption of primary particles was dependent only on G. By analogy to gas-liquid phase transition, the adsorption-desorption equilibrium of primary particles from a floc could be better described as a diffusion process, mathematically similar to the Langmuir adsorption isotherm. Their model made it possible to predict the equilibrium colloidal concentration, i.e. the turbidity obtained when the sludge is stirred for an infinitely long time, in response to sludge solids content and shear level. Furthermore, estimates of the relative interaction energies between primary particles in terms of the thermodynamic concepts $\Delta G/RT$, which is the interaction energy and $\Delta H/R$, which is the enthalpic response to shear could be obtained. The desorption kinetics of activated sludge colloids from the flocs could be approached by using a diffusion model, according to Crank (1975)

$$m_t = m_\infty + (m_0 - m_\infty)\cdot\frac{6}{\pi^2}\sum_{N=1}^{9}\frac{1}{N^2}e^{-N^2Dt}$$

where m_t, m_0 and m_∞ are the dispersed mass (or turbidity) at time t, 0 and at equilibrium, respectively; N is an integer; and D is the effective diffusion constant. In this model, the diffusion constant D is proportional to the turbulent shear, G, but independent of the solids content. The absolute value of D is influenced by the floc structure, while m_∞ is related to the interaction energy within the floc, i.e. by calculating m_∞ for various suspended solids concentrations, estimations of $\Delta G/RT$ and $\Delta H/R$ can be made.

It is difficult to measure floc strength, and to interpret data obtained in diverse studies, because of the non-linear response to shear. In earlier studies the specific resistance to shear (Eriksson *et al.*, 1992) and slope of turbidity versus shear time (Nielsen and Keiding, 1998) have been used as a measure of floc strength. Recently, a standardized method was developed by Mikkelsen and Keiding (1999c).

Low floc strength can be the result of many factors, both physico-chemical and biological. The floc strength at a given wastewater treatment plant changes with time, which causes a varying sensitivity to shear. Various factors that are known to affect the floc stability are summarized in the next section.

2.3.4 Deflocculation

Three *physico-chemical factors* that can initiate deflocculation, caused by low stability of the activated sludge flocs, are outlined here.

Shear
Activated sludge flocs are exposed to different degrees of turbulence in the treatment plant. Koníček and Burdych (1988) found that the type of aeration affects the degree of deflocculation. Diffused air aeration caused much less floc break-up than mechanical aeration devices. Das *et al.* (1993) found that fine bubble aeration caused less floc break-up than large bubble aeration. Galil *et al.* (1991) studied the influence of mixing, expressed in terms of the velocity gradient, G, on the size of sludge flocs. For G values lower than approximately 200 s^{-1}, the size of the flocs was found to be a reciprocal function of the turbulence. The floc size had a bimodal distribution; the number of very small flocs increased at higher G values. Floc break-up can also occur during transport through the treatment plant (Das *et al.,* 1993). Pumping can cause deflocculation (Forster, 1988). During filtration also, in connection with dewatering, the activated sludge can be exposed to high shear levels (Novak and Lynch, 1990). To compare the effects of shear rate (velocity gradient) caused, for example by diverse types of aeration systems can be difficult, since the shear forces on the flocs can be very different locally.

Ion-effects
As mentioned in Section 2.2.3, low ion strength can cause deflocculation, since it weakens the floc strength (Zita and Hermansson, 1994; Keiding and Nielsen, 1997). Zita and Hermansson (1994) explained this by an increase of the electrical double layer thickness according to the DLVO theory (Section 2.2.4). Keiding and Nielsen (1997) suggested that cations determine the surface charge density of the sludge flocs. In particular, Ca^{2+} seems to be important for the flocculation, even though it is not specific in the sense that it would form a special bond, for example, to EPS. It has been found that Mg^{2+} flocculates sludge less well than Ca^{2+}, probably due to its higher degree of hydration. The removal of Fe^{3+}, which is bound very strongly to the flocs, leads to a stronger deflocculation than for Ca^{2+} (Nielsen and Keiding, 1998). The reason is that cations with a higher valency flocculate better than cations with a lower valency (Gregory, 1989).

High concentrations of monovalent cations can cause deflocculation, probably due to the ion exchange of specific adsorbed ions (Bruus *et al.,* 1992; Frølund *et al.,* 1996). This is most likely what happens during the snow-melting period, when high concentrations of NaCl (from the salting of roads) enter the sewerage system. Problems with high effluent turbidities have been reported during the snow melting period in Sweden (Eriksson *et al.,* 1992).

Surface charge
Mikkelsen *et al.* (1996) showed that changes in floc surface charge density can cause deflocculation. An increase in pH, the addition of anionic detergents, or diluting the sludge with distilled water caused an increase in the negative surface charge density. Due to a higher electrostatic repulsion between negatively charged floc components, a rise in the shear sensitivity was observed, i.e. a lowering of the floc strength.

The impact of factors that affect the biology of the microorganisms is poorly investigated. A few observations have been made which indicate that *biological factors* are also of importance; these are outlined here.

Oxygen limitation
The effect of dissolved oxygen (DO) concentration has been studied by Starkey and Karr (1984) who found that conditions of oxygen limitation caused deflocculation. They suggested that two mechanisms could explain these phenomena: (i) the inhibition of the EPS production,

which would reduce the adsorptive capacity as well as the floc strength; and (ii) the inhibition of the eucaryote population, which scavanges the surface of the flocs and consumes free bacteria, and thereby producing a clear effluent. Eikelboom and van Buijsen (1983) explained this by growth of anaerobic or facultatively anaerobic bacteria between the flocs or the dying of the strictly aerobic bacteria. Sürücü and Çetin (1989) observed that, as the DO concentration in a laboratory-scale activated sludge reactor was increased from 0.5 to 5 mg/l, the suspended solids concentration in the supernatant after settling and the specific resistance to filtration gradually decreased. They suggested that the inhibition of EPS production would be the main reason for the deflocculation at low DO concentrations. Another possible reason could also be an incomplete degradation of organics.

Temperature
Rising temperatures have been found to lead to increased deflocculation (Sürücü and Çetin, 1989). The concentration of solids suspended in the supernatant after settling fell as the temperature was increased from 15 to 25 °C, whereas further increases to 30 and 35 °C, led to a higher suspended solids concentration. The specific resistance to filtration rose gradually with temperature. Barr *et al.* (1996) found that rapid temperature shocks (a reduction from 40 - 50 °C down to 30 - 7 °C) in an activated sludge reactor treating wastewater from a bleached kraft paper mill generated a higher suspended solids concentration in the effluent. Temperature affects both the physical properties (e.g. viscosity, structure of EPS) and biological properties (nature and rate of metabolism) of activated sludge.

Anaerobic conditions
Anaerobic storage of activated sludge can cause deflocculation (Rasmussen *et al.*, 1994). An increase in conductivity and turbidity was also observed. Ions such as PO_4^{3-}, Mg^{2+} and K^+ were released, probably from the Poly-P bacteria, since the sludge was taken from a WWTP for biological phosphorus removal. This indicates that an ion exchange took place in the deflocculation process. A fast decrease in total sludge and EPS protein and carbohydrate as a result of degradation processes has been found to take place during anaerobic storage (Nielsen *et al.*, 1996). This may be due to hydrolysis, followed by fermentation and microbial reduction of iron and sulphate.

Activated sludge contains 0.2 - 2 mmol Fe per g of volatile solids, mainly from the addition of iron salts to precipitate phosphorus (Nielsen and Keiding, 1998). Biological iron reduction has been measured in activated sludge treatment plants (Rasmussen and Nielsen, 1996; Nielsen, 1996; Nielsen *et al.*, 1997). Iron reduction may occur in anaerobic process tanks in wastewater treatment plants with biological phosphorus removal, in the settlers or in anaerobic storage tanks prior to dewatering. At low dissolved oxygen concentrations, iron reduction may take place in the interior of sludge flocs. A variety of bacteria are capable of reducing iron during anaerobic conditions (Lovely, 1991). Rasmussen *et al.* (1994) suggested that anaerobic degradation processes such as iron and sulphate reduction can be involved in the disintegration of activated sludge flocs. Caccavo *et al.* (1996) found that the addition of the metal-reducing bacterium *Shewanella alga* BrY to autoclaved activated sludge with H_2 as the electron donor enhanced deflocculation. Nielsen and Keiding (1998) studied the disintegration of activated sludge flocs in the presence of sulphide. The added sulphide reduced the Fe(III) to Fe(II), and floc disintegration was observed soon thereafter. The flocs deflocculated only when they were exposed to shear, which means that this happened as a result of lowered floc strength. Laboratory-grown bacterial flocs containing no iron did not deflocculate in the presence of sulphide, which shows that sulphide does not affect the floc stability. Fe(III) has better flocculating properties than Fe(II), because its valency is higher and its solubility of hydroxides lower than for Fe(II) (Gregory, 1989).

Poisoning
Deflocculation may occur when the aerobic population is poisoned, for example by certain metals (Eikelboom and van Buijsen, 1983; Neufeld, 1976). It is difficult to assess whether this is a change in the inter-particle forces within the aggregate, caused by a metal or toxic chemical, or if it involves the bacterial metabolism directly.

2.3.5 Floc size and size distribution

Settling and dewatering of activated sludge is strongly dependent on the size, size distribution and density of the flocs (Li and Ganczarczyk, 1988; Ganczarczyk, 1994; Dammel and Schroeder, 1991). In the dewatering process, the presence of many small flocs is equally detrimental (Karr and Keinath, 1978; Lotito *et al.*, 1993; Olböter and Vogelpohl, 1993). The size range of activated sludge flocs is very broad and may vary from the size of a single bacteria, approximately 1 μm, up to about 1000 μm. Most flocs are generally smaller than 100 μm. By measuring the size and size distribution of the sludge flocs, changes in the treatment process can be detected; the efficiency of the various separation processes can be evaluated.

Characteristic length
Activated sludge flocs have very heterogeneous shapes, from almost round to very irregular. Accordingly, the size of sludge flocs can be described in various ways. Irregular particles such as sludge flocs can be described by a characteristic length. There are various characteristic dimensions for flocs, e.g. the longest diameter, equivalent surface diameter, perimeter, shortest dimension, and projected surface area (Parker *et al.*, 1971; Sezgin *et al.*, 1978; Ganczarczyk, 1994). The shapes of sludge flocs can also be described by indicators of their deviation from a sphere (Grijspeerdt and Verstraete, 1997; Li and Ganczarczyk, 1990). Due to the irregular shape of activated sludge flocs, it has been found convenient to describe the aggregates with fractal dimensions, which define the relationship between particle size and density (Li and Ganczarczyk, 1989; Li and Ganczarczyk, 1990; Eriksson *et al*, 1992; Zartarian *et al.*, 1994; Grijspeerdt and Verstraete, 1997; Snidaro *et al.*, 1997). The fractal number is between 1 and 3; the lower the fractal dimension, the more open the floc is. Knowledge of the structure and size of activated sludge flocs is still poor; only very simplified models are being used. The floc structure models have been restricted to two dimensions. With the introduction of methods such as digitized microtome sectioning and confocal microscopy, three-dimensional models can be developed (Zartarian *et al.*, 1997; Snidaro *et al.*, 1997).

Measurement techniques
There are several methods available for floc size measurement. During sampling and measurement, it is difficult to avoid deflocculation, reflocculation and other physical alterations of the activated sludge flocs. Various size measurement techniques are summarized in Table 2. Before measurement, the sample has to be either diluted, to minimize flocculation and to distinguish individual flocs, or to be solidified, for example in agar or polymers (Ganczarczyk *et al.*, 1992; Zartarian *et al.*, 1994; Droppo *et al.*, 1996; Snidaro *et al.*, 1997). Individual flocs can also be measured as they settle in a quiescent water column by photographing individual flocs. A thorough summary of different measurement techniques has been made by Li and Ganczarczyk (1986).

Table 2 *Summary of different floc size measurement techniques.*

Equipment	Approximate size range [μm]	References
Photographs of individual flocs	> 50	Magara *et al.*, 1976; Li and Ganczarczyk, 1987; Li and Ganczarczyk, 1991; Leentvaar *et al.*, 1983; Roth and Pinnow, 1981
Coulter counter	> 1	Li and Ganczarczyk, 1991; Andreadakis, 1993
Image analysing	> 1	Zahid and Ganczarczyk, 1990; Li and Ganczarczyk, 1991; Námer and Ganczarczyk, 1993; Grijspeerdt and Verstraete, 1997; Hilligardt and Hoffman, 1997
Optical microscopy	> 1	Parker *et al.*, 1971; Sezgin *et al.*, 1978; Pipes, 1979; Sadalgekar *et al.*, 1988; Galil *et al.*, 1991; Barbusiński and Kościelniak, 1995
Laser beam diffraction	> 1	Jorand *et al.*, 1995
Transmission electron microscopy	> 0.002 - 0.004	Jorand *et al.*, 1995
Scanning confocal microscopy	> 0.002 - 0.004	Zartarian *et al.*, 1994; Droppo *et al.*, 1996; Liss *et al.*, 1996; Snidaro *et al.*, 1997
Filtration through different sized sieves	> 0.001	Karr and Keinath, 1978

Using optical microscopy is easy and it is suitable for studying gross morphology. However, it gives a two-dimensional picture of the flocs and their structure is compressed on the objective glass. It is less convenient for the analysis of floc size distribution, since it is very time consuming to measure the large number of flocs required for statistical relevance. Further, small flocs are difficult to measure correctly. Transmission electron microscopy and confocal microscopy offer the opportunity to study the flocs with a high degree of resolution. The advantage of photographic methods is that the flocs do not have to be touched during measurement. However, small flocs are difficult to measure correctly. Filtration is not as good, since the flocs are sheared and compressed during measurement. The Coulter counter is simple to use and many flocs can be measured fast. The disadvantages are that the flocs, which are rather fragile, have to pass through a small orifice, which that can lead to floc dis-integration; in addition, high NaCl concentrations are usually used. Laser beam diffraction techniques are simple to use and the flocs are generally not subjected to high shear forces. They cannot measure the whole size interval at the same time, which is why it is often necessary to measure small flocs separately. The image analysis technique, which is under fast development, is probably the most promising technique for the analysis of floc size and size distribution.

Floc size distribution

The measurement of size distributions of activated sludge can give more information than the average floc diameter alone. It is an efficient way of detecting changes in the floc properties at the various treatment steps, or shifts in growth conditions in the wastewater treatment plant, as well as how well the different separation processes work.

Size distributions can be expressed as number, surface area, volume and mass of particles within a certain size interval. The frequency of occurrence (distribution by number) is defined as $dP_i = dN_i/N$ where dN_i is the number of flocs within the size interval i, and N is the total number of flocs. The distributions by number can be converted to distribution by volume, surface area and mass under the assumption that the flocs are spherical (Allen, 1981):

distribution by surface area:	$dA_i \propto x_i^2 dP_i$
distribution by volume:	$dV_i \propto x_i^3 dP_i$
distribution by mass:	$dM_i \propto x_i^3 \rho\varepsilon(x) dP_i$

where x_i is the characteristic size of the ith size interval, ρ is the density of the solids, and $\varepsilon(x)$ is solids fraction of the flocs.

Distribution by mass is uncommon for activated sludge flocs since it is very difficult to measure the density of the flocs. Large flocs are often less compact than small flocs and may therefore have a lower density. Andreadakis (1993) measured the floc density by means of interference microscopy. He found a strong correlation between floc density and size: the smaller flocs had a higher density than larger ones. Hilligardt and Hoffmann (1997) made similar observations. Based on experiments with settling of individual flocs, Li and Ganczarczyk (1987) found that the floc porosity increased at two distinctive rates as the floc size increased, with the transition occurring at 200 μm. This means that the smallest flocs may contribute to a significant proportion of the biomass. The larger flocs are much more important individually, with respect to volume and surface area. Andreadakis (1993) suggested that the actual surface area was up to two orders of magnitude higher than the geometric surface area (measured with a dye adsorption technique).

Parker *et al.* (1971) studied the size distribution of activated sludge within the whole size range and found that the distribution *by number* was always bimodal (Figure 9). Very few flocs in the interval 5 - 25 μm were found. In their study, the sludge flocs were impinged onto a filter membrane by vacuum pressure, which could have distorted the flocs in such a way that some of them appeared larger than they actually were.

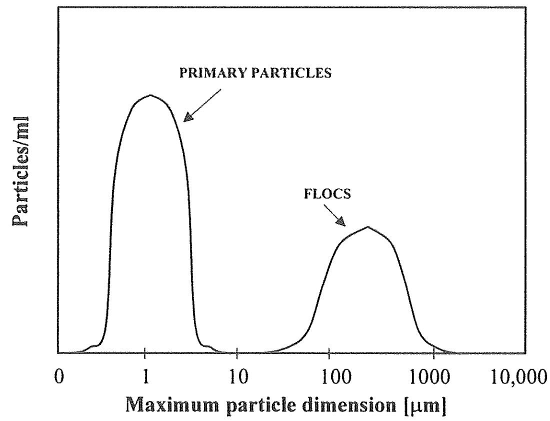


Figure 9 *Typical floc size distribution (redrawn from Parker et al. (1971).*

Li and Ganczarczyk (1991) found that the size distribution of activated sludge flocs, < 10 μm (measured with a Coulter counter), could best be fitted to power functions of the form:

$$\textit{power function: } f(x) = \frac{\Delta N_i}{\Delta x_i} = C x_i^{-\beta}$$

where ΔN_i is the number of flocs within the size interval i, x_i is the floc size, Δx_i is the length of the size interval i, and C and β are constants.

Flocs > 10 μm (measured with image analysis) could best be fitted to log-normal distributions:

$$\textit{log-normal distribution: } f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2\sigma^2}(\ln x - \mu)^2\right]$$

where μ is the average of ln$x$, σ is the standard deviation of ln$x$, and x is the floc size. Flocs larger than 50 μm were found to be the major source of surface area, volume and mass although they were greatly outnumbered by the small flocs. Flocs smaller than 2 μm could be ignored in terms of volume compared to the larger ones. However, calculations showed that they may comprise a significant proportion of the particle mass. The size distribution *by number* across the whole size interval correlated well with the power-law model, which is contradictory to the results of Parker *et al.* (1971). Babusiński and Kościelniak (1995) reported that the distribution *by number* of flocs > 10 μm could be fitted well to log-normal distribution functions. Jorand *et al* (1995) found that the size distribution of sludge flocs *by volume* could also be well fitted to log-normal distribution. Hilligardt and Hoffmann (1997) fitted the whole size interval of flocs (3 - 300 μm) to power-functions. The differing results are probably due to dissimilar measurement techniques used or to differences in the handling of the sludge samples. The usage of microscopy, is likely to underestimate the number of small flocs.

From the literature it seems clear that the whole size range of flocs generally must be divided into two size categories based on measurement technique limitations: flocs < 10 μm and flocs > 10 μm.

Parameters affecting the floc size
Several parameters may influence the size, size distribution and structure of activated sludge flocs.

Shear forces: turbulence can directly affect the floc size. Parker *et al.* (1971) suggested that small-scale eddies (on the same scale as the floc size) causes floc break-up, while larger eddies can cause flocculation. Galil *et al.* (1991) found that the floc size was a reciprocal function of turbulence for G values < 200 s^{-1}.

Types of microorganisms present: Sezgin *et al.* (1978) reported that the presence of large amounts of filamentous bacteria produced large flocs. Parker *et al.* (1971) suggested that activated sludge flocs containing filaments are strong and, when they are subjected to shear, the diameter of the flocs is closely related to the filaments.

<u>*Sludge loading and sludge age:*</u> Li and Ganczarczyk (1993) found that the process operation factors affect the floc size in a very complex way. However, the sludge loading was the most important and statistically significant factor influencing the amount of primary particles (< 2 μm), i.e. at higher organic loadings, the number of primary particles increased. Roth and Pinnow (1981) observed that the amount of small flocs rose in the secondary effluent as the organic loading increased.

In the studies by Knocke and Zentkovich (1986) and Jiwani *et al.* (1997) increasing floc sizes at higher sludge ages were reported. At higher sludge ages, more EPS can accumulate (Keiding and Nielsen, 1997), which could explain the increase in floc size with sludge age. Another possibility is that older flocs are more firmly bound together, which makes them less susceptible to shear (Eriksson *et al.,* 1992). Very low sludge ages (i.e. very high organic loading) as well as very high sludge ages (i.e. very low organic loading) are known to produce dispersed growth (Bisogni and Lawrence, 1971; Chao and Keinath, 1979; Pipes, 1979; Palm *et al.,* 1980; Cashion and Keinath, 1983; Lovett *et al.*, 1983; Knocke *et al.*, 1986; Eriksson *et al.*, 1992). In these studies the changes of the floc size were estimated by microscopy and the dispersion of flocs was measured as the concentration of suspended solids.

A very thorough investigation of the floc size distribution was made by Babusiński and Kościelniak (1995). They found that the size of the activated sludge flocs showed a direct proportionality to the changes of the organic load and that long term changes in the loading caused larger disturbances than short term changes. It was also observed that when the sludge was exposed to increasing organic loads, the flocs became more susceptible to break up, which could be seen as an increased turbidity of the effluent, at the same time as as the average floc size increased. Andreadakis (1993) operated some pilot plants differented by the age of the activated sludge. A sludge age of approximately one day produced the smallest flocs, while sludge ages of 4 - 12 days yielded the largest flocs. If the sludge age was extended to 17 days, the flocs became smaller again.

It would appear that the number of small flocs (primary particles) does not necessarily have anything to do with the size of the larger flocs; a larger organic loading gives an increased number of small particles at the same time as the size of the larger flocs increases. Possibly, the increased organic loading also stimulates the growth of dispersed bacteria in the bulk water.

<u>*Dissolved oxygen concentration:*</u> surprisingly few studies can be found in the literature that deal with the effect of DO concentration on the size and structure of non-filamentous activated sludge flocs. It is, however, well known that the DO concentration affects the growth of filamentous bacteria (Palm *et al.,* 1980; Wanner and Grau, 1988; Jenkins, 1992). Li and Ganczarczyk (1993) studied the influence of different process parameters on the size distribution and dispersion of activated sludge flocs. They concluded that the organic loading and the availability of dissolved oxygen were the two most significant factors influencing the size distribution of activated sludge flocs. It is apparent that the parameters DO concentration and organic loading are so closely related that it can be difficult to distinguish them from each other.

Starkey and Karr (1984) observed that the effluents from both full scale activated sludge plants and lab scale units contained a larger number of small particles (seen as an increase in turbidity) during periods of low DO concentrations. The effects of low DO concentrations were more pronounced at higher organic loadings. Sürücü and Çetin (1989) found that the filterability of activated sludge was reduced at low DO concentrations due to the increased turbidity of the effluent. The flocs were comparatively larger at DO concentrations of 2 - 5 mg/l than they were at DO concentrations of 0.5 - 1.5 mg/l.

In the study by Knudsson *et al.* (1982) a trend towards larger flocs at higher DO concentrations could be found; however, the relationship was not very clear. It was also observed that the maximum utilization rate of oxygen rose with increasing DO concentration over the range 1.5 - 15 mg/l, provided the flocs were larger than 400 μm.

2.3.6 Concluding remarks

The problems caused by activated sludge with poor separation properties can be divided into two major categories:

- the excessive growth of filamentous bacteria (bulking and foaming), and
- poor flocculation properties of activated sludge.

Much research has been carried out in the field of filamentous bacteria. Although far from everything is known about what causes its proliferation, efficient control strategies are available for many types of filamentous bacteria. An exception is the growth of *Microthrix parvicella,* which is one of the most common filamentous bacteria in nutrient removal plants.

Knowledge of what parameters affect the structure, size and size distribution of non-filamentous activated sludge flocs is still poor. One problem has been the lack of effective measurement techniques for tracking changes in the floc properties. Until recently, only two-dimensional structures could be measured: now, a new technique makes it possible to measure three-dimensional structures. New techniques such as confocal microscopy and digitized microtome sectioning are still being worked out and little data can be found in the literature. No suitable techniques are available for measuring floc properties such as size, size distribution and structure *in situ* in the aeration tank.

Relatively few studies about parameters that affect the average size of activated sludge flocs can be found in the literature. The probable reason is that it is difficult to distinguish the effects of various parameters from each other.

The *organic loading* is an important factor influencing the size of the sludge flocs as well as the presence of small particles between the large flocs. High sludge loadings seem to produce larger flocs, however, the number of small flocs between the large ones also increases. The organic loading dealt with here has a diurnal variation, while sludge age extends over a longer period of time.

The *sludge age* affects the floc size in a complex way. Intermediate sludge ages produce large flocs with few dispersed bacteria between them, while either very high or very low sludge ages produce a high frequency of small flocs. At high sludge ages, more EPS (mainly humic substances from the wastewater) accumulate in the flocs and they tend to become more compact than at low sludge ages. The older flocs also become rounder as irregularities of their surfaces are eroded off.

No clear relationship between floc size and *dissolved oxygen concentration* has been found. The effect of DO is closely linked to the effect of organic loading. Since oxygen must diffuse through the flocs, the effect of shifting DO concentrations on the floc size may depend on the diffuseability of the oxygen. At high DO concentrations the oxygen can diffuse deeper into the flocs; they can thus be larger without suffering from oxygen depletion in the interior. Hence, the organic loading and the availability of dissolved oxygen are closely linked.

The applied *shear forces* affect the floc size. The effect of a given shear on the disintegration of a floc depends on its strength. One can also distinguish between erosion of small particles from larger flocs and fragmentation which is the break-up of a large floc into smaller ones. The deflocculation due to erosion appears to be more important than fragmentation for the appearence of high turbidities of the effluent.

The size distribution, by number, of activated sludge flocs is often bimodal with a very large number of small flocs and single bacteria and a high number of larger flocs. The floc size distribution of the whole size range can be described by a power function, while flocs larger than about 10 μm can best be fitted to log-normal distribution functions.

Deflocculation causes a broadening of the size distribution. The known physico-chemical factors leading to deflocculation include: a reduced ion strength, removal of di- and tri-valent cations, alteration of the surface charge density, and shear. Less is known about the biological mechanisms involved. However, some observations indicate that when the microbial activity is reduced, the flocs tend to deflocculate. For example, either anaerobic conditions or oxygen limitation produces more small flocs. The role of the biological activity on the floc stability has not been extensively investigated.

3 EXPERIMENTS

Experimental approach

Little is known about the effects of dissolved oxygen (DO) concentration on the separation and settling properties of activated sludge. The effects of DO concentration can be divided into *long term effects* and *short term effects.*

On a *short term* basis, two processes are probable: (i) poor adsorption (or possibly desorption) of particulate and colloidal material onto sludge flocs, and (ii) deflocculation due to lower floc stability. It was decided to investigate first whether the adsorption of colloidal material onto the activated sludge flocs is affected by the DO concentration (***Paper I***). A large proportion of the contaminants in wastewater is in a particulate or colloidal form, and cannot pass directly through a cell wall. The initial step in removing particulate and colloidal material from the wastewater is therefore adsorption onto the flocs. Consequently, it is essential that the contaminants be adsorbed onto the flocs as the water passes through the aeration tank. In a treatment plant, periods of oxygen limitation could lead to an increased turbidity of the effluent caused by poor adsorption of particulate and colloidal material. Thus, it was essential to study the changes in turbidity of the effluent water, which are generated by fast changes in DO concentration in a continuous activated sludge system. Experiments were carried out in a small pilot plant where the dissolved oxygen concentration was lowered for periods of time that are typical of normal hydraulic retention times. Since there are rather large differences in design and operation between a small pilot plant and a full scale plant, it was decided that similar experiments should also be carried out in a full scale plant. In particular, treatment plants with a high organic loading could be expected to react fast to changed DO concentration, which is why experiments were carried out in a treatment plant with a low sludge age.

Short term changes in DO concentration affect mostly the turbidity of the bulk water between the sludge flocs. Knowledge of the *longer term* effects of DO concentration is poor; accordingly, experiments were carried out in a pilot plant where the sludge was exposed to different DO concentrations for longer periods of time (***Paper II***). The experiments were conducted for sludge of different ages. Besides measuring the turbidity of the effluent, the settling and compaction properties of the sludge were measured.

From the results in ***Paper I***, it is difficult to judge whether the increase in turbidity under anaerobic conditions was due mainly to poor adsorption of contaminants or to deflocculation (or even possibly desorption of adsorbed material). Deflocculation experiments were then carried out in controlled systems to investigate the importance of floc disintegration under anaerobic conditions. Furthermore, it appeared from the previous experiments that the microbial activity could be a significant parameter in the flocculation process. In ***Papers III - VI***, deflocculation of activated sludge subjected to shear under different conditions, was studied. The conditions were such that the microbial activity was affected in various ways, i.e. the experiments were run at aerobic, anaerobic and anoxic conditions and at different temperatures. Experiments were also run in such a way that the effects of physico-chemical and biological factors could be separated.

Methods

The various experimental set-ups are described in brief.

Adsorption tests (***Paper I***): To an activated sludge suspension, differing concentrations of colloidal material (milk) were added. After various contact times (15-120 minutes) between sludge and colloidal-containing wastewater at different DO concentrations, the turbidity of the supernatant after settling was measured. The adsorbed amount of colloidal material per g of suspended solids (measured in terms of turbidity) was calculated and fitted to adsorption isotherms.

Pilot plant studies (***Papers I - II***): A schematic picture of the pilot plant is illustrated in Figure 10. Two identical pilot plants were run in parallel to enable comparison of the effects of one parameter without disturbing effects from other parameters. The pilot plants were inoculated with activated sludge from the Rya wastewater treatment plant (WWTP) in Göteborg, Sweden, which is a conventional activated sludge treatment plant receiving wastewater from approximately 570,000 inhabitants and 220,000 population equivalents of industry. The plant was fed with domestic wastewater to simulate as far as possible the conditions at a full scale treatment plant. A primary settler was installed in the pilot plant because, at the time of this experiment, precipitation chemicals were added to the primary settlers of the Rya WWTP, and it was undesirable to have them in the pilot plant. Return sludge and settled wastewater were mixed in a small selector in front of the aeration tank. This was to decrease the risk of getting filamentous bacteria in the pilot plant, something that often occurs in completely mixed tank reactors.

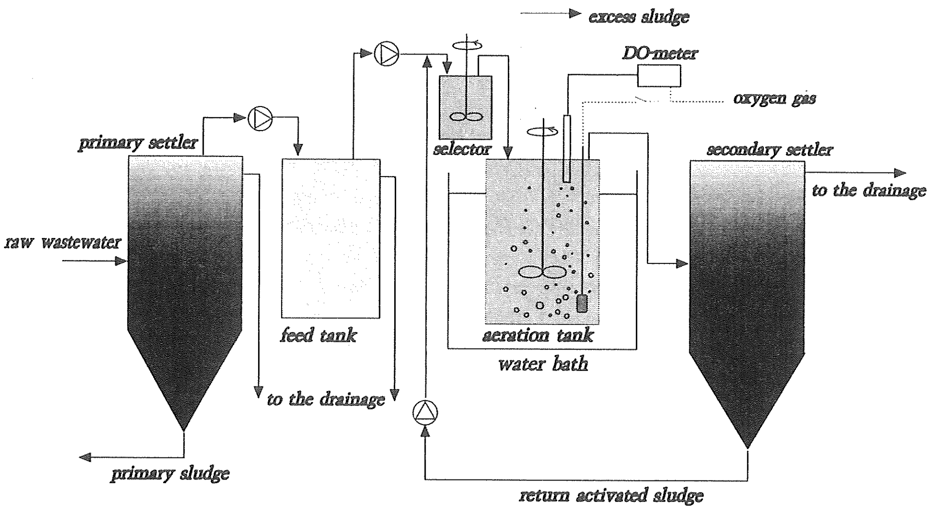


Figure 10 *Schematic drawing of the pilot plant ($V_{primary\ settler}$ = 100 l; $V_{feed\ tank}$ = 20 l; $V_{selector}$ = 0.6 l; $V_{aeration\ tank}$ = 18 l; $V_{secondary\ settler}$= 20 l; $Q_{return\ sludge}$ = 0.8 - 1.0×$Q_{influent}$; $Q_{influent}$ = 2.4-3.6 l/h).*

Full scale experiments (***Paper I***): The full scale experiments were carried out at a rather highly loaded (SRT of 2 days at the time of the experiments) conventional activated sludge treatment plant (the Rya WWTP, Göteborg, Sweden). The four treatment steps include: primary settling (HRT: 1.5 h), simultaneous precipitation of phosphorus with iron sulphate, aeration (HRT: 2-3 h), and secondary settling (HRT: 3-4 h). The experimental layout is illustrated in Figure 11. Both DO and turbidity were measured on-line. Data was given as a 6-

minute average value. In the first section of the aeration tank (1/5 of the total aeration volume), the DO concentration is very low or near zero. Thereafter, the DO concentration gradually increases. In the various experiments, the DO concentration was changed along the aeration tank to give different profiles of oxygen concentration, for a few hours, after which it was changed back to the initial values. The flow into the treatment plant was constant during the experiments (2 - 3 m^3/s). The volume of the aeration tank was 18,900 m^3 and the settler volume was 30,000 m^3 (total volume of 12 parallel lines with settlers in two stories). The temperature in the aeration tank was 17 - 19 °C.

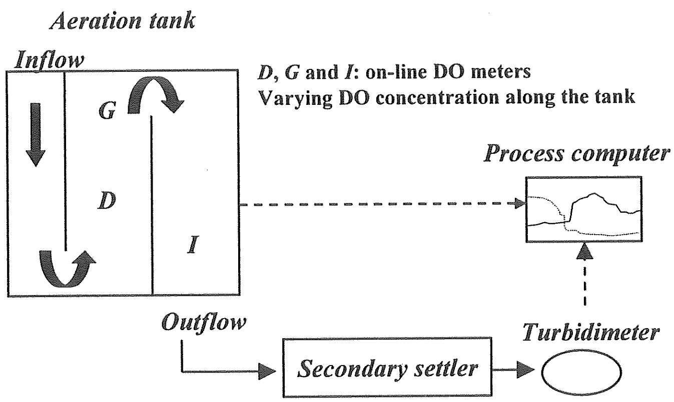


Figure 11 *Schematic drawing of the experimental set-up for the full scale experiments.*

Floc stability experiments *(Papers III - V)*: The flocculation-deflocculation reactors were specially developed to give a homogeneous shear (Figure 12) and the stirring speed was kept high to get a stable development in turbidity (G = 700 - 1000 s^{-1}). The development in turbidity of the supernatant, after a mild centrifugation of sludge samples, (1300 *g* for 2 minutes) at different shear rates looked typically as illustrated in Figure 13. To distinguish effects of stirring from other effects, a reference reactor was always run at aerobic conditions. The sludge was thickened twice to get a higher turbidity in the supernatant during the deflocculation.

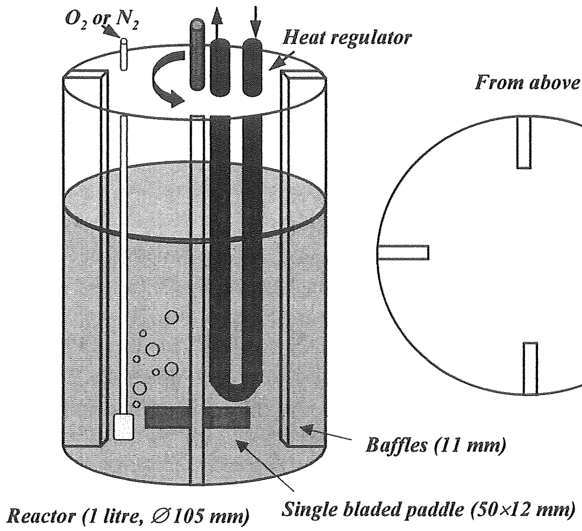


Figure 12 *Schematic drawing of the flocculation-deflocculation reactor.*

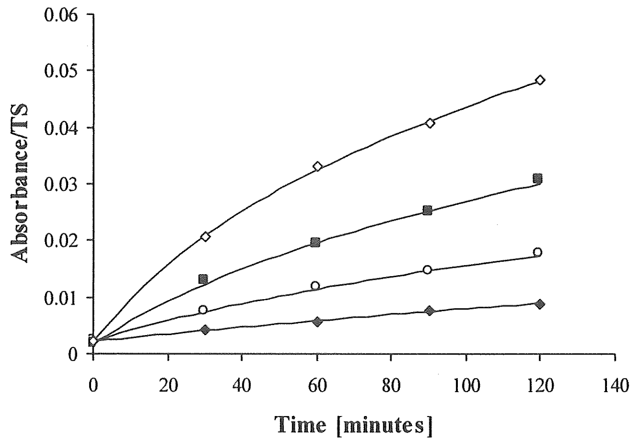


Figure 13 *Development in turbidity under anaerobic conditions when activated sludge is exposed to different shear levels: (◆) 229 s^{-1}, (○) 453 s^{-1}, (■) 725 s^{-1}, and (◇) 1035 s^{-1}.*

4 RESULTS AND DISCUSSION

4.1 Short Term Effects of Dissolved Oxygen (DO) Concentration on Effluent Turbidity (Paper I)

It has been found in several studies that oxygen limitation can cause increased effluent turbidities (Eikelboom and van Buijsen, 1983; Starkey and Karr, 1984; Sürücü and Çetin, 1989). Various reasons for this have been proposed. Eikelboom and van Buijsen (1983) suggested that anaerobic or facultatively anaerobic bacteria start to grow in the bulk water, between the flocs or that strictly aerobic bacteria die. Starkey and Karr (1984), on the other hand, proposed that the production of EPS and/or the eucaryote population become inhibited during oxygen limited conditions. They also observed that the rise in turbidity under low DO conditions was more pronounced at higher colloidal loading of the sludge flocs, which indicates a reduced adsorption capacity.

Pilot plant experiments

Experiments were carried out in a pilot plant with two parallel reactors to evaluate the effects of shorter periods (a few hours) of oxygen limitation on the effluent turbidity (Table 3). The settling properties were similar in all of the experiments (SVI: 100 ml/g), which is important when comparing the separation properties of two sludges. The structure of the flocs (measured as SVI) can affect the fraction of non-settleable solids. This is discussed later in Section 4.2. The sludge flocs were well flocculated and contained low numbers of filamentous bacteria. The pilot plant was operated at an organic loading of 0.9 – 1.0 g COD/g biomass·d and at a temperature of 17 – 18 °C.

Table 3 *Operational conditions during the pilot plant experiment.*

	Conditions
Experiment I	
Reactor A	Adapted to alternating aerobic/anaerobic conditions (4 mg O_2/l)
Reactor B	Adapted to constant aerobic conditions (4 mg O_2/l)
Experiment II	
Reactor A	Adapted to 2 mg O_2/l
Reactor B	Adapted to 5 mg O_2/l

In the first experiment the turbidity increased immediately (reactor A) when the oxygen supply was turned off (Figure 14a), The rise in turbidity corresponded to a suspended solids concentration of approximately 8-9 mg/l; the estimation is based on the following relationship: suspended solids concentration = 1.4 - 1.5 × NTU (Wilén, 1997). Turbidity was used as a measure of non-dissolved compounds since it is easily done. Directly after the oxygen supply was turned on again, the turbidity started to fall. After about two hours the turbidity was almost back to the initial values.

Activated sludge has a large capacity for adsorption of both dissolved and non-dissolved compounds. The EPS production can be inhibited under anaerobic conditions, as was suggested by Starkey and Karr (1984), which would reduce the adsorptive capacity of non-dissolved particles in the wastewater. The observed increase in turbidity during the anaerobic phase could thus be due to a poor adsorption of particulate or colloidal matter onto the sludge flocs. Since there is no aerobic activity under anaerobic conditions, microbial degradation of adsorbed organic material does not take place; i.e. adsorbed organic matter is not continuously removed from the floc surface. After a while, the adsorption capacity is saturated with adsorbed matter and particulate and colloidal matter start to accumulate in the bulk water. This has been used in the contact stabilization process (e.g. Bunch and Griffin, 1992), where the adsorption capacity of the sludge is restored by aerating the return sludge separately.

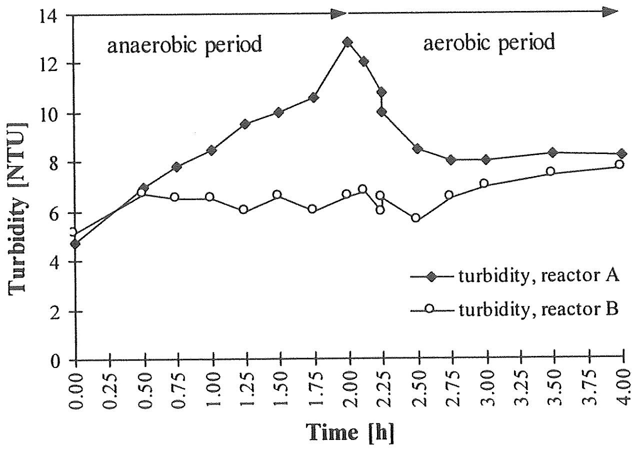
To find out whether or not the rise in turbidity was due only to an accumulation of un-adsorbed matter, the same experiments was carried out without any influent to the reactor. Although similar results were obtained, the increase in turbidity was less. From these results it is not possible to distinguish between lack of adsorption (or desorption of adsorbed material) and floc disintegration. The increase in turbidity could also be explained by an active movement of bacteria to avoid undesired conditions. This is something that has been observed during short term anaerobic storage of activated sludge (Rasmussen *et al.*, 1994).

The G value was calculated to approximately 30 s^{-1}, due to the stirring, and the contribution from the oxygenation was small since pure oxygen gas was used. Hence, the sludge flocs were exposed to such low shear forces that this should not cause erosion of small particles from the flocs (Mikkelsen and Keiding, 1999a). The comparison of the degree of deflocculation under various shear intensities made in ***Paper III*** showed that the turbidity increased much less at low shear (see Section 4.3, Figure 28). There was an increase in turbidity, albeit small, which is in agreement with the results in ***Paper I***.

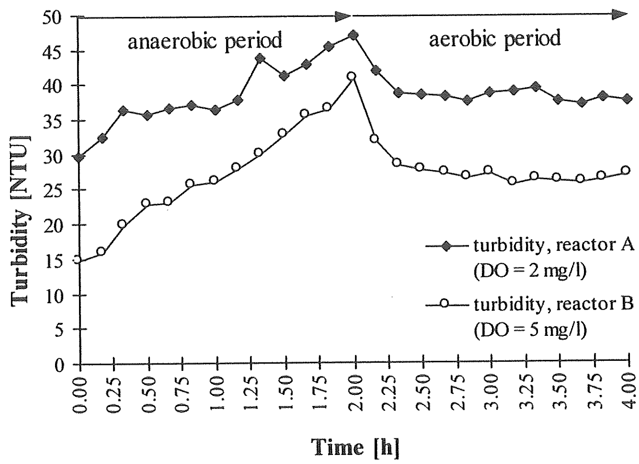
At the time of the writing of ***Paper I*** it was believed that two hours was too short a period for a reduction in EPS production to occur. However, later research (***Papers III to V***) indicates that the floc stability, possibly due to a reduced EPS production, can change fast when conditions are changed.

In the second experiment, the sludges in the two reactors were acclimatized to different DO concentrations. The turbidity rose immediately in both reactors when anaerobic conditions prevailed (Figure 14b). However, the turbidity increased more in the reactor operated at a higher DO concentration. There is no clear explanation for this. One hypothesis is that, since the microbial activity is higher at higher DO concentrations, as found by (Williamson and Nelson, 1981), the response to low DO concentration could be more pronounced. On the other hand, the flocs acclimatized to oxygen rich conditions were more compact and appeared be stronger. The background turbidity was higher for the reactor operated at the lower DO concentration, as shown in ***Paper II***. In some experiments the turbidity did not return to the initial levels after the aeration period. This indicates an irreversibility of the deflocculation, something that was also observed later in ***Papers III*** and ***V***.

The pH rose slightly during the anaerobic period and fell during the subsequent aerobic period. The changes were so small that they should not have significant effects on the floc stability. The surface charge density becomes more negative at increased pH values, which would lead to a reduced floc stability, according to general colloidal theory (Mikkelsen *et al.*, 1996). There was no nitrate present during the anaerobic phase, which may have reduced the degree of deflocculation for these conditions (***Paper III***).



a)



b)

Figure 14 *Change in turbidity under alternating aerobic and anaerobic conditions: (a) experiment I; and (b) experiment II.*

Full scale experiments

The full scale experiments showed that shorter periods (a few hours) of low DO concentrations along the aeration tank caused increased turbidities of the effluent. There was a plug flow through the aeration tank and, normally, the DO concentration started to rise after the first third of the basin length. The results show that it was crucial to keep the DO concentration at the end part of the aeration tank high to get a clear effluent; when the DO concentration was high at the beginning of the aeration tank and thereafter gradually falling, an increased turbidity was observed (Figure 15). When the DO concentration was thereafter increased in the whole aeration tank, the turbidity fell. The effect of the various combinations of DO concentration along the aeration tank on the effluent turbidity is summarized in Table 4.

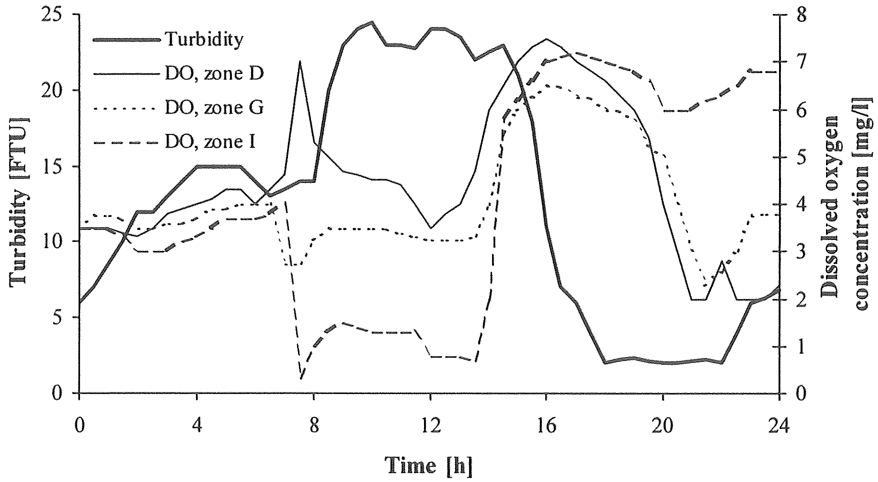


Figure 15 *Full scale studies: change in turbidity of the effluent when the dissolved oxygen (DO) concentration was kept high at the beginning and low at the end of the aeration tank (D, G and I are on-line DO meters).*

At the time of the experiments, the Rya WWTP was highly loaded, which is probably why, the effect of reduced DO concentrations on the effluent turbidities was so pronounced. Generally, the load of organic matter had a diurnal variation with the highest loading during the early morning hours (time zero in Figure 15 corresponds to midnight), which could often be seen as a rise in effluent turbidity. The values of turbidity and DO concentration are average values for 6 minute intervals, which makes them rather reliable. The flow through the treatment plant was kept constant during the experiments; changes in the hydraulic conditions can significantly influence the effluent turbidity, as described by Bergh and Olsson (1996), see Section 2.1.2. The peak in effluent turbidity appeared very soon after the DO concentration was decreased: about half of the usual hydraulic retention time of the settler. This is due to short-circuiting of the streams through the WWTP (Lumley and Horkeby, 1989).

Table 4 *Summary of the full scale experiments.*

Test	DO concentration at the beginning of the aeration tank (DO meter D)	DO concentration at the end of the aeration tank (DO meter I)	Effect on effluent turbidity
I	Low (< 1 mg/l)	High (4 mg/l)	No increased turbidity
II	High (4 - 5 mg/l)	Low (1 - 1.5 mg/l)	Increased turbidity
III	Low (< 1 mg/l)	Moderate (2 - 4 mg/l)	Slightly increased turbidity
IV	Low (< 1 mg/l)	Low (< 1 mg/l)	Increased turbidity

Adsorption of particulate and colloidal matter onto activated sludge
When activated sludge is brought into contact with wastewater, it has been observed that the disappearance of organic material is faster than the consumption of oxygen, which indicates that the organic material is adsorbed onto the activated sludge flocs (e.g. Torrijos *et al.,* 1994). Physico-chemical processes were believed to be involved, e.g. adsorption and the entrapment

of larger particles in the floc matrix. Since the contaminants in wastewater are a mixture of particulate, colloidal and soluble compounds (Levine *et al.,* 1985; Boller, 1993), it is very important that the non-dissolved compounds be adsorbed onto the flocs. Bunch and Griffin (1992) studied the removal of colloidal organics from domestic wastewater. They found that the colloidal fraction (0.03 to 1.5 μm) accounted for 18% of the total amount of COD in the wastewater; the dissolved fraction 28%; particles within the range 1.5 to 10 μm 13%; and particles larger than 10 μm the rest of the COD. They observed that the removal of the colloidal fraction was much faster than the removal of the other fractions; after a contact time of 5 minutes, most of it was removed (under aerobic conditions). The removal was not affected by the viability of the microorganisms in the sludge, since poisoned sludge showed the same removal rate as biologically active sludge. It was then suggested that the removal was a physical process. Pujol and Canler (1992) found that the degree of adsorption (measured as removal of COD from wastewater) was affected by floc structure, organic loading, temperature and aeration, which indicates that physical, chemical and biological mechanisms are involved. Riffat and Dague (1995) found that the adsorption of organic matter onto anaerobic sludge was strongly temperature dependent; they suggested that the adsorption was physical. This literature review shows that it is, at the moment, not known whether the adsorption of particulate and colloidal matter is purely physico-chemical or if biological factors are involved as well. It appears plausible that fractions of the contaminants are adsorbed by different mechanisms as suggested by Bunch and Griffin (1992).

The adsorption of colloidal material (milk) to activated sludge was studied at three dissolved oxygen concentrations. Milk is a colloidal system that contains fat particles within the size range 0.1 to 10 μm. The adsorption (measured as removal of turbidity per g of biomass) was much larger under aerobic than under anaerobic conditions for all of the sludges tested. The difference in adsorption under conditions of high or low DO concentrations was, however, small. Data fitted to Freundlich isotherms are illustrated in Figure 16. Adsorption of organic matter from wastewater has been found to be well described by Freundlich and Langmuir isotherms (Riffat and Dague, 1995). The difference in adsorption, under aerobic versus anaerobic conditions, increased with contact time (Figure 17). The adsorption under anaerobic conditions was less affected by the contact time. For longer contact times, microbial degradation processes of the adsorbed matter take place; thus it can also be expected that the adsorption under aerobic conditions increases relatively more with time than under anaerobic conditions.

The similar variation in adsorption capacity under aerobic and anaerobic conditions was observed for all of the sludges investigated; however, no attempt was made to compare their adsorption capacities. To do this, the loading of colloidal matter onto the sludge flocs would have had to be the same. In this study, the initial colloidal loading varied widely for the individual studies. The activated sludge flocs from the pilot plant had a morphology that differed somewhat from that of the sludge flocs from the full scale plant, which probably affected the adsorption capacity. This was not investigated since it lay outside the scope of this study.

These results show that DO limitation can affect the effluent turbidity in two ways: poor adsorption of organic contaminants in the wastewater or deflocculation (or possibly desorption of colloidal and particulate material). In the pilot plant experiments, the turbidity increased both with and without influent to the reactors, indicating that a combination of these different processes is the reason for the increased turbidity.

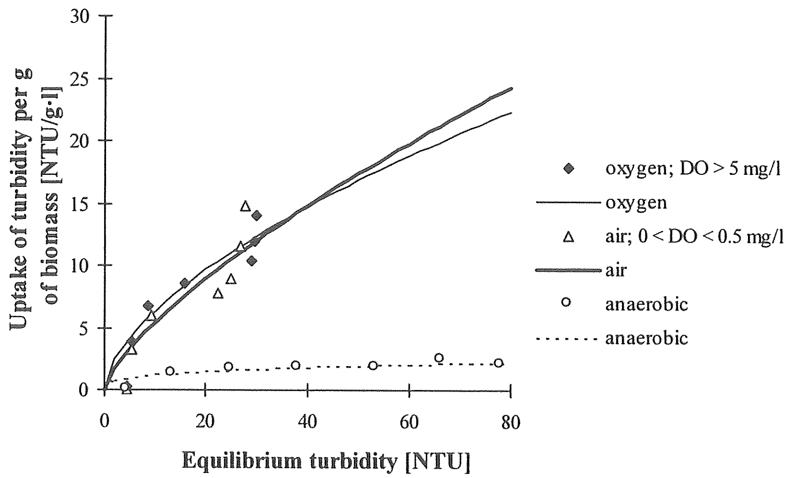


Figure 16 *Adsorption tests (15 minutes contact time) with activated sludge taken from the end of the aeration tank at the Rya WWTP; the data are fitted to Freundlich isotherms.*

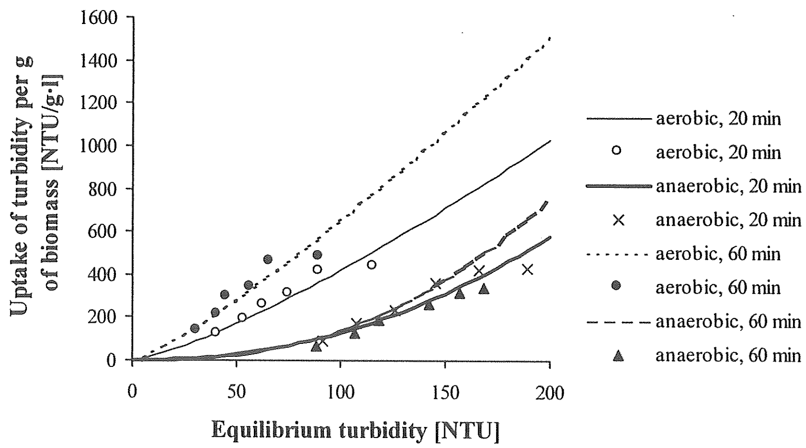


Figure 17 *Adsorption tests with activated sludge taken from the pilot plant; the data are fitted to Freundlich isotherms.*

4.2 The Effects of Dissolved Oxygen (DO) Concentration on Activated Sludge Floc Structure and Size (Paper II)

The settleability of activated sludge is mainly dependent on the structure, size and density of the activated sludge flocs, as was previously discussed in Section 2.3. Both the proliferation of filamentous bacteria and the colloidal characteristics of the flocs (size, density and surface properties) have a large impact on the settling and separation properties of activated sludge flocs. Bulking due to excessive growth of filamentous bacteria often causes acute settling problems; a considerable amount of research has been carried out to understand what stimulates their proliferation (Section 2.3.2). Much less is known about the colloidal properties of the sludge flocs. Several parameters are known to affect the size and structure of non-filamentous activated sludge flocs: (1) solids retention time (Bisogni and Lawrence, 1971; Knocke and Zentkovich, 1986); (2) organic loading (Pipes, 1979; Li and Ganczarczyk, 1993; Barbusiński and Kościelniak, 1995); (3) turbulence (Parker *et al.*, 1971; Konícek and Burdych, 1988; Galil *et al.*, 1991; Wahlberg *et al.*, 1994; Das *et al.*, 1993); and (4) dissolved oxygen (DO) concentration (Knudson *et al.*, 1982; Starkey and Karr, 1984). Some of these parameters are closely linked, which can make it difficult to distinguish them from one another. In particular, the organic loading and the availability of DO are closely interrelated (Li and Ganczarczyk, 1993). Low DO concentrations are known to lead to: larger amounts of filamentous bacteria (Palm *et al.*, 1980; Wanner and Grau, 1988; Jenkins, 1992); smaller flocs; and a more turbid effluent (Knudson *et al.*, 1982; Starkey and Karr, 1984; Sürücü and Çetin, 1989).

The composition and structure of the sludge flocs change with the environment surrounding them, on both short and long term scales. Long term effects of DO concentration could be the selection of certain microorganisms, floc size and compactness (depending on the diffusion depth of oxygen, EPS production etc.). Short term effects could be deflocculation caused by an altered floc stability due to: changed EPS production; changed cell surface hydrophobicity; or the active movement of bacteria away from undesired conditions.

When studying the settling and separation properties of activated sludge flocs it is almost never possible to study the effect of various parameters on the floc forming alone, since filamentous bacteria are nearly always present. Nevertheless, by running two pilot plants in parallel, differences in flocculation properties can be observed even though filamentous bacteria are present.

Pilot plant experiments were carried out to study how DO concentration, as well as alternating aerobic and anaerobic conditions, influence the structure, size and size distribution of activated sludge flocs. Experiments were carried out at various sludge ages and DO concentrations as summarized in Table 5.

Table 5 *Summary of experiments to study long term effects of dissolved oxygen (DO) concentration on floc structure and size.*

Experiment	DO concentration [mg/l]	SRT [d]
I	0.5 - 2	5
II	0 - 4 (alternating periods: 1, 2, 4 h)	5
III	0.5 - 2	1.25 - 5
IV	2 - 5	5

As the size range of the activated sludge flocs investigated was very wide it could not be measured simultaneously. Therefore, the measurements were divided into two intervals: (i) large flocs, > 10 μm to 1100 μm (measured with a Malvern instrument), which, according to Li and Ganczarczyk (1991), contribute to the main part of the volume of activated sludge; and (ii) small flocs in the supernatant after settling, 1 to 100 μm (measured with a Met One instrument). The large flocs affect mainly the settling, while the small fractions mostly affect the clarification.

The flocs in the large size range were generally large in all of the experiments, with an average size of 300 to 400 μm. The flocs measured in the full scale plant were generally smaller (50 - 200 μm). That flocs tend to become larger in small pilot plants than in full scale plants has been reported elsewhere (e.g. Sezgin *et al.,* 1978; Knocke and Zentkovich, 1986). A possible reason could be that the flocs are subjected to shear forces other than those in full scale plants. The G values in the full scale plant are difficult to estimate, since they are caused predominantly by the aeration, although pumping can also cause high shear levels.

Change in floc morpholgy and floc size distribution

In the first experiment (SRT = 5 days), the DO concentration was decreased from 2 to 0.5 mg/l for a period of three months. When the reactor was operated at a DO concentration of 2 mg/l, the flocs were large and compact. As the DO concentration was decreased to one and finally down to 0.5 mg/l, the flocs remained large but they became more porous and contained large quantities of filamentous bacteria (*Sphaerotilus natans* and *Thiothrix* I-II). Palm *et al.* (1980) found that bulking due to excessive growth of filamentous bacteria occurred for certain combinations of DO concentration and organic loading. This means that the term low DO concentration is a relative concept, since the DO concentration associated with the filamentous bacteria, known as low-DO-filaments, is dependent on the organic loading. According to their observations, bulking should occur if the DO concentration is lower than approximately 1 mg/l at an organic loading of 0.5 - 0.6 g COD/g MLSS · d; the same results were also found in this study. Their results are, however, based on a specific wastewater and the same results might not be obtained with another one. As in this study, they observed *Sphaerotilus natans* which is commonly found at low DO concentrations (Jenkins, 1992).

Seeding of activated sludge plants with filamentous organisms can be a large problem when working with small pilot plants, due mainly to the high surface area-to-volume ratio in such systems. Such filaments as *S. natans*, type 1701 and *Thiothrix* are particularly common in pilot plants (Jenkins, 1992). In this study the reactor walls, as well as the tubings, were cleaned (by hypochlorite) daily to minimize wall growth. In spite of this precaution, filamentous bacteria were always present.

In treatment plants designed for biological nitrogen and phosphorus removal, the sludge is exposed to aerobic, anoxic and anaerobic conditions. The effects of alternating aerobic and anaerobic conditions were investigated in the second experiment. Alternating periods of 1 to 4 hours did not affect the size of the flocs. Alternating periods of 4 hours produced, however, slightly less compact flocs, which was seen as slightly higher SVIs. The sludge also settled slower than the sludge operated at constant aerobic conditions for the same sludge volume (Wilén, 1997), indicating less compact flocs. The sludge age was too short for nitrification, but occasionally, some nitrification and denitrification took place in the reactor operated under alternating aerobic-anaerobic conditions. This could have a positive impact on the floc stability as described in ***Papers III to V***.

In the third experiment, the effects of DO concentration were studied at three different solids retention times (SRT): 5, 2.5 and 1.25 days. At 2 mg O_2/l the flocs were slightly larger than at 0.5 mg/l (Figure 18). The average floc diameter decreased in both the reactors as the SRT was decreased from 5 to 2.5 days, whereas a further reduction to 1.25 days produced larger flocs. As the SRT was decreased, the organic loading increased. This should, according to Barbusiński and Kościelniak (1995), produce larger flocs. The low DO concentration produced flocs that were less compact; hence, giving higher SVIs. The initial settling velocities were also lower for the sludge operated at 0.5 mg O_2/l than at 2 mg/l for similar sludge volumes (Wilén, 1997). There were similar numbers of filamentous bacteria (*Sphaerotilus natans* and *Thiothrix* I-II) in the two reactors. Perhaps a DO concentration of 2 mg/l is too low to avoid filamentous bacteria, since the organic loadings were rather high (0.9 - 1.9 g COD/g MLSS/d) in this part of the experiment. The results by Palm *et al.* (1980) showed that DO concentrations of about 3-6 mg/l would be necessary to avoid filaments at similar organic loadings as in this experiment.

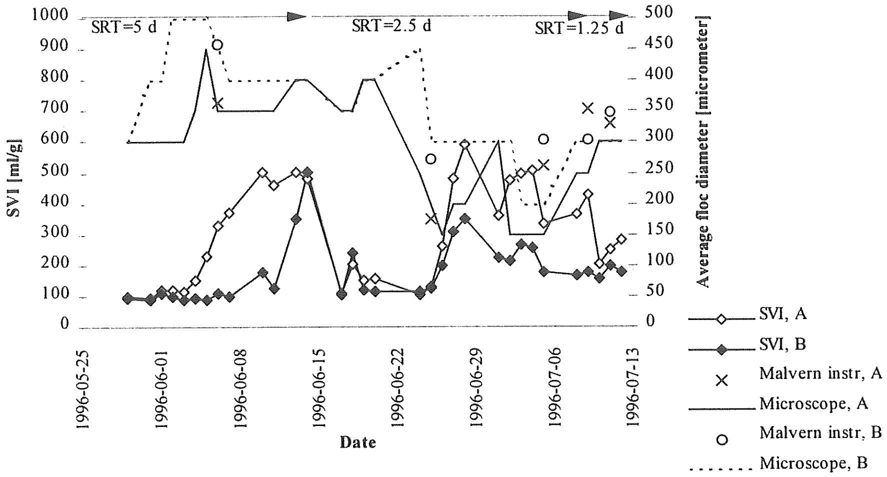


Figure 18 *Part III: variation in average floc diameter and SVI at dissolved oxygen concentrations of 0.5 mg/l (reactor A) and 2 mg/l (reactor B) at SRTs from 5 to 1.25 days.*

Experiments were not carried out at long sludge ages. At longer sludge ages, the flocs generally become larger and the number of smaller flocs reduces (Knocke and Zentkovich, 1986). Besides, the active heterotrophic biomass gets smaller (Gray, 1990). Under such conditions there are reasons to believe that the effects of DO on the floc structure and size distribution are smaller. Advanced treatment plants performing biological N and P removal, are operated at long sludge ages (15-35 days). Therefore it is important to include studies of long sludge ages in future research.

In the fourth experiment, the flocs were larger and contained less filamentous bacteria in the reactor operated at 5 mg O_2/l (reactor B) than at 2 mg O_2/l (reactor A) (Figure 19). The settling properties in reactor A deteriorated gradually due to proliferation of filamentous bacteria. When the DO concentration was increased from 2 to 5 mg/l, the settling properties improved and the SVI fell to the same level as in reactor B. Simultaneously the flocs became more compact, round and large. This shows, contrary to what often is claimed, that a concentration of 2 mg O_2/l in the aeration tank is not always enough to achieve good settling and separation properties.

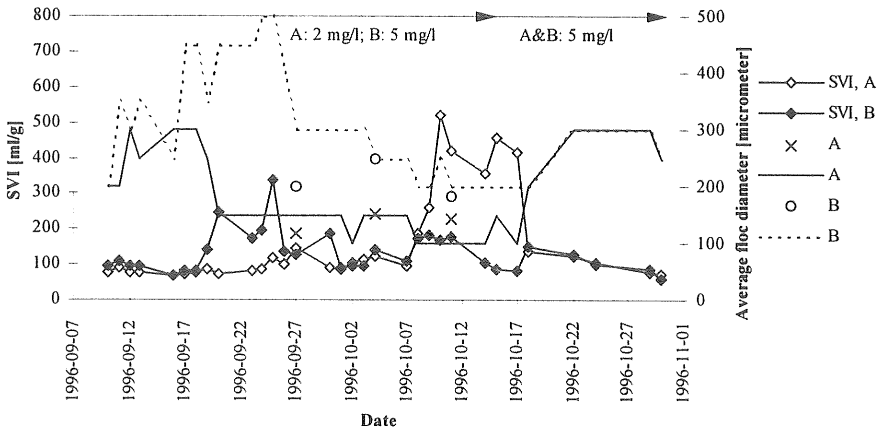


Figure 19 *Experiment IV: the average floc diameter and SVI at a dissolved oxygen concentration of 2 to 5 mg/l (lines = microscope; points = Malvern instrument).*

In all experiments, the size distribution *by volume* of flocs in the range 11.6 - 1128 μm fitted well to log-normal distribution functions (Section 2.3.5). This is in accordance with Jorand *et al.* (1995), who used a laser diffraction measurement device for floc size measurements. Li and Ganczarczyk (1991), as well as Barbusiński and Kościelniak (1995), measured flocs by light microscopy. They showed that the *frequency by number* of flocs (> 10 μm) fitted well to log-normal distribution functions. The difference in results could be due to the different particle analysis techniques used. Microscopic methods underestimate the number of smaller flocs. Examples of floc size distributions from experiment IV are illustrated in Figure 20. The size distribution was shifted towards smaller flocs in reactor A, which was operated at a DO concentration of 2 mg/l, than reactor B which was operated at a DO concentration of 5 mg/l.

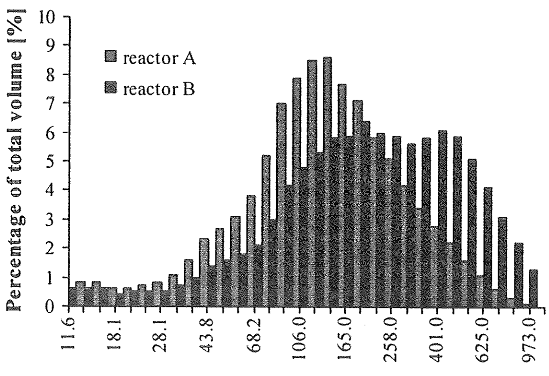


Figure 20 *Floc size distributions (experiment IV).*

Turbidity and size distribution of flocs in the supernatant

In the first experiment no correlation could be found between DO concentration and turbidity of the supernatant after settling. The turbidity was instead a function of the floc structure, measured in terms of SVI (Wilén, 1997) (Figure 21). Irregular flocs, containing filamentous and *zoogloea* bacteria, sweep small flocs and dispersed bacteria with them during settling; thereby producing a clear effluent (Eriksson *et al.,* 1992).

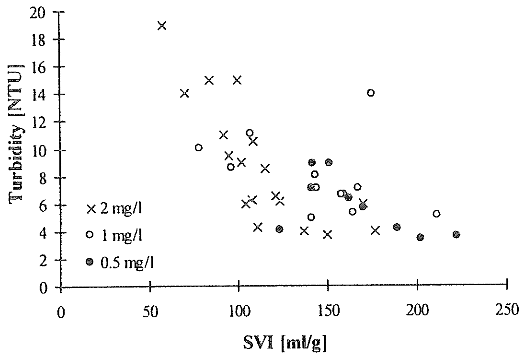


Figure 21 *Experiment I: the turbidity as a function of SVI (at different DO concentrations).*

In experiment II to IV, samples of the supernatant were analysed with a particle analyser (Met One), simultaneously as the turbidity was measured. Parameters such as turbidity and suspended solids concentration are normally used to evaluate the quality of the effluent. They are, however, poorly related to the size distribution of particles; a particle analysis can give some more information about which size intervals of particles affect the effluent quality.

The size distribution of flocs in the supernatant after settling could be fitted to power functions (Section 2.3.5), which is in accordance with Li and Ganczarczyk (1991). More than 80% of the number of flocs were smaller than 2 μm.

In experiment II, the turbidity and particle size distributions were analysed during the alternating aerobic and anaerobic periods. The turbidity and total number of particles/ml of sample, throughout an alternating period of 4 hours, is illustrated in Figure 22. The turbidity remained low in the aerobic reference reactor (reactor B), while it rose gradually during the anaerobic period (reactor A). Immediately, as the aeration was started again, the turbidity fell. The number of particles in the supernatant followed the turbidity closely (r^2 = 0.82). The constant a in the power function, which can be related to total amount of particles in the supernatant, was much higher during the anaerobic period (Table 6). The parameter b increased during the anaerobic period, indicating a shift in size distribution from larger to smaller flocs. Both a and b were much higher in the pre-settled wastewater than in any of the reactors, which reflects the efficiency with which the sludge flocs adsorb particulate material in the wastewater.

There were low nitrate concentrations in the supernatant during the anaerobic phases (0 - 4 mg/l), which might have reduced the turbidity under anaerobic conditions (***Papers III to IV***).

This could explain why hardly any increase in turbidity could be observed during alternating aerobic and anaerobic periods of 1-2 hours.

Table 6 *The parameters of the power function during alternating four-hour periods of aerobic-anaerobic conditions.*

	Parameters in the power function: $f(x) = ax_i^{-b}$	
	a	b
End of aerobic period		
Reactor A	$4.3 \cdot 10^5$	3
Reactor B	$1.5 \cdot 10^5$	3.6
End of anaerobic period		
Reactor A	$41 \cdot 10^5$	4.4
Reactor B	$1.7 \cdot 10^5$	3.7
Pre-settled wastewater	$440 \cdot 10^5$	4.7

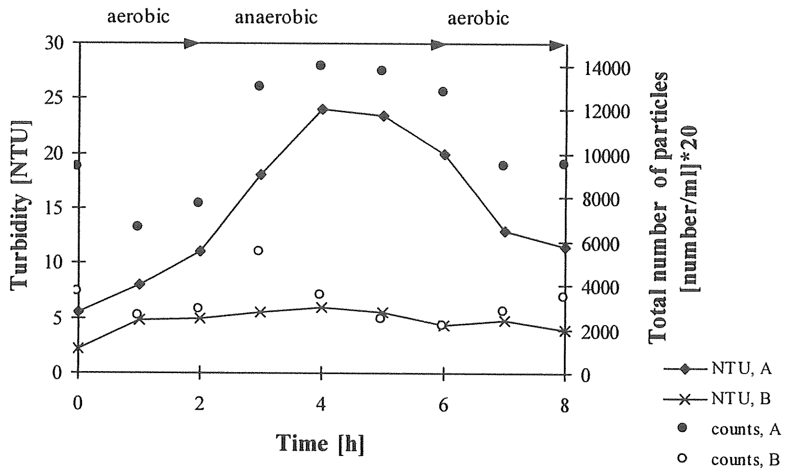


Figure 22 *Experiment II: change in turbidity and total number of particles in the supernatant during an 8 hour cycle for reactor A (operated under alternating aerobic/anaerobic conditions) and reactor B (operated under constant aerobic conditions).*

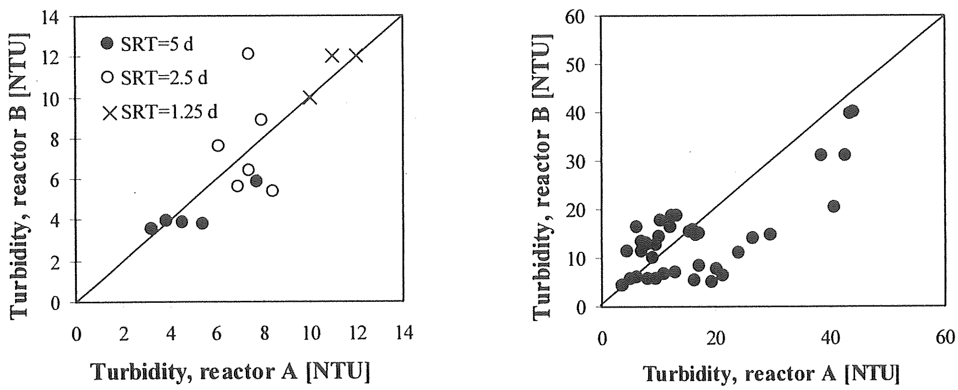
In experiment III, the turbidity was higher in the reactor operated at 0.5 mg O_2/l (reactor A) than at 2 mg O_2/l (reactor B) when the SRT was 5 days (Figure 23a). At shorter sludge ages, no difference in turbidity between the two reactors could be found. The turbidity increased with a reduced sludge age (i.e. increased organic loadings) which is in accordance with Li and Ganczarczyk (1993); Starkey and Karr (1984); and Roth and Pinnow (1981). A larger effect of DO concentration was expected at shorter sludge ages since the demand for oxygen then is higher. The most plausible explanation to why there was no difference in turbidity between the two reactors is that the flocs became more irregularly shaped at shorter sludge ages and large numbers of filaments started to proliferate which produced clear supernatants irrespective of dissolved oxygen concentration (compare with experiment I).

In experiment IV, the turbidity was generally higher at a DO concentration of 2 mg/l than at 5 mg/l (Figure 23b). There was also a good relationship between number of particles/ml and turbidity ($r^2 = 0.81$).

These results show that there is no clear relationship between average floc diameter and DO concentration. When the reactors were operated at higher DO concentrations, the flocs became more compact. Other factors such as sludge age, wastewater composition and turbulence are probably equally important. There was, on the other hand, a clear relationship between DO concentration and turbidity of the supernatant. As discussed in ***Paper I***, the suspended solids in the supernatant could be particles in the wastewater that have not adsorbed onto the flocs, or bacteria and other floc components present in the supernatant as a result of deflocculation. In the various experiments, there was no difference in treatment efficiency in terms of COD removal, i.e. there was no oxygen limitation with respect to degradation of organic material. The increase in turbidity was therefore probably due to differences in flocculation properties.

The measurements of the small particles in the supernatant are probably not very accurate and this could explain why no large differences in parameters *a* and *b* could be found. Also, many of the small particles and colloids, which contribute to the turbidity are too small to be measured with the Met One instrument (i.e.< 1 μm).

Apparently, the effects of DO limitation on the settling and separation properties of activated sludge can be divided into long term effects and short term ones. The long term effects were floc size, density and bacterial population, while the short term effect was the amount of small particles in the bulk phase between the larger flocs. However, in a long term situation also, the amount of small flocs was larger at lower DO concentrations. Long term effects probably have a more dramatic influence on the effluent quality, whereas short term effects can lead to smaller, but nevertheless significant, fluctuations in the amount of non-settleable solids.



a) b)

Figure 23 *A comparison of the turbidity of the supernatant for two reactors operated at different dissolved oxygen concentrations; (a) experiment III: reactors operated at 0.5 and 2 mg O_2/l (reactosr A and B, respectively) at different SRTs; and (b) experiment IV: reactors operated at 2 and 5 mg O_2/l (reactors A and B, respectively).*

4.3 Anaerobic Deflocculation and Aerobic Reflocculation of Activated Sludge (Paper III)

The results in ***Papers I*** and ***II*** were obtained in continuous systems, which means that it was difficult to distinguish between deflocculation or desorption of adsorbed matter (or lack of adsorption of organic matter from the wastewater) during periods of oxygen limitation. The results showed that the turbidity formed during short periods of oxygen limitation (a few hours) can be restored by aerating the sludge. Only a little is known about how deflocculated flocs can be restored although, from a practical point of view, this is of great interest. Parker *et al.* (1971, 1972) and Wahlberg *et al.* (1994) found, as described in Section 2.3.3, that sludge which has deflocculated due to shear effects may be reflocculated by means of gentle agitation (G value of 15 s^{-1}). They observed that the potential for reflocculation varied from one sludge to the other and that the supernatant sometimes remained turbid even after reflocculation. This was found to be due to differences in the polysaccharide content of the sludge (Wahlberg *et al.*, 1992). They did not, however, study sludges that were deflocculated due to anaerobic conditions. In ***Paper III***, the experiments reported were carried out in controlled systems to get detailed information about deflocculation-reflocculation processes. In these experiments, the sludge was subjected to high shear forces; the purpose was to study possible differences in floc strength when the sludge is exposed to anaerobic or aerobic conditions.

When the activated sludge was exposed to anaerobic conditions, at a given shear and temperature, it deflocculated comparatively more than under aerobic conditions (Figure 24). The deflocculation appeared to follow a 1st order curve. Within the experimental time interval (6 - 7 hours), the turbidity of the aerobic reactor reached an equilibrium value, whereas for the anaerobic reactor it was not reached even after 20 hours. If the sludge was not strictly anaerobic but nitrate was present as an electron acceptor (anoxic conditions), less deflocculation was observed. The response to the changed conditions in the reactors was, surprisingly, instantaneous. Although Mikkelsen and Keiding (1999a) studied deflocculation due to an applied shear in an experimental system similar to that used here, they investigated only the physico-chemical aspects. Apparently, the deflocculation process approaches a higher level of equilibrium turbidity under anaerobic conditions than under aerobic conditions. This indicates that microbial processes are involved as well.

Even though the sludge was subjected to high shear forces, only a small fraction of the total amount of suspended solids was released to the bulk phase, i.e. the deflocculation has to be understood as an erosion of primary particles from larger flocs, as decribed by Mikkelsen and Keiding (1999a) (Section 2.3.3). Apparently, the different entities are very firmly bound together in the interior of the sludge floc, while the outer particles are less strongly bound to the floc (Eriksson *et al.*, 1992; Jorand *et al.*, 1995; Keiding and Nielsen, 1997). This also explains why activated sludge flocs never deflocculate completely when a shear force is applied.

When activated sludge was exposed to alternating aerobic-anaerobic conditions, it was observed that a partial reflocculation occurred under aerobic conditions when the sludge had been deflocculated beforehand under anaerobic conditions (Figure 24). These results are in agreement with ***Papers I*** and ***III***. After each anaerobic-aerobic cycle, an accumulation in turbidity was observed. In other words, after each anaerobic phase, the turbidity establishes itself at a new equilibrium turbidity. The difference in equilibrium turbidity between the aerated deflocculated sludge and the aerobic reference sludge increased generally with the length of the anaerobic period. This indicates that a part of the deflocculation was irreversible.

This is very important since it has previously been assumed that dispersed sludge can be made to reflocculate if external factors such as shear level are decreased (Wahlberg *et al.*, 1994). Apparently, this is not true for biological sludges.

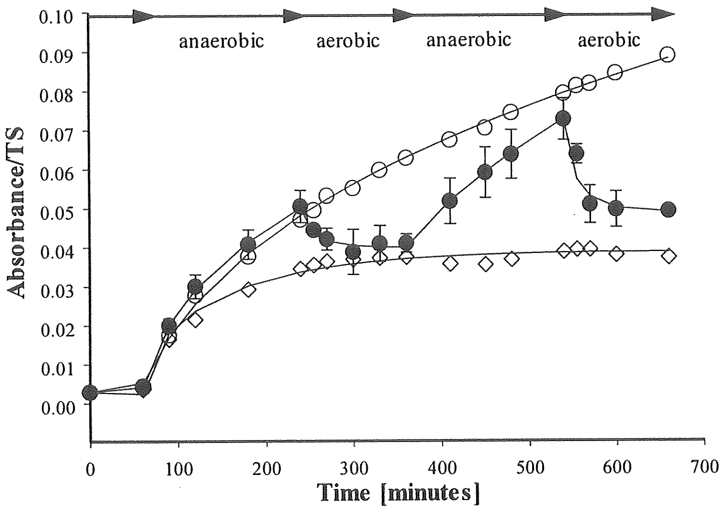


Figure 24 *Deflocculation under anaerobic conditions and reflocculation under aerobic conditions: (◇) aerobic reference reactor, (○) anaerobic reference reactor, and (●) two repeated anaerobic-aerobic periods.*

Two sludges, taken from two different treatment plants, showed the same basic phenomenon, but to a different extent. The Aalborg East wastewater treatment plant (WWTP) is designed for biological N and P removal and the sludge is adapted to aerobic, anoxic and anaerobic conditions. The sludge has also a long sludge age (25 - 30 days). The Rya WWTP is designed for biological N removal, hence adapted to aerobic and anoxic conditions, with a sludge age of only 3-4 days. The young sludge from the Rya WWTP deflocculated much more, especially at anaerobic conditions, which is in accordance with Bruus *et al.* (1993). In Figure 25, the degrees of deflocculations of the two sludges under aerobic and anaerobic conditions are compared. The reflocculation under aerobic conditions was faster for the Rya sludge, but a large amount of particles was left unflocculated. If the biological activity is the primary reason for the difference in deflocculation-reflocculation behaviour observed, the most probable explanation was the larger viable biomass in the sludge with short sludge age (Gray, 1990). Consequently, the difference in response was particularly large under anaerobic conditions. It is apparently more critical with periods of oxygen limitation, if the sludge age is low. It has been proposed that activated sludges of low and high sludge ages have rather different structures (Eriksson *et al.*, 1992), which is something that may affect the floc strength as well. Sludge flocs that have remained in a treatment plant for a long period of time have been exposed to more shear compared to younger flocs, and irregularities have been eroded away. Besides, more EPS such as humic substances have accumulated in the floc matrix, which has made the flocs more compact; the different constituents are firmly "glued" together. Young sludge, on the other hand, has a more fragile surface structure and the different entities appear to be loosely bound to the floc. It is risky to assume that there is always a clear difference in structure between young and old sludge. Other parameters such as type of treatment process, and wastewater compositions etc. are probably equally important for the development of the floc structure.

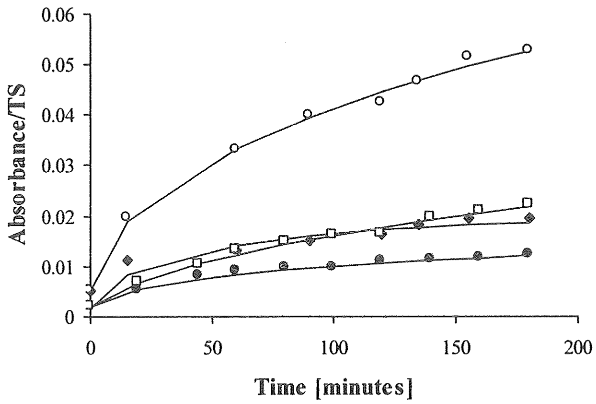


Figure 25 *Deflocculation under aerobic and anaerobic conditions of two sludges with different sludge ages: (◆) Rya-aerobic (4 - 5 days); (○) Rya-anaerobic; (●) Aalborg East-aerobic (25 - 30 days); and (□) Aalborg East-anaerobic. The experiments were carried out at the same total solids concentration (3.7 g/l).*

The chemical composition of the "turbidity" was analysed to see whether the composition of the turbidity corresponded to the one of the entire sludge. The composition of the turbidity, during two repeated anaerobic-aerobic cycles, show that the concentration of the different components, follow the turbidity rather well: $r^2 > 0.9$ (Figure 26). The majority of the deflocculated particles were removed by a strong centrifugation, which means that they were not dissolved but they were particles attached to larger floc components. The composition of the supernatant showed that the deflocculated particles were enriched in number of bacteria and protein, compared to the turbidity of the aerobic reference reactor, indicating a difference in compositions of the deflocculated particles. The relationship between concentration of protein and turbidity in the deflocculated activated sludge, is shown in Figure 27. These bacteria may arise from single bacteria existing in the "cloud" of loosely attached EPS material, or bacteria adsorbed on the more rigid backbone of organic fibers, strong micro-colonies and filamentous bacteria. Protein is the dominating compound of the EPS; its release would enrich the supernatant with protein (Section, 2.2.2). It is, however, not known why these deflocculated bacteria and other floc constituents could not reflocculate. To reestablish the state of equilibrium by aeration, it must be possible both thermodynamically and kinetically for the floc entities. Microbial processes like Fe(III)-reduction, hydrolysis of important components that act as "glue", or due to inhibited EPS production might prevent that.

During the anaerobic period, the turbidity was also enriched in humic substances which was also the dominating compound left in the supernatant after an intensive centrifugation. Large organic molecules like humic substances are adsorbed in the floc matrix and are easily detached upon shear (Keiding and Nielsen, 1997). The amount of organic matter left deflocculated after aeration corresponded to approximately 1-2% of the amount in the whole sludge, which does not seem much, but it can have a large impact on the effluent quality. With a suspended solids concentration of 4 g/l in the aeration tank, this would correspond to an effluent concentration of 25-50 mg/l.

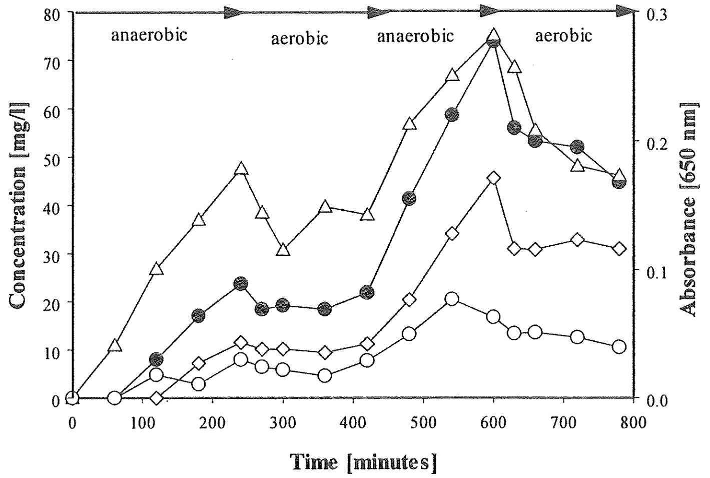


Figure 26 *Chemical composition of the organic fraction of the "turbidity" during alternations of anaerobic and aerobic conditions (given as difference in turbidity between the anaerobic and aerobic reactors): (●) absorbance, (○) carbohydrate, (◇) humic substances, and (Δ) protein.*

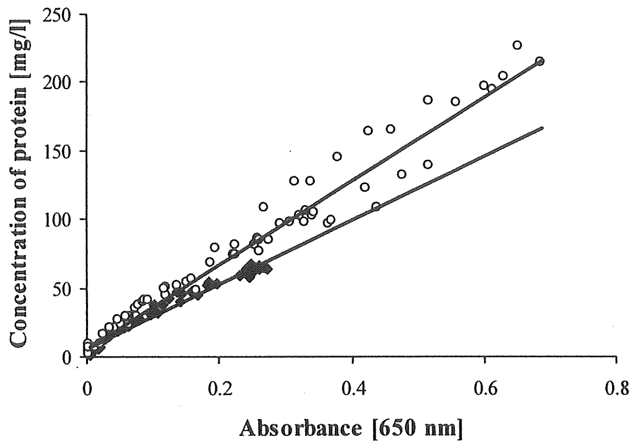


Figure 27 *The relationship between concentration of protein and turbidity in the deflocculated activated sludge: (◆) aerobic conditions, and (○) anaerobic conditions.*

The deflocculation was strongly shear-dependent, which is in accordance with the results obtained by Mikkelsen and Keiding (1999a). Also the reflocculation was shear dependent; at increased shear levels, the amount of particles remaining deflocculated after aeration increased. However, the relative degree of reflocculation seemed to be independent of shear level since the same proportion of the maximum turbidity was removed at all shear levels tested. This also indicates that the same components reflocculated irrespective of shear level. At low shear levels, a very low degree of deflocculation was observed, whereas at higher shear levels, an almost linear relationship between G value and deflocculation was observed (Figure 28). Furthermore, the effect of shear was reinforced at anaerobic conditions. In the results reported in ***Papers I*** and ***II*** the sludge was subjected to low shear levels (G=30 s^{-1}) and the increase in turbidity was, consequently, also low. The increase in turbidity during low shear levels could, as mentioned before, be due to an active transport of bacteria to avoid undesired conditions (Rasmussen *et al.*, 1994). In ***Paper II***, the deflocculation under anaerobic conditions and low shear was partly due to particles > 1 μm, indicating that also larger floc entities deflocculated.

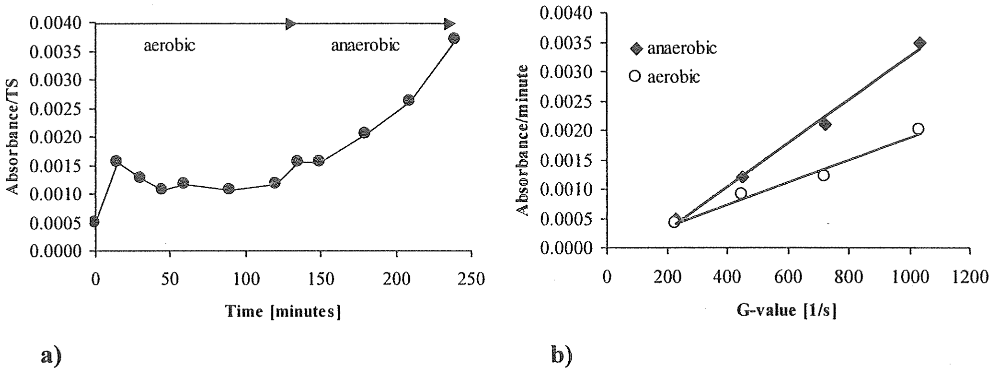
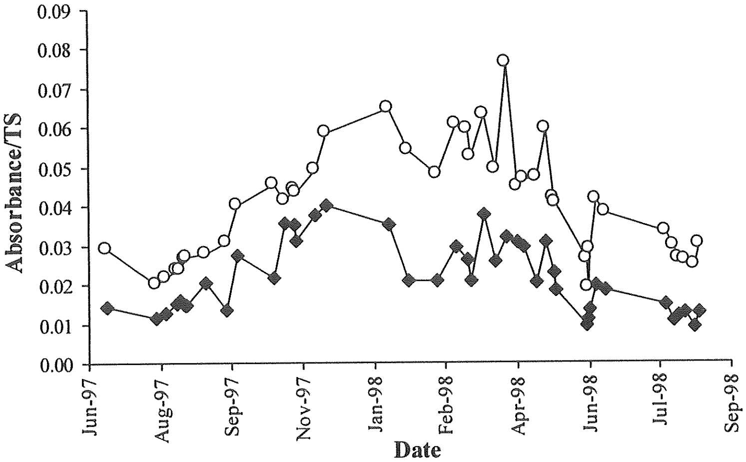


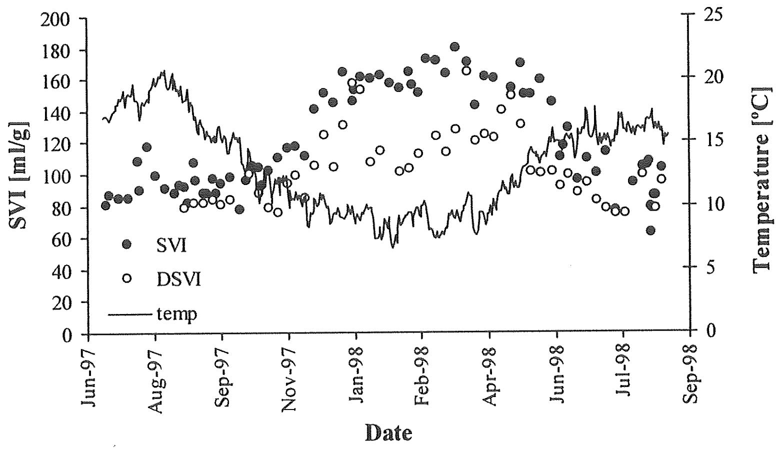
Figure 28 *Deflocculation of activated sludge subjected to: (a) low shear levels (150 rpm; G ≈ 100 s^{-1}; and (b) high shear levels (G values from approximately 200 to 1000 s^{-1}).*

There was a seasonal variation in deflocculation behaviour, with the highest levels of deflocculation during the winter (Figure 29a). In other words, the floc strength was reduced during the winter. The difference in turbidity between the anaerobic and aerobic reactors increased during the winter, which indicates that the weaker flocs had lesser tendency to deflocculate if the conditions were aerobic. It is not known why the floc strength changed throughout the year or if this is typical for other treatment plants. Deflocculation tests showed that there was a temperature dependence of the deflocculation; the degree of deflocculation increased when the temperature decreased (***Paper VI***). The situation is rather different, however, in an activated sludge treatment plant where the bacteria have adapted gradually to decreased temperatures, whereas in deflocculation tests the sludge is exposed to sudden changes in temperature. Some changes in floc morphology could be recorded. During the winter, the sludge contained more filamentous bacteria (*Microthrix parvicella*, Eikelboom index 1 - 2) and the flocs appeared to be less compact. The SVI increased during the winter (Figure 29b). The presence of *M. parvicella* during the winter has been reported elsewhere (Wanner, 1994; Knoop and Kunst, 1998). No clear relationship between reflocculation and season could be found.

The results show that the activated sludge floc stability is strongly dependent on the availability of an electron acceptor, oxygen or nitrate, a phenomenon that can be connected to the microbial activity. External effects like shear intensity and temperature can reinforce these effects. Furthermore, flocs that have deflocculated during anaerobic conditions can be "repaired" by aeration, but only to a certain extent, i.e. a certain fraction of the deflocculation is irreversible. Therefore, when considering the design and operation of full scale plants, anaerobic conditions should be avoided, for instance at high organic loadings, in the secondary settler, in anoxic tanks, or in connection with anaerobic storage. Besides, it is particularly important that sludge, which has been subjected to anaerobic conditions, is not exposed to high shear, since this could deteriorate the solid-liquid separation.



a)



b)

Figure 29 *(a) Turbidity normalized by total solids concentration (TS) under (○) anaerobic and (◆) aerobic conditions after 300 minutes stirring at 750 rpm for a series of experiments carried out over one year; and (b) temperature in the aeration tank, SVI and DSVI at the Aalborg East WWTP (TS = 9.4 ±0.9 g/l).*

4.4 The influence of Microbial Activity on the Stability of Activated Sludge Flocs (Paper IV)

The stability of sludge flocs is determined by their components and the interparticle forces among these (e.g. microorganisms, EPS, organic fibers, adsorbed organic particles from the wastewater, and inorganic components) as described in Section 2.2. The stability of these aggregates has been found to follow general rules for colloidal stability; factors such as ionic strength, ionic composition and surface charge affect the floc stability (e.g. Zita and Hermansson, 1994; Keiding and Nielsen, 1997; Mikkelsen *et al.*, 1996). The results from ***Paper III*** indicate that the bacteria are the driving forces in the deflocculation process. The similarity of the deflocculation under anaerobic conditions and at a low temperature, as well as the reduced deflocculation under anaerobic conditions when nitrate was present, strongly support this.

It is not possible to apply the information obtained from studies of bacterial attachment-detachment in pure culture directly, since the composition of activated sludge flocs (as well as many other natural microbial aggregates) is not quite the same; the biomass constitutes only a minor fraction of the floc and the bacteria are growing in strong microcolonies that are bound together in a matrix of EPS. Due to the large complexity of activated sludge flocs, it is difficult to distinguish between the different mechanisms involved.

In ***Paper IV***, the effect of aerobic microbial activity on the floc stability was investigated. When the easily degradable substrates ethanol and glucose were added to the activated sludge, stronger flocs were formed. This could be seen as a reduced deflocculation compared to the reference reactor, to which no substrate was added (Figure 30). Lactate and ammonium had no effect on the floc stability (data not shown), whereas acetate and propionate *increased* the deflocculation. When the microbial activity was inhibited by the addition of azide or chloramphenicol or by lowering the temperature to 4°C, the turbidity increased in a way similar to that under anaerobic conditions, which further suggests that the aerobic microbial activity is essential to keep the flocs intact.

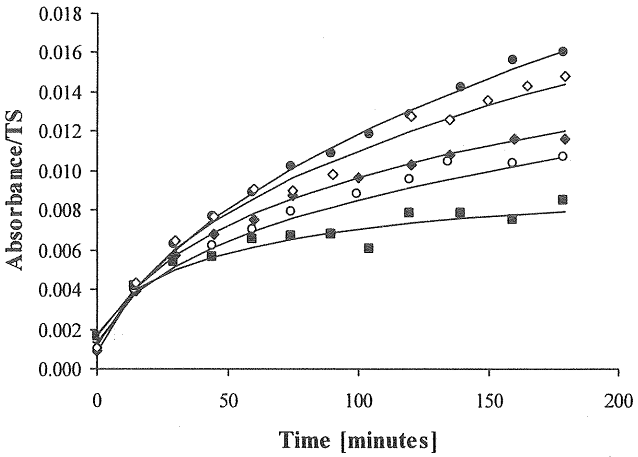


Figure 30 *Stimulation of the microbial activity by addition of substrates under aerobic conditions: (■) ethanol; (○) glucose; (◆) reference; (◇) propionate; and (●) acetate.*

The reduction in floc stability does not necessarily have to be due to a reduction of the *aerobic* microbial activity; it could also be due to a stimulation of the *anaerobic* microbial activity. This is supported by Caccavo *et al.* (1996) who showed that the addition of Fe(III)-reducing bacteria to pasteurized activated sludge enhanced deflocculation. Apparently, the conversion of iron from Fe(III) to Fe(II) causes a reduced floc stability. Other types of anaerobic microbial activity, such as exoenzyme activity (Nielsen *et al.*, 1996) and sulphate reduction (Nielsen and Keiding, 1998), may also weaken the interparticle forces within the sludge floc (Section 2.3.4).

Addition of substrate (glucose and acetate) under anaerobic conditions generally produced a minor increase in deflocculation, indicating a stimulation of anaerobic microbial activity. When the Fe(III)-reducing bacterium *Shewanella alga* BrY was added to the sludge, the deflocculation increased. This was due to the iron reduction since no increase in deflocculation was observed for sludge with heat killed *S. alga*, compared to a control sludge without *S. alga*. A further stimulation of the Fe(III)-reduction by *S. alga,* was achieved by adding lactate as an electron donor; this enhanced the deflocculation even more (Figure 31). The Fe(III) reduction rates were measured several times in the Aalborg East sludge and an additional stimulation of the reduction rates was observed when *S. alga* bacteria were added (Table 7). In general, the increase in Fe(II) concentration was well correlated to the turbidity. The selective removal of Fe(III) from the floc matrix, by precipitation with sulphide, revealed the large effect Fe(III) has on the floc strength. This is also in agreement with earlier observations that Fe(III), not calcium, is the most important cation for the stability of activated sludge flocs, provided Fe(III) is present (Nielsen and Keiding, 1998). The Fe(III)-reducing activity is common in activated sludge treatment plants (Rasmussen and Nielsen, 1996; Nielsen, 1996; Nielsen *et al.,* 1997). It is often assumed that most of the iron in the aeration tank is in the oxidized state. The results reported here show that the amount of Fe(II) in the aeration tank was 15 - 30% of the total amount of iron in the sludge.

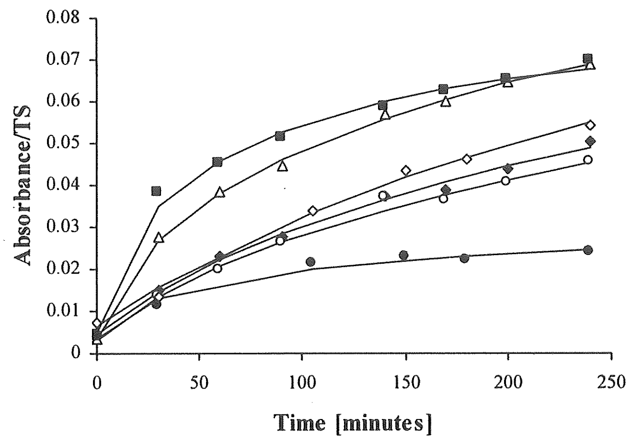


Figure 31 *Deflocculation after stimulation of Fe(III)-reduction by the addition of S. alga BrY bateria: (●) aerobic reference reactor; (○) –BrY + lactate; (◆) –BrY -lactate; (◇) pasteurized BrY; (Δ) +BrY -lacate; and (■) + BrY + lactate. The turbidity caused by S. alga that did not stick to the activated sludge is withdrawn.*

In the Rya sludge, which contained 2 to 3 times more iron than the Aalborg East sludge, substantially higher Fe(III)-reduction rates were measured. One such experiment is illustrated in Figure 32. The Fe(III)-reduction mechanism seems to be particularly significant for prolonged anaerobic conditions or when the population of Fe(III)-reducing bacteria is high. The reason why the reduction rates were so much higher in the Rya sludge is not known; however, the large concentration of iron, as well as the low sludge age with its associated high microbial viability, may stimulate the growth of Fe(III)-reducing bacteria in the sludge. The activated sludge flocs from the Rya WWTP were less well flocculated; i.e. they were more porous and irregularly shaped. Removal of Fe(III) from such poorly flocculated sludge may have a greater impact on the floc strength than removal from a well flocculated sludge as the one from the Aalborg East WWTP. The important issue is apparently the amount of Fe(III) reduced. This was illustrated by addition of sulphide to the sludge; the more iron that was removed by precipitation, the more deflocculation took place.

Table 7 *Summary of iron reduction rates measured in the sludge from the Aalborg East WWTP.*

Parameter	
Total amount of Fe (n = 11)	0.93 - 1.32 mmol/g VS
Iron as Fe(II) at the start of experiment	Approximately 30% of Fe(total)
Iron as Fe(II) at the end of experiment (3 - 5 hours)	35 - 40% of Fe(total)
Fe(III)-reduction rate (n = 11)	4 - 24 μmol/g VS · h
Increase in reduction rate after addition of	
S. alga BrY (n = 4)	0 - 400% (average: 150%)
S. alga BrY + lactate (n = 1)	200%

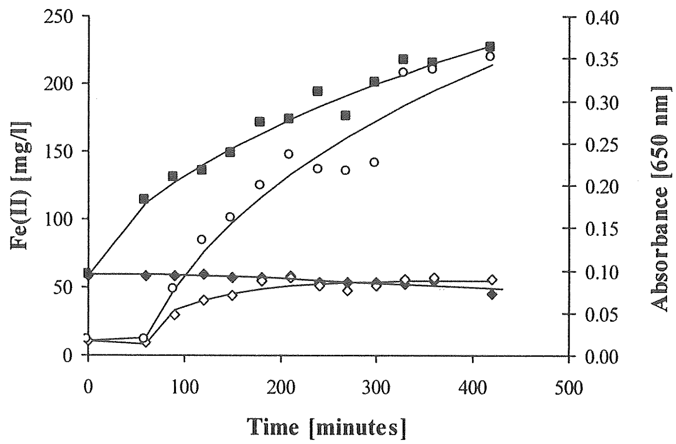


Figure 32 *Deflocculation of activated sludge from the Rya WWTP under aerobic and anaerobic conditions: (◇) turbidity-aerobic; (○) turbidity-anaerobic; (◆) Fe(II)-aerobic; and (■) Fe(II)-anaerobic (VSS = 4 g/l).*

To illustrate the difference in floc strength for the various conditions investigated in this study, a recent method developed by Mikkelsen and Keiding (1999a), described in Section

2.3.3, was adopted. This method makes it possible to estimate the relative interaction energies between floc components from the equilibrium turbidity. From deflocculation experiments carried out under different conditions (Figure 33), the equilibrium turbidity could be estimated from the diffusion model by Mikkelsen and Keiding (1999a). The maximum turbidity levels normalized to the suspended solids concentration are summarized in Table 8. The largest change from a stable aerobic floc, was obtained by sulphide addition. The diffusion constants for the anaerobic reactors did not differ statistically from each other. When the average value of the diffusion constants for the three anaerobic reactors was used, a larger difference in equilibrium turbidity was obtained, as well as smaller standard deviations. This shows that there is a statistically significant difference in interaction energy between the different anaerobic reactors, as well as between the various aerobic reactors and the anaerobic reactors. The absolute value of the diffusion constant may be influenced by the floc structure. The high values for the aerobic reactors may reflect an active metabolism of the bacteria. A difference in equilibrium turbidity has been interpreted as a difference in the composition of the components leaving the flocs (Mikkelsen and Keiding, 1999). Their experiments did, however, not take the microbial activity into account, which could affect the equilibrium turbidity too. This method enables a better quantitative description of the flocculation-defloccualaion processes, and makes it easier to compare different conditions.

Table 8 *Equilibrium turbidity and diffusion constants for sludge deflocculated under different conditions (standard deviations in brackets).*

Conditions	Equilibrium turbidity	Diffusion constant (10^{-5})	Equilibrium turbidity $D = 1.48 \cdot 10^{-5}$
Aerobic with added ethanol	0.007 (0.00013)	40.2 (5.32)	
Aerobic with added glucose	0.012 (0.001)	13.0 (2.33)	
Aerobic	0.015 (0.001)	10.2 (2.24)	
Anoxic	0.054 (0.006)	1.09 (0.25)	0.046 (0.0007)
Anaerobic	0.069 (0.006)	1.18 (0.56)	0.061 (0.0007)
Anaerobic with added sulphide	0.079 (0.004)	2.16 (0.24)	0.094 (0.009)

These results, together with the ones in ***Paper III***, clearly show that aerobic microbial activity is essential to keep the flocs intact. It is, however, not possible to say whether the changes in stability under anaerobic conditions are due to a change in microbial production of exopolymers, a change in surface hydrophobicity, a change in membrane potential, or due to other changes. The important conclusion is that the bacterial activity can change the interaction forces between the different entities within the flocs almost instantaneously.

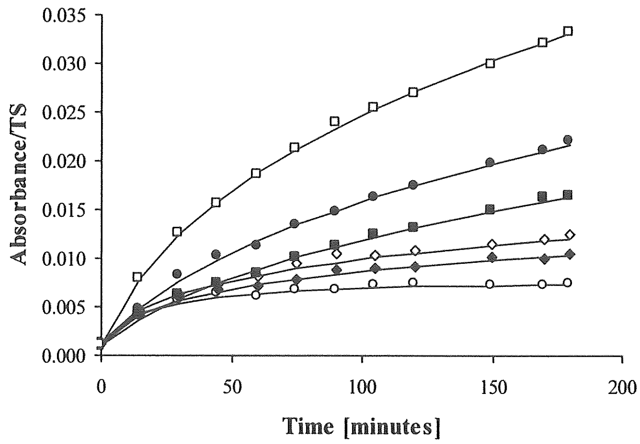


Figure 33 *Comparison of the relative effects of six parameters on the deflocculation of sludge from the Aalborg East WWTP: (○) aerobic with ethanol; (◆) aerobic with glucose; (◇) aerobic; (■) anoxic; (●) anaerobic; and (□) anaerobic with 0.9 mM sulphide.*

Sludge flocs contain only to a minor extent microorganisms (10-20%), while the EPS constitute approximately 50-60% of the organic matter. It could be so that a continuous EPS production is required to keep the flocs intact by "glueing" the different entities together, and that even shorter periods with anaerobic conditions could affect the EPS production. Especially at the outer surface of flocs where the particles are bound with weak forces (Eriksson *et al.*,1992, Section 2.2.4), and where the particles are exposed to the highest hear forces, this could be critical.

Nitrification is a slow process which yields little energy and the nitrifying population is small compared to the heterotrophic. This could be the reason why no improvement in flocculation could be observed when ammonium was added. Acetate and propionate could affect the composition of the EPS produced in such a way that the flocs become weaker. However, more research is needed to determine why the floc stability is affected differently by different substrates. Acetate did not stimulate the growth of single bacteria between the flocs in the bulk phase, since the addition of acetate to activated sludge subjected to low shear levels (150 rpm) did not cause increased turbidity of the supernatant. Ethanol did not affect the deflocculation of pasteurized sludge, which shows that the effect of ethanol was not purely chemical.

In pure culture studies, it has been found that the EPS production is affected by the types as well as by the concentrations of substrates present; different species do not respond the same way to different substrates (e.g. Knox *et al.*, 1985; McEldowney and Fletcher, 1986; Jorand *et al.*, 1994) (Section 2.2.4). Oxygen limitation has been found to reduce both hydrophobicity and EPS production in pure culture (Palmgren *et al.*, 1998; Palmgren and Nielsen, 1998). It is not yet clear whether the production of EPS is necessary for cell adhesion to surfaces. Allison and Sutherland (1987) found that, for two strains of bacteria investigated, EPS were not necessary for the initial attachment to a surface, but they were necessary for the formation of microcolonies. Another cell surface property that has been reported as particularly important for the attachment of bacteria to activated sludge flocs is the hydrophobicity (Zita and

Hermansson, 1997a, 1997b; Olofsson *et al.*, 1998). Bacteria are often regarded as being colloidal particles; however, they are not inert and their cell surface properties can change with alterations in environmental conditions.

4.5 Reflocculation of Activated Sludge by Stimulating the Biological Activity (Paper V)

Paper V reports an investigation of the importance of aerobic microbial activity in the reflocculation process. The sludge flocs that had been deflocculated beforehand under anaerobic conditions, reflocculated partly when the sludge was aerated. It was also observed that, when the aerobic microbial activity was inhibited by azide or chloramphenicol, or by cooling to 4 °C, deflocculation increased and reflocculation was inhibited (Figures 34 and 35). This shows that aerobic microbial activity is essential to obtain good reflocculation of activated sludge sludge flocs deflocculated under anaerobic conditions. The reflocculation was inhibited only when the conditions were strictly anaerobic or when the aerobic microbial activity was inhibited. Some reflocculation was obtained when nitrate was added, which shows that nitrate can be used as an electron acceptor by some, but not all, aerobic heterotrophic bacteria.

From the results obtained in studies of adhesion and flocculation of pure cultures, it is not clear whether microbial activity is important for cell adhesion. Viability has been reported important for attachment to surfaces in some studies. Paul (1984) found that viable cells of an estaurine *vibrio* sp. adhered better than dead cells to polystyrene. The addition of protein synthesis inhibitors (e.g. chloramphenicol) and uncouplers (e.g. azide) led to some inhibition of cell adhesion. In other studies the viability has not been found significant for attachment of cells; e.g. McEldowney and Fletcher (1986) found that chloramphenicol did not inhibit the attachment of aquatic bacteria to solid surfaces. They suggested that the synthesis of fresh cell wall material is not required to maintain adhesion over short periods of time. Similarly, Hantula and Bamford (1991) found that azide did not cause deflocculation of two microbial strains isolated from wastewater. However, inhibitors such as chloramphenicol and azide can inhibit the production of certain enzymes that are involved in the production of adhesive molecules. McEldowney and Fletcher (1986) found that the addition of agents that denature the exopolysaccharides at the cell surface led to detachment. Hermanowicz and Filho (1992) found that disinfectants reduced the ability of a mixed bacterial population, isolated from well water, to attach to surfaces. Heterogeneous species of bacteria have dissimilar surface properties and differing mechanisms are involved in the attachment to surfaces and aggregation (Mozes *et al.*, 1989). Therefore it is not possible to simply transfer the results of pure culture studies directly to such complex systems as activated sludge.

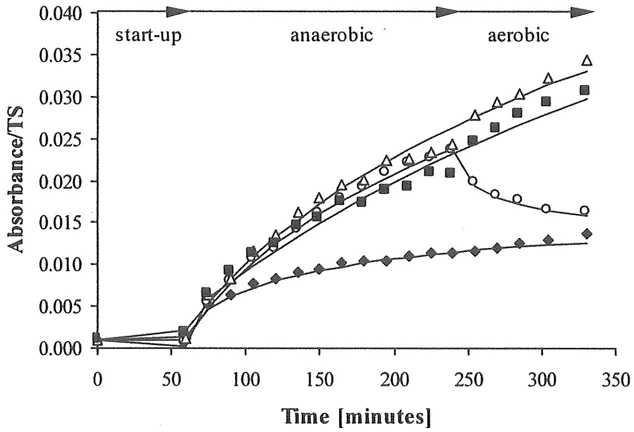


Figure 34 *Deflocculation and reflocculation of activated sludge in the presence of azide: (◆) aerobic reference; (○) no azide added; (■) aerobic with azide added at the start of the experiment; and (Δ) azide added at the beginning of the reflocculation phase.*

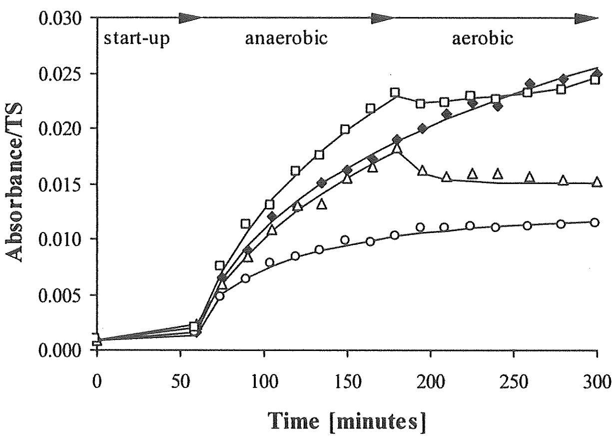


Figure 35 *Deflocculation and reflocculation at different temperatures: (○) aerobic reference 20 °C; (Δ) test 20 °C; (◆) aerobic reference 4 °C; and (□) test 4 °C.*

The reflocculation was further improved when the aerobic microbial activity was stimulated by adding substrate at the onset of the reflocculation phase. It was also observed that the effect of the various substrates varied; glucose, in particular, had in some cases a very large effect on the reflocculation (Figure 36), but in some cases, it had no or little effect. Interestingly, acetate had always a deteriorating effect on the reflocculation while ethanol, on the other hand, always improved reflocculation. The two sludges investigated responded in a similar way to the different substrates, but to a somewhat different degree. This reflects that different populations of bacteria exist in the two treatment plants.

Stimulation of the reflocculation by some substrates and deterioration by others, might depend on a change in the production of certain surface biomolecules, which are important for the attachment of cells to surfaces (Allison and Sutherland, 1987; Fletcher, 1996). As discussed in Section 4.4, the production of EPS can be affected differently by different substrates.

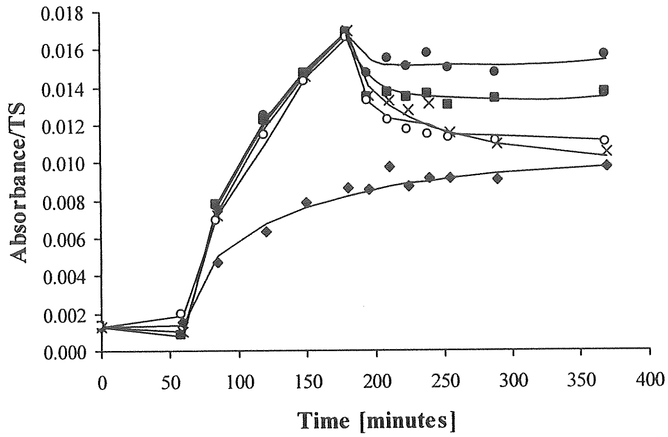
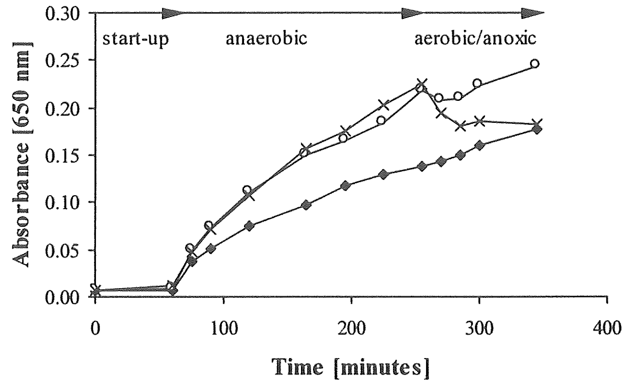


Figure 36 *Deflocculation under anaerobic conditions and reflocculation under aerobic conditions with addition of glucose: (◆) aerobic reference; (●) no glucose; (■) 0.1 g/l; (×) 0.25 g/l; and (○) 0.5 g/l.*

Even though the aerobic microbial activity seems to be important for improved reflocculation, other processes could be important as well. During the anaerobic phase, Fe(III) is reduced to Fe(II) (***Paper IV***). Especially at extended periods of anaerobic conditions, or when the population of Fe(III)-reducing bacteria is high, significant concentrations of Fe(II) can accumulate. The Fe(II), formed during the anaerobic phase, was found to be easily oxidized back to Fe(III) during aerobic conditions. At aerobic conditions, the amount of Fe(II) decreased gradually. Nielsen (1996) found that even if sludge was aerated for more than 6 days, still some Fe(II) remained which was probably bound tightly to the sludge flocs. When nitrate was present at anaerobic conditions, no Fe(III)-reduction took place. When nitrate was added to sludge that beforehand had been subjected to anaerobic conditions, the concentration of Fe(II) decreased in the same way as under aerobic conditions; simultaneously some reflocculation was observed (Figure 37). The reflocculation at anoxic conditions was, however, much smaller than at aerobic conditions. It appeared therefore that the oxidation of Fe(II) is less important than to the aerobic microbial activity.

To further investigate how important the Fe(II)-oxidation is for the reflocculation of activated sludge, the concentration of Fe(II) was measured during reflocculation experiments at which various substrates were added. The oxidation rate was found to be similar for all substrates, while the degree of reflocculation differed markedly. In another experiment, azide was added at the onset of the reflocculation; the accumulated amount of Fe(II) was oxidized to Fe(III) even though no reflocculation was observed. These results show that the oxidation of Fe(II) is less important than the effects of aerobic microbial activity on the reflocculation of activated sludge. If a large amount of Fe(III) is reduced in the sludge, which might happen if the wastewater contains large quantities of sulphides, oxidation of iron sulphide would be important for the reflocculation. The results in ***Paper IV*** showed that when such high concentrations of sulphide was added that approximately 80-90% of the iron was reduced, the flocs deflocculated significantly. At such situation it is essential that the Fe(II) is oxidized to reflocculate the sludge. Experiments indicate, however, that oxidation of iron at such situations is slow (Wilén and Nielsen, unpublished data).



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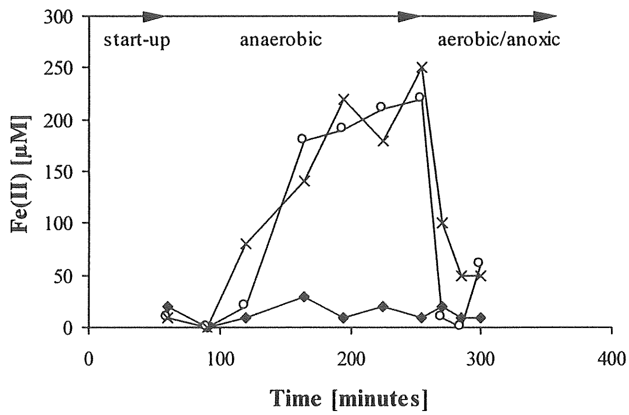

b)

Figure 37 *Deflocculation under anaerobic conditions and reflocculation under aerobic and anoxic conditions; (◆) anoxic reference, (×) reflocculation with oxygen, and (○) reflocculation with nitrate: (a) turbidity as absorbance at 650 nm; and (b) concentration of Fe(II) [µM].*

These results do not explain the reasons to the lack of complete reflocculation. From pure culture studies it is known that bacteria do not always exhibit reversibility (Rijnaarts *et al.*, 1995). One possibility is that hydrophilic bacteria, which are embedded in the floc matrix, are released to the bulk phase during the deflocculation under anaerobic conditions; these bacteria cannot adhere again to the flocs during aeration. Olofsson *et al.* (1998) studied the adsorption of different bacterial strains marked with green fluorescent protein to activated sludge. They observed that hydrophilic bacteria adhered poorer to the sludge than hydrophobic ones. They adhered also spatially different; the hydrophilic bacteria adhered predominantly at the outer surface of the flocs whereas hydrophobic bacteria adhered also in the interior of the flocs. Zita and Hermansson (1997a) found that most bacteria present in the effluent water from a wastewater treatment plant were hydrophilic. Furthermore, bacteria differ in their surface structure which can change for one specific species as a consequence of different growth conditions. It is possible that some bacteria change their surface structures during anaerobic conditions and that they, for some reason or other, cannot regain a surface structure that is beneficial for adhesion. This needs more attention in future research. Molecular methods could be used to identify the dominating bacterial species in the microbial community in the bulk phase between the flocs as compared to the total sludge.

Only a few studies have been carried out to investigate the importance of microbial activity on the attachment and aggregation of bacteria. In most studies the bacteria have been harvested and washed before the experiments. The problem is that bacteria can change their surface properties and EPS production when their environmental conditions are changed. Hence, to get relevant results, the studies should be carried out *in situ*.

4.6 Temperature Effects on Deflocculation (Paper VI)

In previous studies it has been found that activated sludge treatment plants operated at high temperatures often have problems with poor settling properties (Sürücü and Çetin, 1989; Krishna and Loosdrecht, 1999). Sürücü and Çetin (1989) observed a rise in the SVI, as well as increased concentrations of suspended solids in the effluent, at raised temperatures (15 - 35 °C). Krishna and Loosdrecht (1999) observed that there were more EPS in the sludge at elevated temperatures and that the SVI rose, partly due to the proliferation of filamentous bacteria and Zoogloeal bacteria. These results reflect the long term effects of different operational temperatures on the settling properties. Little is known about the effects of temperature on the floc stability on a short term scale.

The temperature affects both the metabolic activity of the microorganisms and the physical properties of the sludge flocs (e.g. viscosity, physical characteristics of the EPS). According to the assumption that the aerobic microbial activity is a key parameter in the flocculation process, it can be expected that the temperature influences the floc stability significantly. In ***Paper VI***, the effects of temperature on the degree of deflocculation were investigated.

For sludge acclimatized to 11 °C, a reduction in deflocculation under aerobic conditions was observed at 20 °C, whereas the deflocculation increased when the sludge was cooled to 4°C (Figure 38). When the sludge was heated to 30 °C, the flocs deflocculated substantially more than at 20 °C under both aerobic and anaerobic conditions (Figure 39). At higher temperatures, the production rate of EPS should increase; however, it is also plausible that the speed of hydrolysis of the EPS matrix, as well as the cell decay rate, increases. It is also probable that the physical properties of EPS are changed by different temperatures.

At elevated temperatures (45 °C) there was no difference in deflocculation under aerobic and anaerobic conditions. The supernatant also contained much higher concentrations of humic substances than at ambient temperatures, indicating a “dissolution” of the flocs. There should be little or no bacterial activity at 45 °C, since the bacteria in the activated sludge were acclimatized to much lower temperatures.

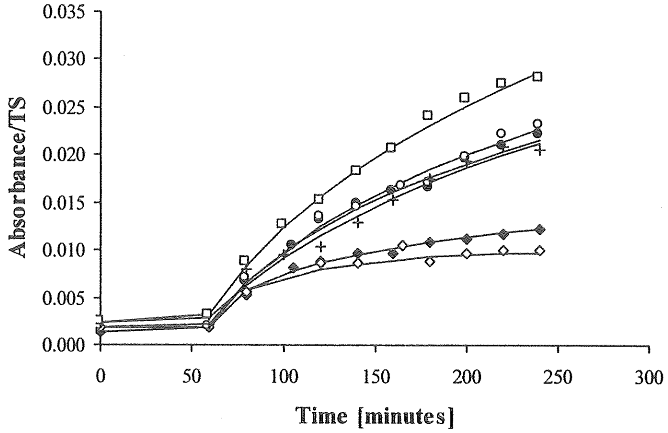

Figure 38 *Deflocculation under aerobic and anaerobic conditions at different temperatures (sludge acclimatized to 11 °): (◇) aerobic 20 °C; (○) anaerobic 20 °C; (◆) aerobic 12 °C; (●) anaerobic 12 °C; (+) aerobic 4 °C; and (□) anaerobic 4 °C.*

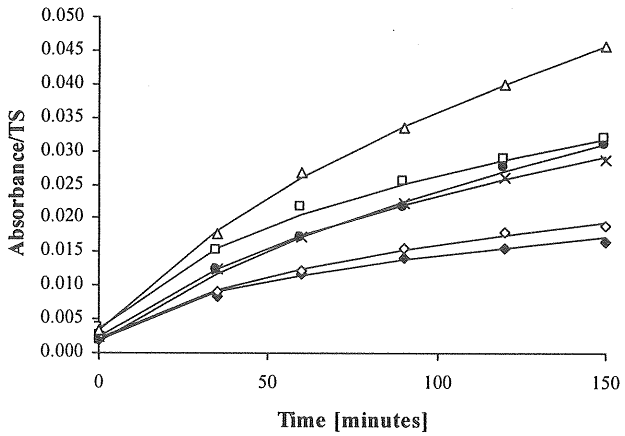

Figure 39 *Deflocculation under aerobic and anaerobic conditions at different temperatures (sludge acclimatized to 9 °C): (◇) aerobic 8 °C; (×) anaerobic 8 °C; (◆) aerobic 20 °C; (●) anaerobic 20 °C; (□) aerobic 30°C; and (Δ) anaerobic 30 °C.*

The results also show that the temperature to which the sludge is acclimated makes a difference in floc stability response to temperatures changes. This means that, when measuring floc strength, the experiment should be carried out at a temperature close to the one to which the sludge is acclimatized. The seasonal variation in floc strength observed in ***Paper III*** indicates that the floc strength is temperature dependent. Whether the floc strength is directly related to the temperature or if it is an indirect effect of a changed floc structure is not known. Other parameters, such as initial floc stability, must also be taken into account when interpreting the results.

The results in ***Paper VI*** show also that it is important that deflocculation-reflocculation experiments are carried out with fresh sludge. Apparently, a reduction in microbial activity occurs if the sludge is stored at 4 °C, which can be seen as a larger tendency to deflocculate. In other words, deflocculation experiments should be carried out directly after harvesting of the sludge, and the experients should be carried out at a temperature close to the one in the treatment plant; this will give results that better reflect the conditions in the full scale plant.

5 CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

Good solid-liquid separation properties of activated sludge flocs are a key parameter for the performance of an activated sludge treatment plant. Small flocs and free bacteria, which cannot be separated by settling, cause a high turbidity of the effluent. Poor settling and thickening properties of the activated sludge may also cause discharge of sludge from the treatment plant, due to build-up of sludge in the settlers. The aim of the thesis was to increase the understanding of what processes affect the solid-liquid separation properties of activated sludge. The research included studies of (i) the effects of dissolved oxygen (DO) concentration on the effluent turbidity and flocculation, as well as on the settling properties of activated sludge, and (ii) the influence of aerobic microbial activity on the stability of activated sludge flocs.

The results of the investigations presented show that the oxygen conditions in the aeration tank of an activated sludge treatment plant affect the solid-liquid separation properties of the sludge flocs. Two types of separation processes take place in the secondary settler: settling and clarification. These two processes are not necessarily interrelated; sludge flocs with poor settling properties can have good clarification properties and vice versa. Both the settling and the clarification properties are affected by the oxygen conditions in the aeration tank. The effects of dissolved oxygen (DO) concentration can be divided into short term effects (from minutes up to a few hours) and long term effects (from days up to weeks). Factors that are important for the development of floc structure on a long term scale include bacterial species and activity. In a short term situation, the balance between deflocculation and reflocculation of small floc entities, such as single bacteria and EPS, is significant.

When the long term effects of different DO concentrations on floc structure were examined, it was found that low DO concentrations (0.5 - 2 mg/l) gave flocs with poorer settling properties than high DO concentrations (2 - 5 mg/l). The inferior settling was due mainly to excessive growth of filamentous bacteria and the formation of porous flocs. This could be seen as higher SVIs and lower initial settling velocities. Furthermore, the turbidity in the supernatant was higher at lower DO concentrations. However, when large numbers of filaments are growing, this sometimes produces low turbidities at low DO concentrations also; this is probably due to the sweeping effect of the network of cross-linked filaments during settling. At lower sludge ages (or at higher organic loadings), the turbidity after settling rose, irrespective of DO concentration (0.5 – 2 mg/l) in the aeration tank.

There was a trend towards larger flocs at higher DO concentrations. The size distribution of larger flocs (10 - 1100 μm) could be fitted well to log-normal distributions, while the smaller flocs remaining in the supernatant after settling (1 - 100 μm) could be fitted to power functions.

From a short term perspective, oxygen limitation had a pronounced effect on the amount of small flocs in the supernatant after settling, although the size and structure of the larger flocs was not significantly affected. In a continuous completely mixed pilot scale reactor, oxygen limitation generated an instantaneous rise in effluent turbidity. This could also be seen in the bulk phase as an increased number of small flocs between the larger ones, and as a shift in the size distribution towards smaller particles. Increased effluent turbidities were observed during periods of oxygen limitation, both with and without influent to the reactor; the turbidity increased more, however, when there was influent to the reactor. This shows that the increased turbidities were due both to deflocculation (or possible desorption of adsorbed particulate and colloidal material from the flocs) and to a poor adsorption of non-dissolved material in the wastewater onto the sludge flocs.

Batch experiments showed that the adsorption of particulate and colloidal material, onto the sludge flocs, was indeed greater under aerobic than under anaerobic conditions. The results show that, during high organic loadings, it is very important to supply the activated sludge with enough oxygen, to ensure that all non-dissolved contaminants are adsorbed onto the flocs during the transport of wastewater through the aeration tank. This was also shown in full scale experiments: changed DO concentrations (for a few hours) along the aeration tank of a highly loaded activated sludge treatment plant caused significant response in effluent turbidity. In both pilot scale and full scale experiments, the increase in turbidity during periods of oxygen limitation could be reduced by aerating the sludge.

Controlled deflocculation tests showed that under conditions of reduced aerobic microbial activity the floc strength decreased. The similar increase in deflocculation under anaerobic conditions, or cooling of the sludge to 4 °C, or adding the inhibitors azide and chloramphenicol, indicates that aerobic microbial activity is essential to maintain the floc structure intact when the sludge flocs are subjected to shear forces. The floc strength could be further increased by adding substrates to the flocs under aerobic conditions. Reducing the aerobic microbial activity was not the only way to decrease the floc strength, since a stimulation of the anaerobic microbial activity could also enhance deflocculation under anaerobic conditions. This was shown by adding the Fe(III)-reducing bacterium *S. alga* BrY, in the presence of the electron donor lactate, to activated sludge.

Activated sludge flocs that have been deflocculated under anaerobic conditions can be only partly reflocculated by aeration. Although the addition of the electron acceptor nitrate yielded some reflocculation, this was less efficient than adding oxygen. The degree of reflocculation can be increased by adding easily degradable substrates to stimulate the aerobic microbial activity. When activated sludge was cooled to 4 °C or inhibitors were added, it did not reflocculate upon aeration. This shows that the aerobic microbial activity is the driving force in the reflocculation process also. The chemical oxidation of Fe(II), formed under anaerobic conditions, was found to be less important for the reflocculation than the aerobic microbial activity. This could be seen in the similar Fe(II)-oxidation rates for different reflocculation rates (e.g. by adding substrates, azide and nitrate during the reflocculation). Both deflocculation and reflocculation were shear dependent with more deflocculation and less reflocculation at higher shear levels.

There was a seasonal variation in floc strength at the Aalborg East wastewater treatment plant (WWTP), with more weak flocs present during the cold winter months. Whether this was caused by the temperature alone or by variations in floc structure also is not known. There was a temperature effect on the deflocculation, especially under aerobic conditions; at lower temperatures the flocs deflocculated more than at higher temperatures. However, at elevated temperatures (e.g. 20-30 °C) an increased deflocculation was observed.

Two activated sludges taken from different treatment plants responded similarly to the diverse conditions, which indicates the generality of the observations. Young sludge deflocculated 2 to 3 times more under anaerobic conditions than the old sludge, which points to higher biological activity in the young sludge.

The observation that the anaerobically deflocculated sludge could reflocculate by aeration only partially, has implications for the design and operation of treatment plants. This means that any period of oxygen depletion in a treatment plant, e.g. in the secondary settlers, in anoxic tanks or in connection with anaerobic storage, may irreversibly damage the flocs. Therefore, precautions should be taken to minimize the duration of anaerobic conditions. The most important factors to pay attention to are the length of anaerobic periods, sludge type (age), temperature and its fluctuations, and shear. These factors differ from plant to plant as well as over the year. Furthermore, the composition of the wastewater, i.e. the concentration and type of organic substrates and the amount of sulphides, can have significant effects on the floc stability.

This doctoral study was undertaken to contribute to the understanding of the flocculation properties of activated sludge. At a wastewater treatment plant, the DO concentration is one of the few parameters that can be readily changed. In spite of this, surprisingly little is known about its effect on the separation properties of activated sludge and on the quality of effluent. The results of this study show that, for the short term situation in particular, it is important to control the DO concentration in the aeration tank so that there are no periods of oxygen limited conditions. The studies of adsorption of colloidal and particulate material onto the flocs were indicative only, which shows that this is a key research topic for the future. The effluent quality can be further improved by increasing the understanding of what parameters affect the adsorption of pollutants from the wastewater onto the flocs, and how anaerobic and aerobic conditions affect the surface properties of the activated sludge flocs. The surface properties of the sludge flocs affect both the flocculation and adsorption properties. It is much more difficult to know the optimal DO concentration needed to obtain good separation properties in the long term, since other parameters are interrelated in a complex way. Hence, more research is required to futher optimize the activated sludge process with respect to the separation properties.

The results emphasize that aerobic microbial activity is critical to maintain the adhesion of bacteria and other floc components to the flocs. Biological aggregates cannot be regarded simply as organic aggregates obeying the general colloidal-chemical laws, since there are also physical, chemical and biological mechanisms that interact. The bacterial activity can change fast, thereby affecting, directly or indirectly, the interaction forces between the entities within the floc. This is fundamental, not only for the solid-liquid separation in wastewater, but also to our understanding of the stability of microbial aggregates. Nevertheless, the results do not explain the mechanisms involved. To do that, other methods such as molecular techniques have to be used to identify the types of bacteria present in the bulk phase between the flocs and to detect changes in the floc matrix. It is important that the studies be carried out under dynamic conditions, as activated sludge flocs are always exposed to such situations. Most previous studies have been conducted with washed cells in pure culture which, according to the present results, is not representative of the conditions in activated sludge. Another important topic for future research is the aerobic microbial activity: Does the formation of extracellular polymeric substances (EPS) determine the floc stability or are other processes involved as well?

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