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LICENTIATE THESIS

The Effect of Dissolved Oxygen Concentration on the Settling Properties of Activated Sludge

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CONTENTS

PREFACE

This thesis consists of seven chapters. In chapter one and two an introduction and the objectives and scope are given. In chapter three the more extensive literature review *Effect of different parameters on the settling properties of activated sludge* (Wilén, 1995) is summarized. Chapter four describes the experimental design. In chapter five the results are described and the paper in Appendix I is based on chapter 5.2. Chapter six and seven give the conclusions and some topics for future research.

This licentiate thesis is a part of a project in Sweden called "STAMP- Control and Operation of Wastewater Treatment Plants-New Methods and New Process Technology". The project has been supported by the Swedish National Board for Industrial and Technical Development (NUTEK) and it was initiated 1991 and finished 1996. The purpose was to increase the knowledge about wastewater treatment in Sweden. The need for this was large since the authorities called for stricter standards with respects to the concentration of effluent nitrogen by the end of the 1980s. New treatment methods and more advanced treatment plants increased the demand for highly educated people.

STAMP has included many disciplines: process technology, biotechnology, microbiology and process control. Researchers at universities and wastewater treatment plants have worked in four different consortia: Uppsala, Stockholm, Göteborg and Malmö/Lund. My project has been a part of the Göteborg consortium which has been a co-operation between Göteborg Regional Sewage Works, Department of Sanitary Engineering and Control Engineering, Chemical Reaction Engineering at Chalmers University of Technology and General and Marin Microbiology at Göteborg University.

Most of my experiments have been carried out at the Rya wastewater treatment plant in Göteborg.

I would like to thank Peter Balmér for guidance and support and for giving me an opportunity to work at the Rya wastewater treatment plant, which I have enjoyed a lot. I also would like to thank Torsten Hedberg for his support throughout this project.

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Britt-Marie Wilén

ABSTRACT

In wastewater treatment, the effectiveness of gravity separation (settling) of activated sludge from the treated mixed liquor, is mainly dependent on the ability of the activated sludge to form flocs. This settling is often the last treatment step before discharge to the recipient and it must, apart from producing a supernatant containing low enough concentrations of suspended solids to satisfy the given effluent standards, produce a settled sludge which is thickened enough to maintain a desired concentration of activated sludge in the aeration tank. These two processes which are occurring in the settling tank are called clarification and thickening and they were, in this study, quantified as turbidity and size distribution of the particles in the supernatant and sludge volume index (SVI), initial zone settling velocity and size distribution of the larger flocs in the activated sludge suspension.

Long term effects of dissolved oxygen (DO) concentration on the settling properties of activated sludge were studied in continuous, completely mixed pilot plant reactors. Low DO concentrations (0.5-2.0 mg/l) gave poorer settling properties and higher turbidities of the effluent than high DO concentrations (2.0-5.0 mg/l). The main reasons for the deteriorated settling properties were excessive growth of filamentous bacteria and porous flocs. Alternating oxic and anoxic conditions (1-4 hours) did not affect the settling properties to a large extent: longer alternating oxic and anoxic conditions (2-4 hours) produced slightly less compact flocs than constant oxic conditions did. Turbidity increased dramatically during the anoxic period and decreased during the oxic period. No clear relationship between DO concentration and average floc diameter could be found. There was a trend towards a decreased average floc diameter as the sludge age was decreased from 5 to 1.25 days; both at a high DO concentration (2 mg/l) and at a low DO concentration (0.5 mg/l). Turbidity increased as the sludge age was decreased, and there was a small difference between the reactors operated at a DO concentration of 0.5 and 2.0 mg/l. The volume distribution of flocs in the size range 11.6-1128 μm fitted well to log-normal distribution functions. Flocs in the supernatant, after 20 minutes settling, had a diameter up to 70-80 μm. More than 80% of the number of flocs were ≤ 2 μm in most of the measurements. The size distribution by number of flocs in the supernatant could best be fitted to power functions. The number of flocs in the supernatant after 20 minutes settling could be related relatively well to the turbidity.

Short term effects of DO concentration were studied in batch tests in which the adsorption of colloidal and particulate material was followed in terms of a decrease in turbidity. The adsorption capacity of colloidal material in the wastewater onto the activated sludge flocs was larger at aerobic than at anaerobic conditions. When the SVI was high, the difference in adsorption capacity between aerobic and anaerobic conditions was smaller. The difference in adsorption capacity between high (≥5 mg/l) and low (<1 mg/l) DO concentrations was small. Pre-aeration or periods of anaerobic conditions (0.5-2.5 hours) prior to mixing of activated sludge and pre-settled wastewater affected the adsorption capacity slightly.

To verify the above described phenomenon, a few full scale experiments were performed at the Rya wastewater treatment plant in Göteborg, Sweden. The aerators were adjusted to change the DO concentrations along the aeration tank (plug flow reactor) for periods of 2-3 hours. DO concentrations below 1 mg/l by the end of the aeration tank produced a turbidity increase of the effluent, while low DO concentrations in the first half of the aeration tank did not affect the turbidity to a large extent.

Key words - wastewater, activated sludge, settling, dissolved oxygen concentration, floc size distribution, settling properties, filamentous microorganisms, biosorption.

SAMMANFATTNING

Vid avloppsvattenrening beror separationsgraden av aktivt slam från det behandlade vattnet i huvudsak på förmågan hos det aktiva slammet att bilda flockar. Sedimenteringen är oftast det sista reningssteget innan utsläpp till recipient. Under sedimenteringen måste ett klart vatten med tillräckligt låga halter av suspenderat material för att klara utsläppskraven samt ett tillräckligt förtjockat slam för att upprätthålla en önskad koncentration av aktivt slam i luftningstanken produceras. Dessa två processer som äger rum i luftningstanken är klarning och förtjockning. Som mått på dessa egenskaper användes i denna studie turbiditet och storleksfördelningsmätningar av partiklarna i supernatanten samt slamvolymindex (SVI), initial sjunkhastighet och storleksfördelning av de större flockarna i det aktiva slammet.

Långtidseffekter av syrekoncentrationen på sedimenteringsegenskaperna hos aktivt slam studerades i kontinuerliga totalomblandade reaktorer i pilotskala. Låga syrekoncentrationer (0.5-2.0 mg/l) gav sämre sedimenteringsegenskaper och högre turbiditeter i utloppet än höga syrekoncentrationer (2.0-5.0 mg/l). Huvudanledningen till de försämrade sedimenteringsegenskaperna var en ökad tillväxt av filamentbakterier och bildandet av porösa flockar. Alternerande oxiska och anoxiska förhållanden (1-4 timmar) påverkade inte sedimenteringsegenskapern nämnvärt: långa alternerande oxiska och anoxiska förhållanden (2-4 timmar) producerade mindre kompakta flockar än vad konstanta oxiska förhållanden gjorde. Turbiditeten ökade dramatiskt under de anoxiska perioderna och minskade under de oxiska perioderna. Inget klart samband mellan syrehalt och genomsnittlig flockstorlek kunde uppnås. Det fanns en trend mot en minskad genomsnittlig flockdiameter när slamålder sänktes från 5 till 1.25 dagar, både vid en hög syrehalt (2 mg/l) och vid en låg syrehalt (0.5 mg/l). Turbiditeten ökade när slamåldern sänktes och skillnaden mellan reaktorerna i vilka syrehalterna var 0.5 och 2.0 mg/l, var liten.

Volymsfördelningarna av flockar i storleksintervallet 11.6-1128 µm kunde väl passas till log-normalfördelningar. Flockarna i supernatanten efter 20 minuters sedimentering hade en diameter på upp till 70-80 µm. Mer än 80% av antalet flockar var ≤ 2 µm i de flesta mätningarna. Storleksfördelningarna baserade på flockantal, kunde bäst passas till potensfunktioner. Antalet flockar i supernatanten kunde relativt väl relateras till turbiditeten.

Korttidseffekter av syrehalten studerades i burkförsök i vilka adsorptionen av kolloidalt och partikulärt material till aktivslamflockarna mättes som en minskning i turbiditet. Adsorptionskapaciteten av kolloidalt material i avloppsvattnet på aktivslamflockarna var högre vid aeroba än vid anaeroba förhållanden. När SVI var högt var skillnaden i adsorptionskapacitet mellan aeroba och anaeroba förhållanden mindre. Skillnaden i adsorptionskapacitet mellan en hög (≥ 5 mg/l) och en låg (< 1 mg/l) syrehalt var liten. Förluftning och perioder med anaeroba förhållanden innan det aktiva slammet och det försedimenterade avloppsvattnet blandades påverkade adsorptionskapaciteten.

För att verifiera de ovan beskrivna fenomenen, utfördes några fullskaleförsök vid Rya avloppsreningsverk i Göteborg, Sverige. Luftarna justerades för att ändra syrehalten längs luftningsbassängen (pluggflöde) under 2-3 timmar. När syrehalterna var lägre än 1 mg/l i slutet av luftningsbassängen ökade turbiditeten i utflödet, medan en låg syrehalt i den första hälften av luftningsbassängen inte påverkade turbiditeten nämnvärt.

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GLOSSARY OF ABBREVIATIONS

C_x	concentration of suspended solids [g/l]
COD	chemical oxygen demand [mg/l]
COD_p	chemical oxygen demand for pre-settled wastewater [mg/l]
DLVO	Derjaugin, Landau, Verwey and Overbeek theory
DO	dissolved oxygen concentration [mg/l]
ECP	extracellular polymer
F/M	Food-to-microorganisms ratio [mg COD/g biomass]
HRT	hydraulic retention time [h]
MLSS	mixed liquor suspended solids [g/l]
NTU_p	turbidity for pre-settled wastewater [NTU]
PHB	poly-3-hydroxy butyrate
SRT	solids retention time [d]
SSVI	stirred sludge volume index [ml/g]
SV	sludge volume [ml]
SVI	sludge volume index [ml/g]
V_p	volume of pre-settled wastewater [litre]
V_s	volume of return sludge [litre]
VSS	volatile suspended solids [g/l]
WWTP	wastewater treatment plant

1 BACKGROUND

In biological treatment of wastewater, dissolved and colloidal organic material are degraded by means of microorganisms which spontaneously form flocs. The major objectives are to reduce the amount of biologically degradable organic material and, in some cases, to remove nutrients like nitrogen and phosphorus. The activated sludge process was developed in England by Arden and Lockett in 1914 and the process has been subjected to many improvements throughout the years. The conventional activated sludge process consists of an aerated suspension of a mixed bacterial culture which carry out the biological conversion of the contaminants in the wastewater. The suspension is supplied with oxygen by means of compressed air, pure oxygen or by mechanical aeration. After a certain contact time between the wastewater and microorganisms, the suspension is transferred to a settler where the microorganisms are separated gravimetrically from the wastewater to produce a clear effluent. One part of the settled biomass is recycled back to the inlet of the aeration tank and one part is wasted as excess sludge. The recycling ensures a continuous sludge inoculation and extends the sludge residence time to give the microorganisms a chance to adapt to the new environment and enables the adsorbed organic material to be oxidized.

The effectiveness of gravity separation of activated sludge from the treated mixed liquor is mainly dependent on the ability of the activated sludge to form flocs. Settling is often the last treatment step before discharge to the receiving waters and it must, apart from producing a supernatant containing enough low concentrations of suspended solids to satisfy given effluent standards, produce a settled sludge which is thickened enough to maintain a desired concentration of activated sludge in the aeration tank. Organic material discharged from activated sludge plants is mainly particulate. Apart from exerting an oxygen demand, particles in the effluent may contain particulate bound phosphorus which can cause eutrophication in receiving waters. Further, heavy metals, toxic non-biodegradable organic compounds and pathogenic microorganisms are generally associated with suspended solids and can thus be discharged with the effluent. The effluent can be polished by means of filtration, flotation etc but this is not common.

In the past, much research has been focused on the design and operation of the secondary settlers to improve the separation process. In recent years, more effort has been put into understanding the flocculation process and on what parameters affect the structure and size of activated sludge flocs.

The settling process is associated with many problems such as the formation of flocs with poor settling properties and floating sludge which can cause loss of sludge from the settler into the effluent. The efficiency of the settling process is dependent on both physical (e.g. design and operation of settlers, turbulence in the aeration tanks), and chemical/biological factors (e.g. degree of bioflocculation, wastewater characteristics and composition of the microflora). The process is very complex and in spite of a considerable research within this field, the factors which affect the settling properties are still poorly understood. The reason for this is the many interdependent factors.

The introduction of nutrient removal in activated sludge plants has, in many cases, lead to deteriorated settling properties (e.g. Andreasen and Sigvardsen, 1996; Hoffman, 1987), mainly

due to excessive growth of filamentous bacteria. In these processes the activated sludge is subjected to alternating oxic and anoxic conditions which may affect the structure and size of the activated sludge flocs. Little is, however, known about this. Nitrogen removal systems require high solids retention times in the aeration tank. By keeping high concentrations of suspended solids, the aeration tank volumes can be minimized. An expansion of the settlers is not always possible due to high costs and limited space available. The settlers will be highly loaded and therefore it is important to optimize the settling process. To be able to do this, knowledge about which factors affect the settling properties has to be increased.

Basically, there are two types of settling properties: the thickening properties which often are quantified using SVI and initial zone settling velocity and the clarification properties which are often measured in terms of turbidity and floc size distribution. The clarification properties have the largest impact on the quality of the effluent. Small flocs (pin-point) and dispersed bacteria won't settle gravimetrically and will remain in the supernatant after settling. Poor thickening properties can cause flooding of the settlers and large amounts of suspended solids will escape with the effluent. The thickened sludge must be easy to handle. The particle size distribution and floc structure have a large impact on the dewatering process (Bruus *et al*, 1992).

Most research within this field has focused on the growth of filamentous bacteria, while less attention has been given to the size, size distribution and structure of non-filamentous activated sludge flocs. The size as well as size distribution of activated sludge flocs may change as a result of changes in the process conditions. Therefore, monitoring the variations in floc size may be a valuable tool in understanding what process conditions affect the size of activated sludge flocs.

Besides poor flocculation and break-up of flocs, high concentrations of particulate material in the effluent may be the result of poor adsorption of particulate material in the wastewater onto the activated sludge flocs. A special property of activated sludge is its adsorption capacity of suspended material. Some organic molecules are oxidized immediately while others are more slowly oxidized. The initial step in removing suspended material from wastewater is attachment onto the flocs. This process is dependent on the adsorption capacities of the flocs. Wastewater consists of a soluble fraction with a molecule size of < 0.1 μm, a colloidal fraction (0.1-50 μm) and a particulate fraction (> 50 μm) (Levine *et al*, 1985; Boller, 1993; Torrijos *et al*, 1994). 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 it is adsorbed onto the activated sludge flocs (Torrijos *et al*, 1994). Larger molecules are first adsorbed physically onto the floc surface and thereafter they are, by means of enzymes, broken down to smaller constituents which can pass through the cell wall for oxidation. These processes may be affected by fast changes in the environment surrounding the activated sludge flocs.

2 OBJECTIVES AND SCOPE

2.1 Objectives

The aim of this project was to study how the dissolved oxygen (DO) concentration affects the following properties of the activated sludge:

1) the settling and thickening properties;
2) the floc size, size distribution and morphology;
3) the clarification properties.

2.2 Scope

Two types of DO concentration effects were studied:

1) Short term effects: High concentrations of organic material in the effluent could be a result of poor adsorption of colloidal and dissolved organic compounds in the wastewater onto the activated sludge flocs, floc dispersion or desorption of colloidal and dissolved organic compounds in the wastewater.

2) Long term effects: Different DO concentrations may affect the floc size, size distribution and morphology of activated sludge flocs.

Short term effects of DO concentration were studied in batch tests. The batch tests were divided into two parts:

- Return activated sludge and pre-settled wastewater were mixed. The suspension was oxygenated with pure oxygen gas or with compressed air to produce different DO concentrations. The change in turbidity of the supernatant was used as a measure of adsorption of colloidal material from the wastewater. The adsorption at high and low DO concentrations was compared with the adsorption at anaerobic conditions.

- Colloidal material in the form of milk was added to three types of activated sludge mixtures: a) activated sludge taken from the end of the aeration tank at the Rya WWTP; b) return sludge taken from the Rya WWTP mixed with pre-settled wastewater; c) activated sludge taken from the pilot plant. Otherwise, the experiment was performed as the previous ones.

A few full scale experiments were made to investigate how the turbidity in the effluent will be affected during periods with low DO concentrations.

Long term effects of dissolved oxygen concentration were studied in continuous completely mixed pilot plant reactors fed with domestic wastewater. The experiment was divided into four parts:

- Part I: effect of low DO concentrations (0.5-2.0 mg/l);
- Part II: effect of alternating oxic and anoxic conditions (1-4 hours);
- Part III: effect of DO concentration at different solids retention times (1.25-5 days);
- Part IV: effect of high DO concentrations (2-5 mg/l).

The settling and thickening properties were quantified as sludge volume index (SVI), initial settling velocity and floc size/size distribution measurements and the clarification properties were quantified as turbidity and particle size distribution of the supernatant. The floc morphology was studied in a microscope.

3 SETTLING OF ACTIVATED SLUDGE - A LITERATURE REVIEW

3.1 Introduction

This literature review is a short summary of the more extensive literature review *Effect of different parameters on the settling properties of activated sludge* (Wilén, 1995) which is a part of this licentiate thesis work.

3.2 Separation of activated sludge

Normally, gravity settling is used to separate the activated sludge from the treated wastewater. The separation of activated sludge from the treated water is a critical part of the activated sludge process. The settlers have three functions: to produce a supernatant containing low enough concentrations of suspended solids to satisfy given effluent standards, to produce settled sludge which is thickened enough to maintain a desired concentration of activated sludge in the aeration tank and to act as a buffer for sludge at high hydraulic loadings. The thickening process is favoured by large, regularly shaped and compact flocs, while the clarification process is favoured by more irregularly shaped flocs which sweep smaller flocs with them during settling. The suspended solids which escape the separation in the settling tank contribute to most of the BOD in the effluent and they may contain particulate bound phosphorus. The effectiveness of the separation process is dependent on the degree of bioflocculation, the physical characteristics of the activated sludge flocs and on the design and operation of the settling tanks. It is also important that the particles in the wastewater are adsorbed properly onto the activated sludge flocs. The basic principles of the settling process are descried by Wilén, 1995.

Long-term effects like solids retention time (SRT) organic loading, reactor configuration, turbulence, DO concentration can affect the flocculation process. In a wastewater treatment plant, the conditions are continuously changing due to variations in the wastewater composition. Activated sludge flocs can be treated as colloidal particles (Busch and Stum, 1968; Loosdrecht *et al*, 1989; Zita and Hermansson, 1994) and, by changing the environment surrounding them, the floc stability can be altered quickly.

There are several types of settling problems. Some are directly related to the structure of the sludge flocs and some are related to substances coming in with the raw wastewater or which are produced during the metabolism.

pin-point flocs: The formation of small flocs (pin-point flocs) and dispersed bacteria have the largest impact on the clarification process. Poor clarification gives rise to turbid effluents and is caused by inefficient flocculation, dispersed growth of bacteria or break-up of flocs due to high shear forces (Pipes, 1979).

bulking sludge: Sludge with poor thickening abilities is normally caused by the excessive growth of filamentous bacteria. Most activated sludge flocs contain moderate or small numbers of filamentous bacteria which may contribute to stronger flocs (Sezgin *et al,* 1978) and a more clear effluent since small flocs can adhere to the network of filaments during settling. There are many types of filamentous bacteria which can thrive in different environments (Eikelboom and van Buijsen, 1981).

Zoogloea bulking: Zoogloea bacteria form finger-like colonies and excrete exocellular slime which can give the flocs a voluminous character. It can also lead to foaming and scumming (Novák *et al*, 1993, 1994).

rising sludge: As a result of biological denitrification, nitrogen gas is formed. The nitrogen gas is poorly soluble and gas bubbles can adhere to the activated sludge flocs and float them to the surface of the settler. This gives high concentrations of suspended solids in the effluent (Henze *et al*, 1993).

scumming: Another common problem is the formation of foam caused by non-degradable surfactants or by the presence of Nocardia sp., Actinomycetes or Microthrix parvicella (types of filamentous bacteria). The sludge is then transported to the surface and it can overflow the settlers (Kappeler and Gujer, 1994; Blackall, 1994).

3.3 Structure and composition of activated sludge flocs

Relatively few studies of the structure of activated sludge flocs can be found in the literature due to difficulties in finding suitable analysis techniques which do not alter the flocs structure during handling. Contradictory results are often encountered due to the inaccuracy in the various measurement techniques. Activated sludge has a very complex structure and there are various techniques available to describe their physico-chemical structure. The most common factors to study include: number of filamentous microorganisms, floc size, surface charge, amount of extracellular polymers, amount of divalent cations (e.g. Ca^{2+}, Mg^{2+}), settling velocity, floc strength, floc density and hydrophobicity.

3.3.1 The flocculation process

Activated sludge is a complex mixture of different microorganisms (mainly different types of bacteria), dead cells and particulate organic and inorganic material. For the conventional activated sludge process, the population of microorganisms present depends on the composition of the wastewater and on the operation of the WWTP. In activated sludge, the bacteria grow in three different forms: free (or dispersed) bacteria, floc-forming bacteria and

filamentous bacteria. The mechanisms behind the flocculation processes are not fully understood but there are a few probable ones. In the polymer bridging model (Busch and Stumm, 1968; Pavoni *et al*, 1972) it is suggested that the bacteria excrete exocellular polymers which join the cells together by means of divalent cations like Ca^{2+} and Mg^{2+} (electrostatic forces). The filamentous backbone model (Sezgin *et al*, 1978) suggests that filamentous bacteria create a backbone onto which exopolymer producing bacteria can attach. In a third model it has been suggested that the interactions between the floc components can be described by the DLVO theory (Derjaugin, Landau, Verwey and Overbeek theory) in which the degree of interaction depends on the surface potential and on the thickness of the electrical double layers (Zita and Hermansson, 1994; Loosdrecht *et al*, 1989), described later in this section. Probably, a combination of these mechanisms are responsible for the flocculation process. A schematic drawing of an activated sludge floc is illustrated in Figure 3.3-1.

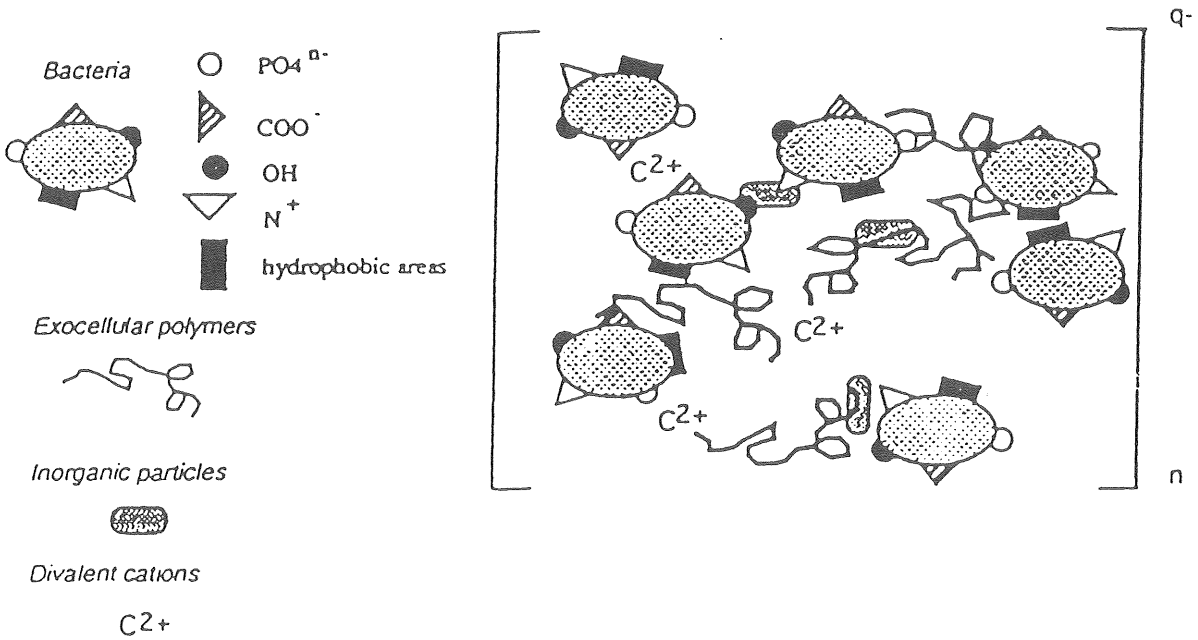


Figure 3.3-1 Schematic presentation of an activated sludge floc (Urbain *et al*, 1993).

The bacteria are the microorganisms which carry out the degradation of organic material but other microorganisms are important for an effective treatment. Microorganisms like protozoa and rotifers consume small biological flocs and dispersed bacteria and keep the effluent clear. High concentrations of protozoa usually indicate that the activated sludge is in balance.

Activated sludge flocs have a very complex structure and there are various techniques available to describe their physico-chemical structure. The most common factors to study are: number of filamentous bacteria, floc size, surface charge, amount of exocellular polymers, amount of divalent cations (e.g. Ca^{2+}, Mg^{2+}), settling velocity, floc strength, floc density and hydrophobicity (Urbain *et al*, 1993). A more detailed description of the parameters which are important for the flocculation process follows.

surface charge: Microorganisms acquire a surface charge through ionization of carboxyl and aminogroups. At the pH of wastewater, the sludge flocs acquire a net negative charge. When a particle is charged, ions of the opposite charge are attached to the surface. The potential at the surface of this cloud of counter ions is called the zeta-potential (measured as electrophoretic mobility). Several studies have been made to relate the surface charge to the settling properties of activated sludge flocs. Contradictory results have, however, been obtained. Forster, 1968, Steiner *et al*, 1976, and Goodwin *et al*, 1985, found that there was a linear relationship between electrophoretic mobility and SVI. The variation in zeta potential was explained to be due to the nature of the surface polymers. Pavoni *et al* (1972) and Chao and Keinath (1979) showed that there was no relationship between surface potential and settling properties.

Other studies show that the ionic strength of the wastewater plays an important role in the flocculation process (Loosdrecht *et al*, 1989; Zita and Hermansson, 1994). The reason to this is that bacteria are usually negatively charged and this leads to a repulsive electrostatic interaction between cells. The degree of interaction depends on the surface potential and the thickness of the electrical double layers (which are inversely proportional to the square root of the ionic strength). If the electrolyte concentration is high or if there are polyvalent counter ions present, the electrostatic interaction will be reduced and the bacteria can easier adhere to each other. These types of colloidal interactions can be described by the DLVO theory. In this theory, van der Waals attraction and electric double layer repulsion are assumed to be additive and combined to give the total energy of interaction between particles as a function of the separation distance. If the repulsion outweighs the attraction, there will be a potential energy barrier which will hinder contact between particles. When the ionic strength is increased and/or the zeta potential is reduced, the energy barrier between the particles becomes lower and the particles can adhere to each other. The interactions between colloidal particles are described in more detail by Gregory (1993).

extracellular polymers: Extracellular polymers (ECP) are compounds excreted by the bacteria and the reason for its production is not fully understood. The ECPs are important in the flocculation process of activated sludge (e.g. Pavoni *et al*, 1972; Brown and Lester, 1980; Eriksson and Härdin, 1984). ECPs consist of high molecular weight compounds (polysaccharides, proteins, nucleic acids and lipids) and it has been suggested that they affect the surface properties of the flocs. The production of exocellular polymers depends, among other things, on the organic loading and solids retention time of the activated sludge plant. Nowadays it is believed that the ECPs are coming from the metabolism and lysis of microorganisms as well as from the wastewater (Urbain *et al*, 1993).

To be able to measure the amount of ECPs, the polymeric material must be extracted from the activated sludge. The problem is to find a method that extracts the polymers you want to investigate. Various extraction methods such as heat extraction, ultracentrifugation, sonication and chemical extraction have been used (Forster, 1971; Brown and Lester, 1980; Novak and Haugan, 1981; Horan and Eccles, 1986; Sanin and Vesilind, 1994).

Several studies have been performed to relate the amount of ECPs to the settling properties of activated sludge. Many contradictory results have been obtained, probably due to the difference in extraction methods (Magara *et al*, 1976; Forster, 1985a; Goodwin *et al*, 1985; Morgan *et al*, 1990; Wahlberg *et al*, 1992; Eriksson *et al*, 1992; Urbain *et al*, 1993; Andreadakis, 1993; Frølund *et al*, 1994). Pavoni *et al* (1972) found that excretion of ECPs took place only after that the microorganisms had entered the endogenous growth phase.

Eriksson *et al* (1984) suggested that up to a certain level, ECP synthesis produces stronger flocs while a further synthesis causes a dispersion of cells.

polyvalent metal ions: That metal ions interact with activated sludge is a well known fact. This is important in removing metals from the wastewater, but it may also play an important role in the formation of activated sludge flocs. Steiner *et al* (1976) found that polyvalent ions could form bonds between exopolymers, probably by binding to carboxyl and hydroxyl groups. Various studies indicate that Ca^{2+} is the most important ion in the flocculation of activated sludge (Eriksson and Axberg, 1981; Turakhia *et al*; 1983; Kakii *et al*, 1990; Eriksson *et al*, 1991; Bruus *et al*, 1992).

hydrophobicity and hydrophilicity: A molecule that is charged or polar is hydrophilic (strongly adhering to water) and a nonpolar molecule is hydrophobic (not adhering to water). Bacteria and other microorganisms like algae, protozoa and viruses can be considered to be hydrophilic biocolloids (Tenney and Stumm, 1965). Several studies have shown that that the degree of hydrophobicity of bacteria influences the cell adhesion (Tenney and Stumm, 1965; Loosdrecht *et al*, 1987). Other studies show that hydrophobic and hydrophilic bacteria coexist in activated sludge flocs (Jorand *et al*, 1994) and that hydrophobic bondings inside the flocs are important in the flocculation process (Urbain *et al*, 1993).

floc porosity and density: It is difficult to measure the porosity of activated sludge flocs since the floc structure is very fragile. Dammel and Schroeder (1991) determined the density to be 1.02-1.06 g/ml, i.e. slightly higher than the density for water. The porosity of the flocs is believed to affect the diffusion of compounds like oxygen and other substrates within the floc.

3.3.2 Filamentous bacteria

Extensive research has been carried out during the last 20 years and even though the knowledge of the control of bulking sludge has increased considerably, many plants suffer from this phenomenon. Small numbers of filamentous bacteria give stronger flocs while excessive numbers normally lead to bulking sludge (i.e. sludge with poor settling properties). There are many types of filamentous bacteria and about 30 different types have been distinguished (Eikelboom and van Buijsen, 1981; Jenkins *et al*, 1986). The various filamentous bacteria can grow in different forms (e.g. rigid, straight and coiled). The settling properties are affected by number as well as by type of filaments.

Different studies have shown that many factors can influence the growth of filamentous bacteria such as organic loading (Chiesa and Irvine, 1985; Gabb *et al*,1991; Wanner, 1992; Jenkins, 1992), dissolved oxygen concentration (Wanner and Grau,1988; Jenkins, 1991), high sulphide concentrations (Echeverría *et al*, 1992), lack of certain nutrients like N and P, high concentrations of readily degradable compounds like saccharides, alcohols, low fatty acids, long chain fatty acids and amino acids and feed pattern (Rensink, 1966, 1974; Chudoba *et al*, 1973; Chudoba, 1985). Filamentous bacteria have advantage over floc forming bacteria at low substrate concentrations due to their higher affinity for the substrate (Chudoba *et al*, 1973). It is, however, difficult to determine which is the limiting substrate. Probably, more than one type of substrate is limiting (Wanner, 1992).

A common way to avoid the proliferation of filamentous bateria is to install a selector, in which return sludge and influent wastewater are mixed to create a zone with high organic loading, which has been found to promote the growth of floc forming bacteria (Rensink and Donker, 1991; Chudoba, 1985; Chudoba and Pujol, 1994). Most filamentous bacteria are not able to carry out anaerobic respiration (Wanner and Grau, 1988) using nitrate as the electron acceptor or to use polyphosphate as a source of energy. Therefore, it has been suggested that anaerobic and anoxic selectors could be used to prevent filamentous growth (Jenkins, 1991).

Wanner (1994) has made an extensive summary of the most important aspects of filamentous bacteria.

3.3.3 Floc size and size distribution

The variations in particle size, density and porosity (Li and Ganczarczyk, 1988; Dammel and Schroeder, 1991) have a large impact on the settling properties of activated sludge suspensions. It is also an important physical factor in the sludge dewatering process (Karr and Keinath, 1978; Bruus *et al,* 1992). The activated sludge flocs can be characterized in terms of diameter, surface area and volume. Many of the chemical and microbiological contaminants in wastewater are adsorbed on the surface of activated sludge flocs. The relative distribution of these contaminants between different size fractions of the flocs depends on the surface properties and surface chemistry of the flocs, floc shape and size distribution. The addition of chemical flocculants like Al and Fe salts (Boller, 1993) changes the surface chemistry, density and size of the flocs.

Activated sludge flocs are very heterogeneous and the size range is very broad. This makes size measurements difficult. The flocs are also fragile which makes it difficult to avoid physical alteration during sampling and measurement. There are various methods for particle size measurements available like direct microscopic observation with an eyepiece micrometer (Barbusinski and Koscielniak, 1995; Sadalgekar et al, 1988; Barber and Veenstra, 1986), photographs of individual flocs (Li and Ganczarczyk, 1987; Magara *et al*, 1976), image analysis system (Zahid and Ganczarczyk, 1990; Li and Ganczarczyk, 1991; Namér and Ganczarczyk, 1993), Coulter counter (Li and Ganczarczyk, 1991; Andreadakis, 1993) and laser beam diffraction (Jorand *et al*, 1995).

The size distribution expressed as frequency of occurrence across the whole size spectrum has been found to correlate well with power-law and Rosin-Rammler models and flocs ≥ 10 μm can best be fitted to log-normal distribution functions (Li and Ganczarczyk, 1991, 1993). More references concerning the size and size distribution of activated sludge flocs can be found in the literature report (Wilén, 1995).

3.3.4 Sludge volume index (SVI)

The most common method to evaluate how well a sludge settles and compacts is to measure the SVI. The SVI is the volume occupied by 1 g of sludge after 30 minutes settling. Various

parameters affect its value like suspended solids concentration, cylinder diameter and height, temperature and stirring (Dick and Vesilind, 1969; Eriksson and Härdin, 1984; Daigger and Roper, 1985). High SVI is normally a result of excessive growth of filamentous bacteria. A sludge is considered as bulking when the SVI >150 ml/g.

The SVI has often been criticized because of its inconsistency in performance. SVI's from different plants cannot easily be compared with each other. Its advantage is that it is easy to measure and it is a valuable tool in following changes in the settling properties.

3.4 Factors influencing the settling characteristics of activated sludge

The process operation factors as well as the composition of the wastewater coming in to the WWTP affects the settling characteristics of the activated sludge flocs in a very complex way. Due to the interdependency of many variable factors, it is very difficult to judge which parameters affect the settling properties of activated sludge.

3.4.1 Solids retention time (sludge age) and organic loading

It is well known that solids retention time (SRT) affects the settling properties to a large extent. Very short SRT's (i.e. very high organic loadings) produce dispersed growth of bacteria and very long SRT's produce pin-point flocs (Bisogni and Lawrence, 1971; Chao and Keinath, 1979; Pipes, 1979; Cashion and Keinath, 1983; Lovett *et al*, 1983; Knocke *et al*, 1986; Barbusiñski and Koscielniak, 1995). Bulking sludge has been observed at very short SRT's, i.e. at very high organic loadings, and at very long SRT's, i.e. at very low organic loadings (e.g. Chao and Keinath, 1979; Pipes, 1979; Chiesa and Irvine, 1985; Gabb *et al*, 1991).

Eriksson *et al* (1992) proposed a general model for activated sludge properties at different sludge ages or sludge loadings (Figure 3.4-1). They claimed that higher sludge ages produce strong flocs which settle fast, but their surface is too smooth to sweep smaller particles with them during settling.

Most studies into the effect of organic loading and on the physical properties of activated sludge have concentrated on the settleability and the SVI. In recent studies the effect of the organic loading on the size distribution of flocs have been investigated (Li and Ganczarczyk, 1993; Barbusiñski and Koscielniak, 1995). They found that higher organic loadings produced larger flocs.

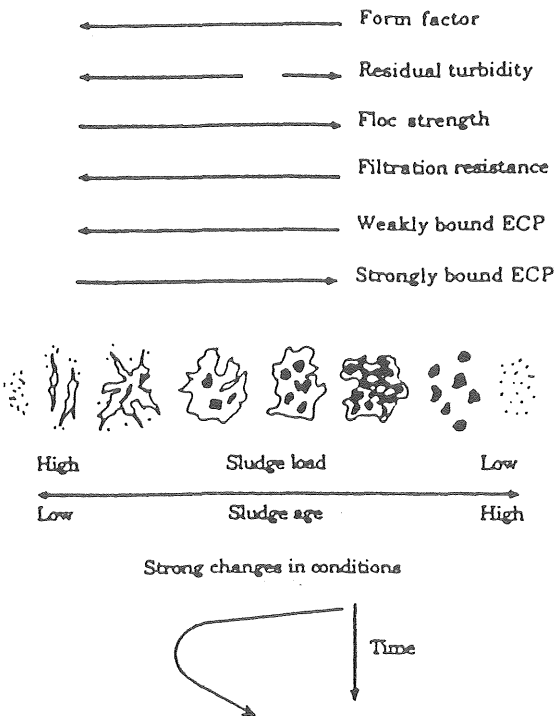


Figure 3.4-1 A general model for flocs built by floc forming activated sludge bacteria (Eriksson *et al*, 1992).

3.4.2 Turbulence

Much work has been made in the past to relate the degree of turbulence to the amount of dispersed bacteria in the effluent. Parker *et al* (1971) suggested that small scale eddies (within the same scale as the floc) caused floc break-up while larger eddies could promote flocculation. The degree of turbulence has a large impact on the number of dispersed bacteria and small flocs in the effluent. Depending on the shear forces, mixing can result in both aggregation and floc break-up. It has been suggested that a mildly stirred flocculation step between the aeration tank and the clarifier may promote flocculation (Parker *et al*, 1971; Wahlberg, 1992).

Depending on the type of aeration, the activated sludge flocs are subjected to different degrees of turbulence which can damage the floc structure (Konícek and Burdych, 1988; Galil *et al*, 1991; Das *et al*, 1993).

3.4.3 Dissolved oxygen concentration

It is a well known fact that some types of filamentous bacteria exhibit a large affinity to dissolved oxygen (e.g. *Sphaerotilus natans*, *H. hydrossis*, Type 1701). Therefore they can out compete the floc forming bacteria at low dissolved oxygen (DO) concentrations. The DO concentration necessary to avoid proliferation of filamentous bacteria depends on the organic loading of the activated sludge plant (Palm *et al*, 1980).

Less information can be found in the literature regarding the effect of DO concentration on the floc size and morphology. Knudson *et al* (1982) found that there was a trend towards larger flocs with increased DO concentrations. It has also been observed that low DO concentrations can lead to high turbidities of the effluent (Starkey and Karr, 1984). They hypothesized that two mechanisms could explain this phenomenon: inhibition of exocellular polymer production and inhibition of eucaryote population (protozoa and rotifers).

Sürücü and Çetin (1989) found that low DO concentrations reduced the filterability of activated sludge as a result of an increased turbidity of the effluent.

3.4.4 Flocculants

The settling properties of activated sludge change when chemicals are added. The type as well as dosing point is decisive for the settling properties. When Al or Fe salts are added, hydroxides are precipitated onto which colloidal particles can adsorb. Synthetic polymers (polyelectrolytes) create bridges between particles to form larger flocs. There are three types of synthetic polymers: anionic, cationic and non-ionic and they are added under high turbulence in the transport channel between aeration tank and settler.

Boller (1993) compared the influence of adding Fe^{2+} and Fe^{3+} salts at different dosing points. Simultaneous precipitation with Fe^{2+} salts gave the best results (in removing colloidal matter).

Eriksson and Alm (1993) studied the addition of cationic polymers to activated sludge dewatering purposes. The addition of cationic polyelectrolytes (which are the most common) improved the dewatering process considerably by creating larger floc aggregates and reducing the number of small particles. Extensive research has been done in this field and this is only treated in brief in this report.

Some experiments have also been made to improve the settling properties of activated sludge by adding weighting agents (inorganic compounds) (Novak *et al*, 1977; Mirzadeh, 1977; Rasmussen *et al*, 1996). This is something that has received much interest the last years.

3.4.5 Wastewater composition

Pollutants in the wastewater can directly affect the settling and flocculation characteristics by adsorbing onto the activated sludge flocs and indirectly by affecting the bacterial metabolism.

Some pollutants are not degraded in the aeration basin and pass into the sedimentation basin dissolved or adsorbed onto the flocs. Little data can, however, be found in the literature.

Eriksson and Axberg (1981) studied the influence of different wastewater pollutants on flocculation and sedimentation of a model system containing E. Coli B. Non-ionic surfactants, Ca^{2+}ions and bicarbonate increased the sedimentation rate, while sodium silicate (hydrophilic and negatively charged polymer) and colloidal particles decreased the sedimentation rate.

Oils, fats and grease promote the growth of filaments like Nocardia which can cause foaming (Blackall, 1994). High concentrations of sulphides can also promote the growth of filamentous bacteria (Echeverría *et al*, 1992).

The composition of the wastewater affects the metabolic activity of the microorganisms and also their surface structure. Wu (1978) found that activated sludge microorganisms grown in nitrogen and phosphorus restricted media possess exceptionally large capsules and produce a higher surface electric charge per unit of dry weight. Horan and Shanmugan (1986) studied the effect of starvation and nutrient depletion on the settling properties of activated sludge. Starvation caused a rapid utilization of internal storage polymer (PHB=poly-3-hydroxy butyrate) as well as a decline in sludge respiration rate. At the same time, the stirred sludge volume index (SSVI) increased. Ericsson and Eriksson (1988) and Echeverría *et al* (1993) found that extreme pre-precipitation causes deteriorated settling properties.

3.4.6 Nutrient removal plants

Many existing WWTPs are now being upgraded to be able to remove nutrients (N and P). Many plants have after the upgrading experienced deteriorated activated sludge settling properties. The most common problem encountered is bulking sludge. Andreasen and Sigvardsen (1996) made a study of about 100 Danish WWTPs and found that the SVIs generally became higher after the introduction of nutrient removal. The WWTPs with biological P removal had the best settling properties while the plants with simultaneous denitrification had the poorest settling properties. Selectors improved the settling properties in some cases.

Holmström *et al* (1996) have reported severe bulking and foaming problems at a Swedish WWTP rebuilt for N removal (pre-denitrification).

Hoffman (1987) has studied the influence of oxic and anoxic mixing zones in compartment systems on substrate removal and sludge characteristics in activated sludge plants.

3.5 Conclusions

The factors affecting the settling process can be divided into physical and biological/chemical factors which interact in a complex way. The settler and the aeration tank have to be seen as an interacting system. Even though a considerable amount of research has been conducted the

last few years, the mechanisms involved in the flocculation process are poorly understood. The probable reason for this is the many interdependent factors in a wastewater treatment plant.

In the past much effort was put into investigating bulking sludge. Today our knowledge about bulking is much greater and there are ways to combat the proliferation of filamentous bacteria. In spite of this, many WWTPs suffer from this phenomenon. The problem with bulking sludge is very complicated due to the big variety of filamentous bacteria and that many of them can thrive in different environments.

The clarification process has received much attention. It seems clear that polyvalent cations play an important role in the flocculation process of activated sludge. Information is, however, scarce about how much cation is necessary for optimal flocculation. More recent studies show that the activated sludge flocs can be treated as colloidal particles and that the flocculation process can be explained in terms of the DLVO theory in which the ionic strength of the solution plays an important role. Other binding forces like hydrophobic interaction and steric interaction and bridging of extracellular polymers by means of cations may also play an important role.

The process parameters which most affect the settling properties are sludge age, turbulence, hydraulic regime in the aeration tank and dissolved oxygen concentration. New types of WWTPs for nutrient removal have shown deteriorated settling properties. Nitrogen removal seems to lead to deteriorated settling properties while biological phosphorus removal often leads to improved settling properties. The reasons to this are not known.

4 EXPERIMENTAL DESIGN

4.1 Continuous experiment

The continuous experiments were performed in a small pilot-plant situated at the Rya wastewater treatment plant (WWTP) in Göteborg.

4.1.1 Experimental set-up

Pilot plant

The activated sludge pilot plant (Figure 4.1-1) consisted of a completely mixed aerated tank (18 l) coupled with a secondary settler (volume 20 l; surface area 0.049 m^2). Return sludge was pumped continuously from the settler to the aeration tank with a ratio of 0.8-1.0 times the influent flow. Excess sludge was withdrawn directly from the aeration tank. The DO concentration was automatically regulated by means of a valve connected to a DO-meter and a tube with pure oxygen gas. The DO-regulator was set at an on/off mode with a hysteresis of ±0.4 mg/l. Mixing was performed by means of a propeller stirrer (diameter 10 cm; 148 rpm). A selector (volume 0.6 l; HRT 6 minutes) was installed in front of each reactor in Part II-IV of the experiment. To avoid warming of the reactors, they were placed in a water bath through which tapwater was run.

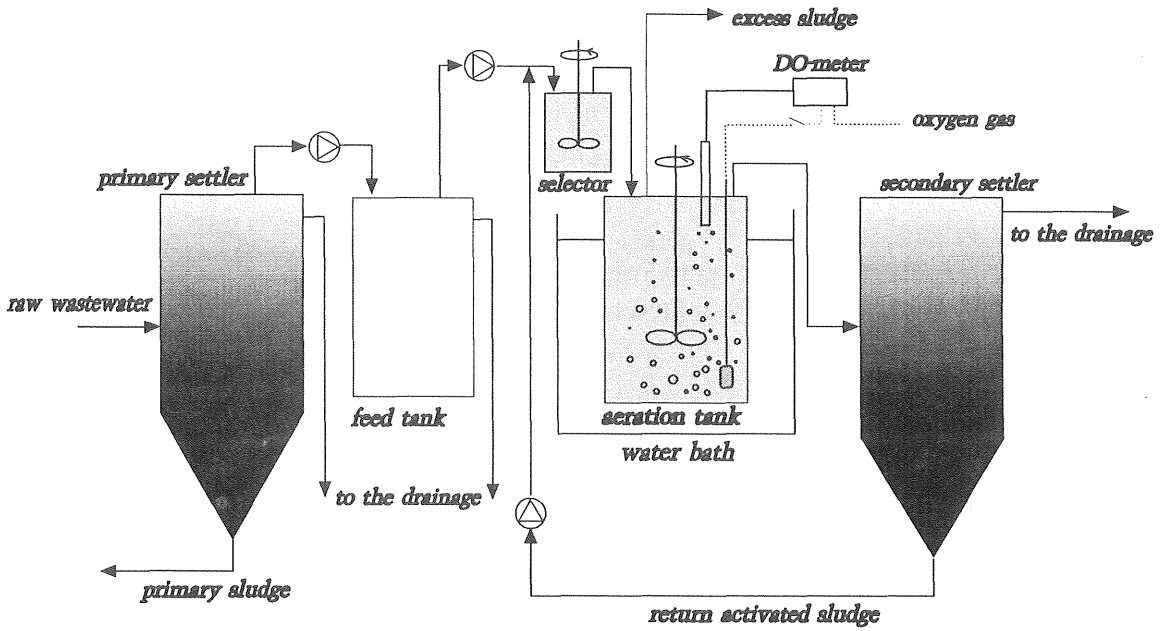
In Part I of the experiment, one pilot plant was operated, while in Part II-IV, two pilot plant set ups were operated in parallel: operating conditions were changed in reactor A while reactor B served as a reference.

Wastewater

The pilot plant was fed with domestic pre-settled wastewater from the Rya WWTP. The Rya WWTP is a conventional activated sludge plant receiving wastewater from approximately 550,000 inhabitants and 220,000 equivalents of industry. A more detailed description of the WWTP can be found in section 4.3. To simplify the pumping of wastewater to the aeration tank, the pre-settled wastewater was continuously pumped to a feed tank (with a hydraulic retention time (HRT) of ca 1.2 hours), placed in a refrigeator at a temperature of 4°C. In Part II-IV of the experiment, a pilot plant pre-settler (volume 0.1 m^3; HRT 30 minutes; overflow rate 2 m/h) was installed in front of the feed tank into which raw wastewater was pumped. This was to avoid precipitation chemicals, which were dosed to the influent at the Rya WWTP at that time, entering into the pilot plant.

Start-up

Initially, the pilot plant was inoculated with activated sludge from the Rya WWTP. The pilot plant was run for at least three sludge ages to establish steady-state conditions. Chemical analyses of the influent and effluent as well as respiration rate measurements were performed 5 times per week during the start-up period to survey the reactors performance.



$V_{primary\ settler}$ = 100 litre
$V_{feed\ tank}$ = 20 litre
$V_{selector}$ = 0.6 litre
$V_{aeration\ tank}$ = 18 litre
$V_{secondary\ settler}$ = 20 litre
$Q_{influent}$ = 2.4-3.6 l/h
$Q_{return\ sludge}$ = 0.8-1.0·$Q_{influent}$

Figure 4.1-1. Schematic drawing of the pilot plant.

4.1.2 Analytical methods

Analyses: The performance of the reactors was followed by regularly (about 5 times per week) measuring chemical oxygen demand (COD) in unfiltered influent and in filtered effluent (Munktell filter paper no. 413 005) according to Swedish Standard SIS 02 81 42; ammonium and nitrate in filtered influent and effluent with a Technicon II auto-analyzer (Zander & Ingeström: industrial method no. 857-871); alkalinity in unfiltered influent and in filtered effluent by titrating with 0.1 M HCl to pH 4.0; total phosphorus in unfiltered influent by adding Oxisolv and Phosver 3 reagents and measuring with a DR2000 Hach spectrophotometer; mixed liquor suspended solids concentration (MLSS) and volatile suspended solids (VSS) concentration according to Swedish Standard SS 02 81 12; turbidity after 20 and 60 minutes settling with a Hach turbidimeter. The analyses of the influent composition were made on 24-hour samples and the analyses of the effluent were made on grab samples. Sludge volume index (SVI) and initial settling velocity were measured in a 1 litre graduated cylinder (∅ = 60 mm) according to Standard Methods (nr 213). The sludge volume (SV), which is the volume (in ml) occupied by sludge after 30 minutes settling in a 1 litre graduated cylinder, was diluted if it exceeded 300 ml, before measuring the initial settling

velocity. Temperature, DO and pH were measured continuously (Satron Instruments: POT200 and PH200). The instruments were calibrated twice a week.

In this study, turbidity was used instead of suspended solids concentration because of its simplicity and sensitivity. Several tests were made in which turbidity and suspended solids concentration were measured simultaneously to be able to estimate a correlation factor. Good linear correlations were found with regression coefficients (r^2) of 0.90-0.99. The suspended solids concentration was in the range 1.2-2.1 times the turbidity. The results are described in Appendix III.

Respiration rate: Respiration rate was measured regularly, directly in the aeration tank by increasing the DO level to about 5 mg/l and then turning the oxygen off and following the decrease in DO level over time. The surfaces of the reactors were not covered during measurement. This meant that oxygen could diffuse through the surface between wastewater and air. This was not taken into consideration and the main aim of the measurements was to compare reactor A and B. At the same time as the measurements took place, a grab sample of the influent was taken and analysed for COD.

Microscopic investigation: The flocs were observed daily in a phase contrast microscope (Olympus BX40) equipped with an eyepiece micrometer for average floc size estimation. Only flocs with a longest dimension of about 50 μm were considered. Wet samples were directly investigated and carefully treated to avoid damaging the floc structure. The morphology of the flocs was judged by means of a standard classification scheme in which characteristics such as floc form, compactness, average size, amount of dispersed bacteria, amount of Zoogloea bacteria, amount of spiral bacteria, amount of protozoa, amount of rotifers and nematodes were classified on a scale from 1-5. Photos were regularly taken of the activated sludge flocs for documentation. Further, special characteristics of the sludge such as dominating type of protozoa etc. were recorded. In Appendix II, a more detailed description of the classification scheme can be found.

The main aim of this project was not to study filamentous bacteria, but when they occurred it was of interest to know which types were dominating. Identification of filamentous bacteria is very difficult and the risk of misjudgements is high. Conventional staining techniques were used to be able to identify the dominating types of filaments in the activated sludge (performed a few times when there were excessive amounts of filamentous bacteria present). The staining methods used were Gram, Neisser and Poly-β-hydroxibutyrat (Eikelboom and van Buijsen, 1981). The staining techniques are described in Appendix II.

Floc size measurements: The floc sizes were measured with two types of laser beam diffraction apparatuses: flocs in the supernatant after settling (1-100 μm) were measured with a Met One (WGS-260) instrument and the larger flocs in the activated sludge suspension (11.6-1128 μm) were measured with a Malvern instrument. The samples for the Met One instrument were always taken 10 cm below the water surface of the 1 litre cylinder and analysed directly after that they were harvested and the samples for the Malvern instrument were analysed within 30-60 minutes from harvesting (had to be transported to another laboratory).

The Met One instrument uses a light blocking system: as the liquid sample with particles pass a sensor, they are illuminated with an intense laser beam. The particles block light and the

amount of light blocked is proportional to the size of the particle. Larger particles block more light than smaller ones. A solid-state photo diode detects the momentary decrease in light and creates a corresponding electrical pulse that is proportional to the particle size. The pulses are then counted and categorized by size in a separate particle counting instrument and the data is given as *frequency by number*. This instrument measure dark particles better than light ones. Thus, there is a risk that the outer surface of the activated sludge flocs will not be detected. It is, however, difficult to check if this is happening. The Met One instrument could measure a maximum of 16,000 particles per ml, which made it necessary to dilute the samples with distilled water before measurement (20-100 times). This will change the ion strength of the liquor. This could have been avoided by diluting the samples with filtrated wastewater. It was, however, difficult to produce a particle free water by filtrating wastewater.

The Malvern instrument is a light scattering instrument. As the laser beam falls on a particle, the light will be spread and give rise to a diffraction pattern. This phenomenon is called *Fraunhofer diffraction*. Depending on the size of the particle, the diffraction pattern has different appearances. If different particles are illuminated simultaneously, the diffraction pattern from the different particles will be summarized and a new pattern will be formed. This diffraction pattern will be caught by a light detector consisting of a number of light sensitive rings. From the detector, a description of the diffraction pattern will be sent to the computer for treatment. The computer makes an assumption of the particle distribution. Thereafter the computer decides how its diffraction pattern should look like and compare this result with the one obtained with the detector. If the both patterns correspond with each other the assumed pattern is correct. Otherwise, the programme makes a new assumption until the best agreement is obtained. With the assumption that the particles are round, the *frequency by volume* is calculated by the computer. By changing lens, different size intervals can be measured: the smallest lens has a focal diameter of 63 mm and it can measure particles between 1.2-102 μm and the largest lens has a focal diameter of 600 mm and it can measure particles between 11.6-1128 μm. In this study, the 600 mm lens was found to be the most appropriate one.

4.2 Batch tests

The batch tests were performed in two 19 litre vessels operated in parallel or in a jar tester in which 6 to 7 one litre beakers could be operated in parallel. The experiments were performed in room temperature and they were initiated directly after that the sludge was harvested.

4.2.1 Effect of DO concentration on supernatant turbidity

Short term effects of DO concentration were studied in batch tests. Return activated sludge and pre-settled wastewater were mixed in vessels (19 l) of a ratio of about 1:3.75 (4 litre return sludge plus 15 litre pre-settled wastewater). The mixing speed was kept low to avoid high shear forces (148 rpm). The suspension was oxygenated with pure oxygen gas or with compressed air to produce different DO concentrations. Pure oxygen gas was used to be able to reach high DO concentrations without creating high turbulence due to high gas flows.

Samples of the suspension were removed during the experiment to settle in 1 litre graduated cylinders for 20-60 minutes. The experimental length was 2-3 hours which is comparable to the typical hydraulic retention time in an aeration tank. Two vessels were run in parallel to be able to compare the effect of different DO concentration at otherwise identical conditions.

The turbidity of the supernatant was measured with a Hach turbidimeter. The pH was monitored throughout the tests. COD and suspended solids concentrations in the supernatant were measured in some of the tests. The analyses are described in section 4.1.2.

It was difficult to define the turbidity and COD for t=0, since the adsorption of dissolved, colloidal and particulate material is very fast. The turbidity and the COD at t=0 was defined as:

$$turbidity_0 = \frac{NTU_p \cdot V_p}{V_s + V_p} \text{ [NTU]} \qquad (1)$$

$$COD_0 = \frac{COD_p \cdot V_p}{V_s + V_p} \text{ [mg/l]} \qquad (2)$$

where

NTU_p = turbidity for pre-settled wastewater [NTU]
COD_p = COD for pre-settled wastewater [mg/l]
V_p = volume of pre-settled wastewater [litre]
V_s = volume of return sludge [litre]

The loading of organic material onto the activated sludge (F/M) was defined as:

$$F/M = \frac{COD_0}{C_x} \text{ [mg/g]} \qquad (3)$$

where

C_x = concentration of sludge in the vessel [g/l]

4.2.2 Effect of DO on the adsorption of colloidal material

The adsorption studies were performed in 1 litre beakers with slow stirring (30 rpm; Kemira flocculators). Activated sludge was taken from: I) the end of the aeration tank of the Rya WWTP (1000 ml); II) return sludge from the Rya WWTP (300 ml) mixed with pre-settled wastewater (600 ml) and III) from the pilot plant (500-900 ml). Colloidal material in the form of milk was added in different concentrations: 500; 1000; 1500; 2000; 2500; 3000; 3500 µl/litre. The stock solution was prepared by adding 100 g milk powder diluted to 1 litre with distilled water. Three different types of milk were used: fresh containing 1.5 and 3 % fat and powder milk. The adsorption tests were performed at different DO concentrations (0; ≤1 mg/l; > 5 mg/l) to study how this would affect the adsorption capacity. Several tests were run in parallel at different concentrations of colloidal material to be able to fit the data to adsorption isotherms. Samples for turbidity measurements were taken after different aeration periods (measured in the same way as in the previous experiment) to be able to calculate the

adsorption isotherms after different periods. The turbidity was measured after 60 minutes settling.

To be able to get the turbidity for t = 0, the mixture of return sludge and pre-settled wastewater was left to settle for 60 minutes. A part of the supernatant was removed and milk was added in the same proportions as to the test beakers. After the addition of milk, the turbidity was measured.

4.2.3 Effect of pH on turbidity

Activated sludge was poured into 1 litre beakers with slow stirring (30 rpm; Kemira flocculators). The pH was adjusted to pre-selected values in the range of 6-8 by adding 3 M HCl and 3 M NaOH, respectively. The suspension was stirred for a short period (ca 15 minutes). The turbidity was measured after 20 and 60 minutes settling.

4.3 Full scale experiments

To investigate the short term effects of DO concentration on the turbidity of the effluent, a few full scale tests were performed at the Rya WWTP in Göteborg, Sweden.

4.3.1 The Rya wastewater treatment plant

The Rya WWTP is a conventional activated sludge plant receiving wastewater from approximately 550,000 inhabitants and 220,000 equivalents of industry. The sewer system transports, on the average, 3.8 m^3/s wastewater to the treatment plant. The variation in inflow can, however, vary considerably: from about 2 m^3/s at dry weather conditions up to 15 m^3/s at extreme wet weather conditions (about 25% of the sewer system is combined).

At the time of the experiments, the Rya WWTP was undergoing an expansion (introduction of nitrogen removal). Only a part of the future aeration volume was in operation and this led to a high organic loading of the plant (solids retention time (SRT) ≈2 days).

The treatment steps include: bar screens for removal of coarse material; primary settling (hydraulic retention time ≈1.5 h); simultaneous precipitation of phosphorus with iron sulphate (dosage of 10 g Fe^{2+}/m^3); aeration (aeration volume: 17000 m^3; hydraulic retention time: 2-3 hours); secondary settling (volume: 30000 m^3; hydraulic retention time: 3-4 hours). The aeration basin is supplied with oxygen by compressors (capacity: 14,000 Nm^3/h at 12.0 m water head per compressor) and a pipe distribution system with rubber membrane diffusers placed on the basin bottom (depth: 10m). The secondary settlers are built in two stories. By the time of this experiment, the half of the future number of secondary settlers were in operation: 24 (12 lines with settlers in two stories).

4.3.2 Experimental design

The aeration tank at the Rya WWTP has a plug flow. The DO concentration is measured on-line at three different points (D, G and I) along the aeration tank. A schematic presentation of the aeration tank is illustrated in Figure 4.3-1 . An example of an oxygen profile is given in Figure 4.3-2. In the first section of the aeration tank (about 1/5 of the total aeration volume), the DO concentration is very low or near zero. Thereafter the DO concentration gradually increases as the mixed liquor passes through the aeration tank. The operators of the Rya WWTP have reported in personal communication that they are convinced from their operation experience that there exists a relationship between DO concentration and effluent turbidity. Therefore they often keep the DO concentration at high levels in the aeration tank (7-9 mg/l at the end of the aeration tank). The DO concentration is measured on-line, but the compressors are adjusted manually to obtain the desired DO levels. Therefore, the DO concentration decreases during the night when the organic loading of the plant is highest.

The purpose of this experiment was to see how the turbidity in the effluent is affected when the DO concentration in the aeration tanks changes for shorter periods (2-3 hours). The DO concentration was regulated by reducing the compressors or by manually reducing the air pressure through the diffusers. This was, however, rather difficult since the aerators are connected to each other: if the air pressure in some diffusers is reduced, the pressure will increase in the other ones. Therefore, it was difficult to get exactly the desired DO concentrations in the different parts of the aeration tank. The change in DO concentration was kept for 2-3 hours after which it was returned to the initial value. The turbidity was measured on-line by means of two different turbidimeters: I) Sigrist; II) BTG MET-3010. They were not calibrated in the same way so their absolute values of turbidity differed. The two turbidimeters are constructed differently and this might affect the result they give. In this experiment, the *change* in turbidity was of interest and not the absolute values. The measurement signals were sent to the process computer at the Rya WWTP.

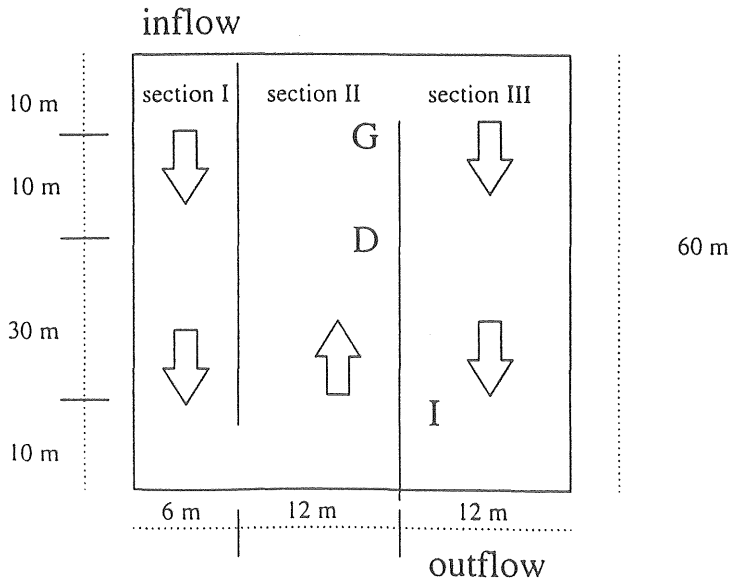


Figure 4.3-1 Schematic presentation of the aeration tank (not drawn to scale): D, G and I are on-line DO-meters; the arrows represent the flow direction of the water.

Four different tests were made in which the DO concentrations at the beginning (DO-meter D) and at the end (DO-meter I) of the aeration tank were changed according to Table 4.3-1.

Table 4.3-1 Summary of full scale tests.

Test	DO conc. at the beginning	DO conc. at the end
I	low (< 1 mg/l)	high (4 mg/l)
II	high (4-5 mg/l)	low (1-1.5 mg/l)
III	low (< 1 mg/l)	moderate (2-4 mg/l)
IV	low (< 1 mg/l)	low (< 1 mg/l)

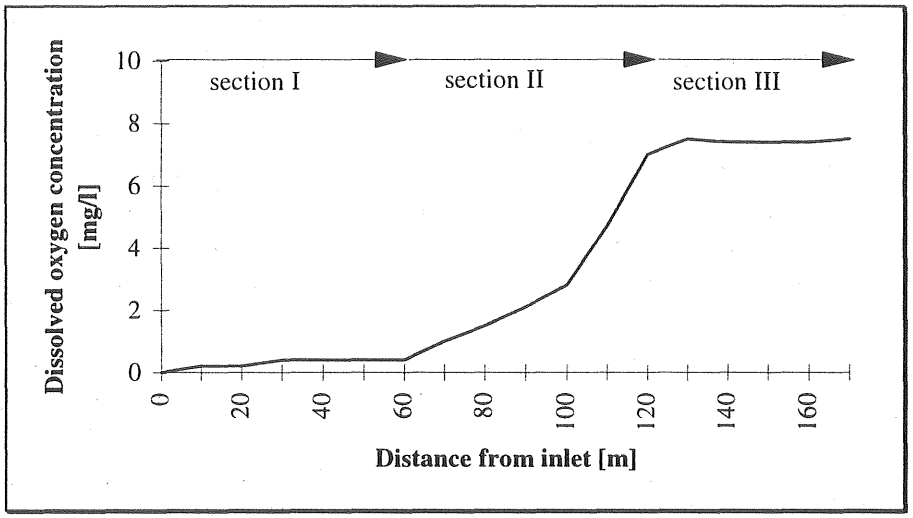


Figure 4.3-2 Example of a DO concentration profile.

5 RESULTS AND DISCUSSION

5.1 Short term effects of dissolved oxygen concentration

Short term effects of DO concentration were studied in batch tests. The experimental length was a few hours which corresponds to the HRT of an aeration tank.

5.1.1 Supernatant turbidity

A series of batch tests were made to investigate how the turbidity in the supernatant after settling changes with DO concentration. Prior to mixing with pre-settled wastewater, the return activated sludge was treated in three different ways: no treatment, pre-aeration or kept under anaerobic conditions for different periods of time to investigate how this would affect the removal of colloidal and particulate material from the wastewater.

Untreated return sludge: In the first tests, the return activated sludge was untreated before mixing with pre-settled wastewater. Two vessels were operated in parallel: one aerobic with a DO concentration of 8 mg/l (by means of oxygen gas) and one anaerobic. One litre samples were removed after 1, 2, and 3 hours and the initial settling velocity, SVI, and turbidity as well as suspended solids concentration after 60 minutes settling were measured. The turbidity decreased by about 30 % in the anaerobic vessel while it decreased by about 85 % in the aerobic vessel. The change in supernatant suspended solids concentration followed the same pattern (Figure 5.1-1). The organic loading (F/M ratio) is defined according to equation 3 in chapter 4.2. The SVI did not change with time. On the other hand, the initial settling velocity increased slightly with stirring time and the increase was larger for the aerobic (10-20%) than for the anaerobic (3-6%) vessel (Figure 5.1-2). However, when a 1 litre cylinder is used for settling tests, the wall effects are significant and relatively large deviations between measurements can be found. This could explain the difference in settling velocity between the two vessels. In most experiments, the initial settling velocity increased with stirring time and it increased sometimes more for the vessel with a high DO concentration and sometimes more for the vessel with a low DO concentration. Therefore, the increase in initial settling velocity is probably a result of mechanical flocculation of the sludge. This has been reported elsewhere (Parker *et al*, 1971; Wahlberg, 1992).

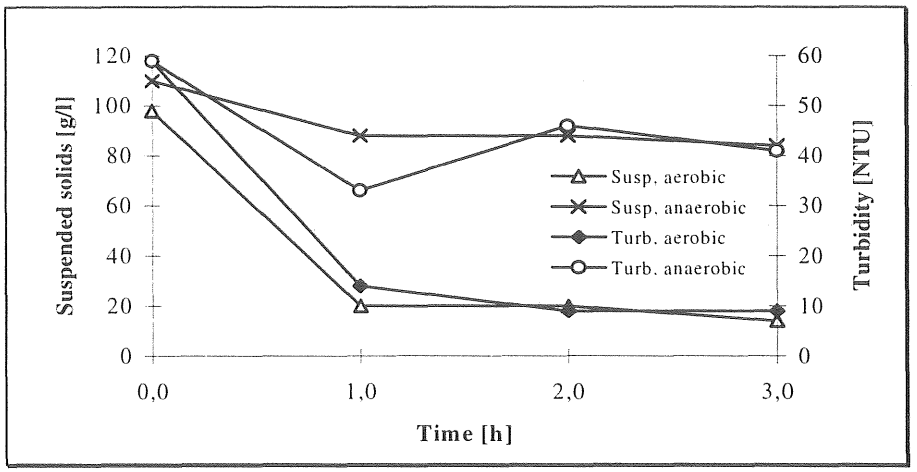


Figure 5.1-1 Turbidity and suspended solids concentration versus time (F/M=34 mg COD/g biomass; temp=15.5 °C; SVI=100 ml/g).

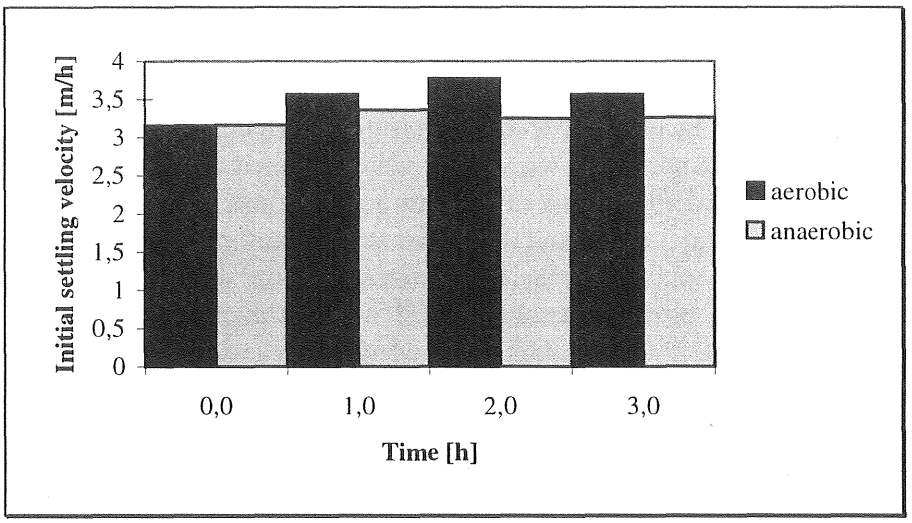


Figure 5.1-2 Change in initial settling velocity with time.

A similar test is illustrated in Figure 5.1-3, and 5.1-4. Besides turbidity, COD was measured in this test. Turbidity was measured after different settling periods: 6, 20 and 60 minutes. It was noticed that for the anaerobic vessel, the turbidity decreased by 15-20 % after 20 minutes settling, while it did not change at all after 60 minutes settling. The probable reason is a mechanical effect of the stirring which causes a flocculation of the activated sludge; i.e. smaller flocs coalesce to larger ones. This was also verified by an increased initial settling velocity of about 10-15 % in both vessels. A microscopic investigation of the sludge after about 4 hours of stirring showed that there were more dispersed bacteria present in the anaerobic vessel than in the aerobic vessel.

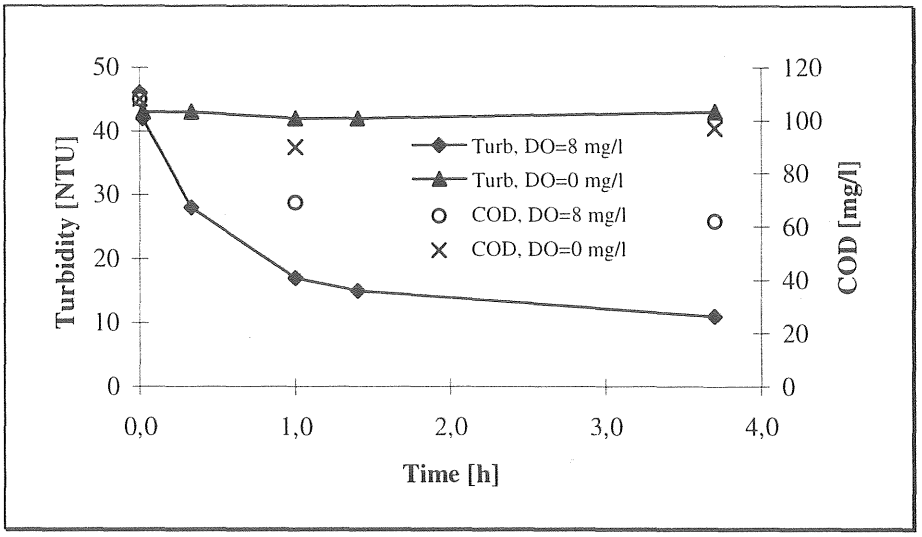


Figure 5.1-3 Change in turbidity and COD versus time (F/M=46 mg COD/g biomass; temp=10.5 °C; SVI=83).

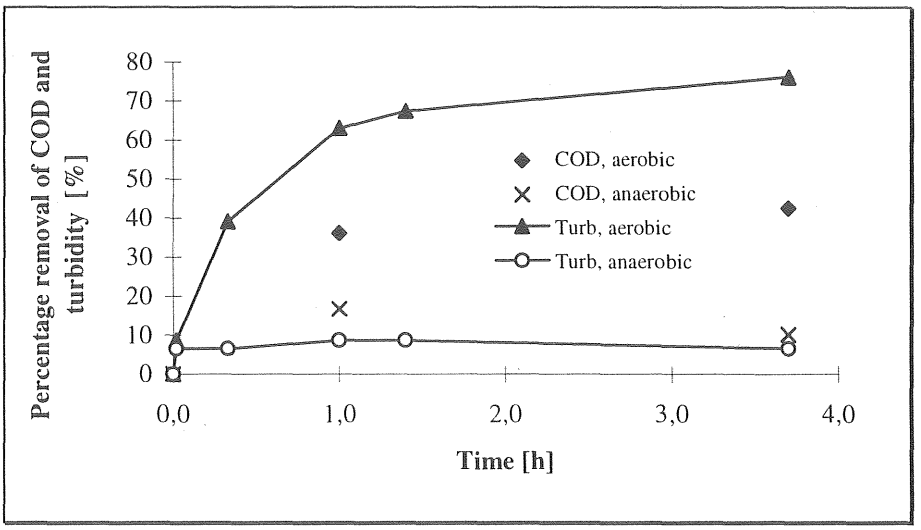


Figure 5.1-4 Percentage removal of turbidity and COD versus time (F/M=46 mg COD/g biomass; temp=10.5 °C;SVI=83).

In an other experiment, high (8 mg/l) and a low (≤1 mg/l) DO concentration were compared. The turbidity decreased faster at a DO concentration of 8 mg/l (oxygenated with pure oxygen gas) than at ≤1 mg/l (oxygenated with compressed air). After about two hours, the turbidity was almost the same in the two vessels. The pH was measured simultaneously and followed the same pattern as the turbidity. The results are illustrated in Figure 5.1-5. The gas flows were not measured but they were low in both reactors and roughly the same amount of bubbles passed through the reactors.

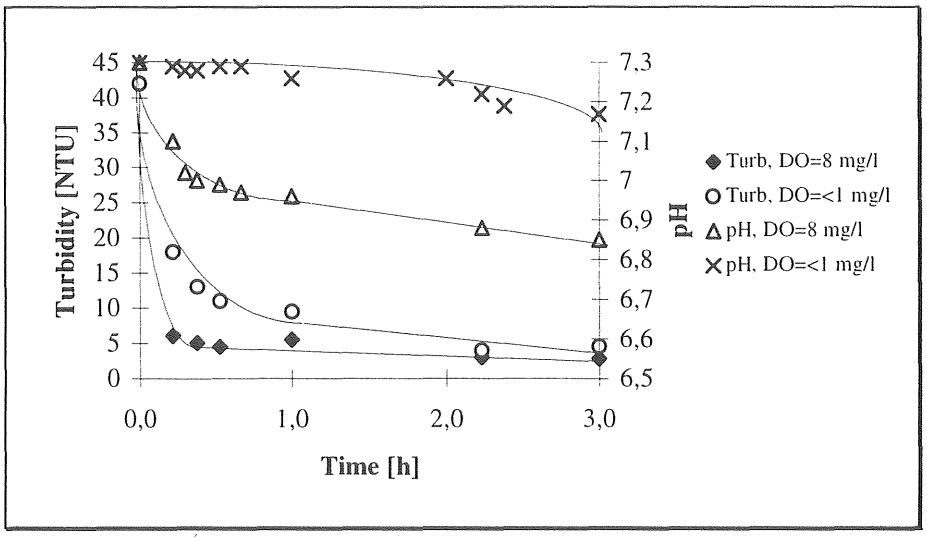


Figure 5.1-5 Change in turbidity and pH for a DO concentration of 8 and ≤1 mg/l (F/M=19 mg COD/g biomass; temp=15 °C; SVI=80 ml/g).

Pre-aerated return sludge: The same test was made as the previous one except that the return sludge was pre-aerated (without stirring) for 30 minutes prior to mixing with pre-settled wastewater. The difference in turbidity was now much smaller (Figure 5.1-6). The pre-aeration seemed to restore the adsorption capacity. This is a well know phenomenon which is used in the contact stabilization process (Metcalf and Eddy, 1995). In Figure 5.1-7 and 5.1-8, a similar test is illustrated. Besides the turbidity, the COD in the supernatant was measured.

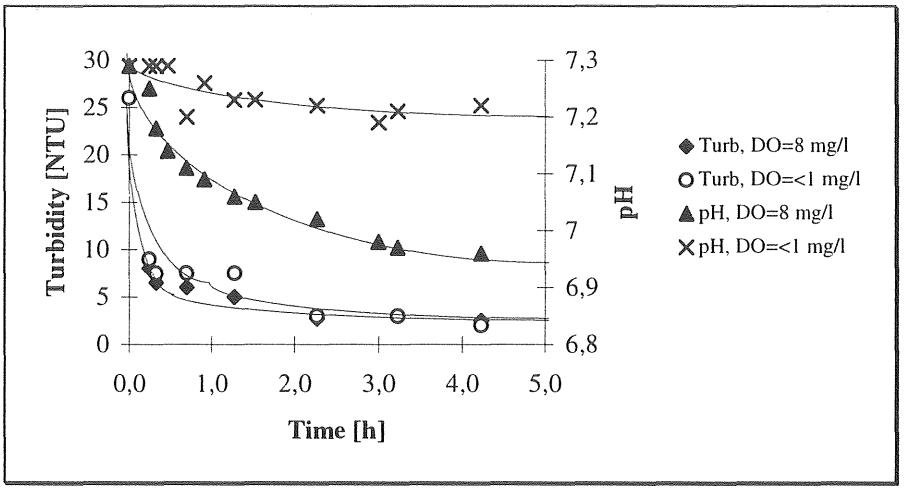


Figure 5.1-6 Change in turbidity and pH versus time (F/M=20 mg COD/g biomass; temp=15.5 °C; SVI=100 ml/g).

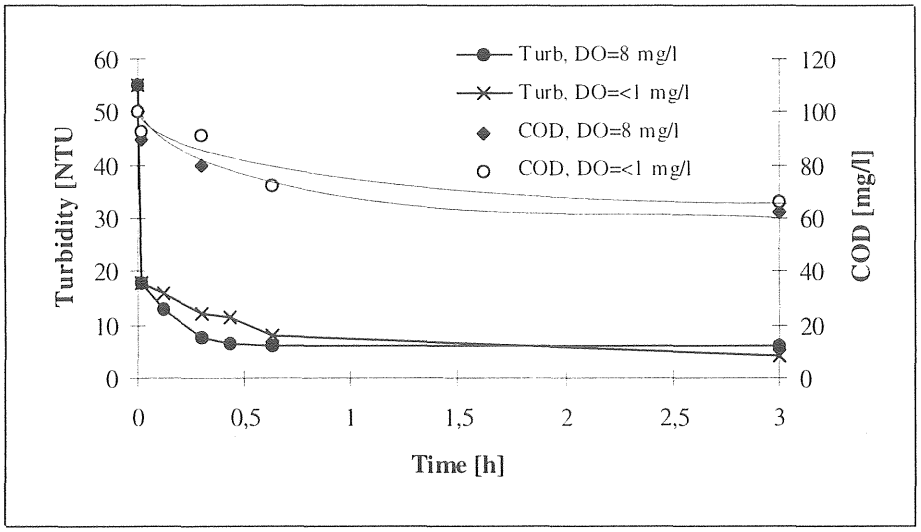


Figure 5.1-7 Change in turbidity and COD versus time (F/M=40 mg COD/g biomass; temp=13 °C; SVI=112 ml/g).

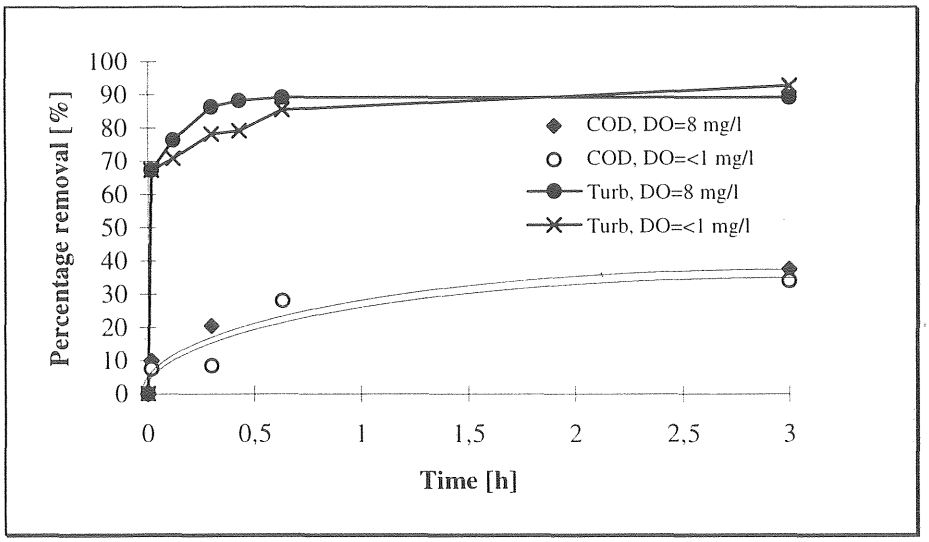


Figure 5.1-8 Percentage removal of turbidity and COD versus time (F/M=40 mg COD/g biomass; temp=13 °C; SVI=112 ml/g).

In a later test, the suspension of return activated sludge (pre-aerated for 30 minutes) and pre-settled wastewater was brought in contact for about three hours before oxygen was supplied (8 mg/l and ≤ 1 mg/l, respectively). During the anaerobic period, the turbidity decreased by 30 %. As the oxygen was supplied, the turbidity as well as the pH decreased immediately (Figure 5.1-9). The turbidity decreased faster when the DO concentration was high.

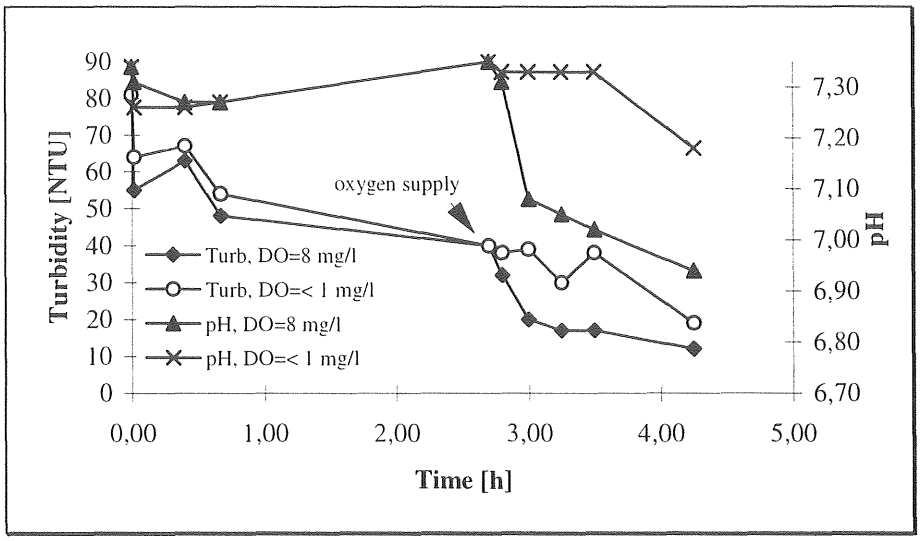


Figure 5.1-9 Change in turbidity and pH versus time (F/M=65 mg COD/g biomass; temp=11 °C; SVI=65 ml/g).

Return sludge with different pre-history: Return activated sludge was pre-aerated with compressed air or kept anaerobic for different periods of time prior to mixing with pre-settled wastewater. The suspension was aerated (DO conc. > 2 mg/l) and the turbidity was measured after 60 minutes settling. First return sludge was kept anaerobic (without stirring) for 0.5-2.5 hours. A slight decrease in adsorption capacity could be noticed when the sludge had been stored anaerobically for 2.5 hours compared to 0.5 hours (Figure 5.1-10 and 5.1-11). Pre-aeration for a period of 2.5 hours gave a better adsorption capacity than a pre-aeration of 0.5 hours. However, after about one hour of aeration there was no difference any more (Figure 5.1-12).

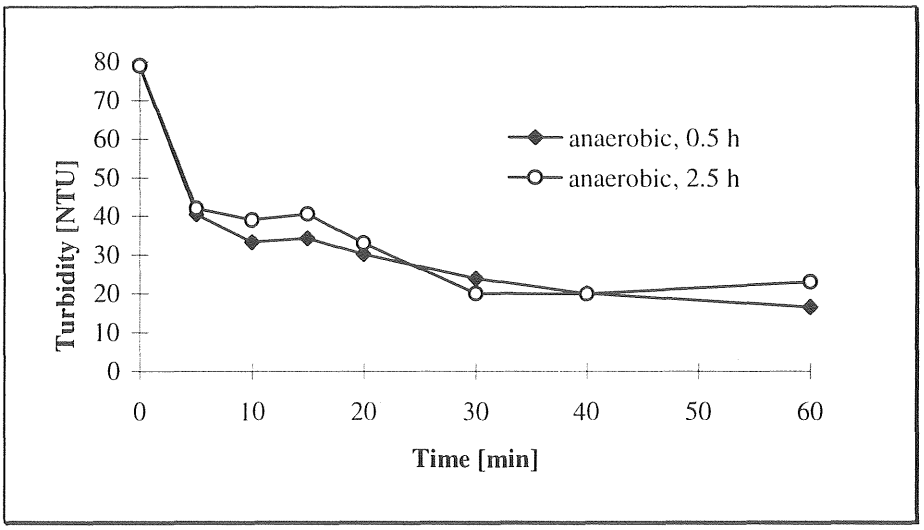


Figure 5.1-10 Change in turbidity versus time for return sludge which has been stored anaerobically for 0.5-2.5 hours (F/M=29 mg COD/g biomass)

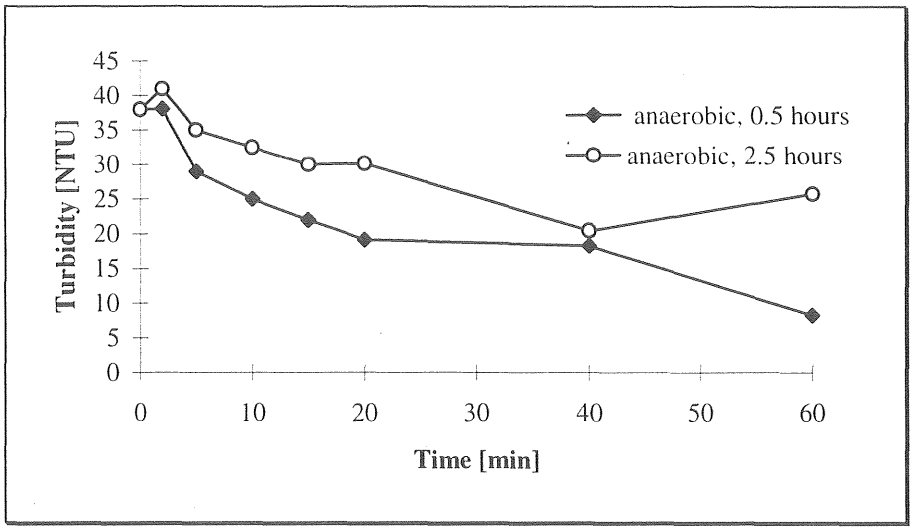


Figure 5.1-11 Change in turbidity versus time for return sludge which has been stored anaerobically for 0.5-2.5 hours (F/M=28 mg COD/g biomass)

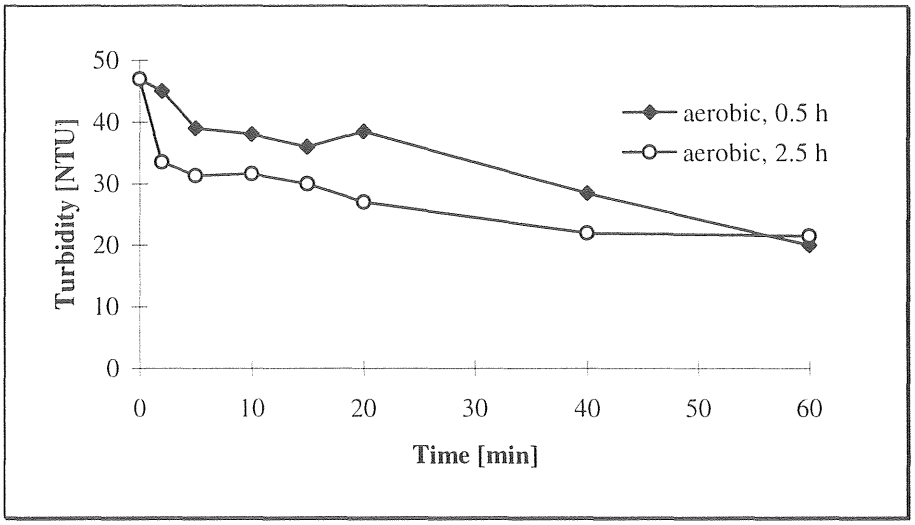


Figure 5.1-12 Change in turbidity versus time for return sludge which has been stored aerobically for 0.5-2.5 hours (F/M=92 mg COD/g biomass)

5.1.2 Adsorption of colloidal material

To further verify the results from the previous section, adsorption tests were made to compare the adsorption of colloidal material onto activated sludge at aerobic and anaerobic conditions. Milk was used as a colloidal material. Milk is a colloidal system containing fat particles within the size range 0.1-10 μm. These particles are too large to pass the cell wall and are therefore suitable for use in adsorption tests. Three types were used: fresh containing 3% fat, fresh containing 1.5% fat and powder milk containing 1% fat. The composition of the milk is given in Table 5.1-1. The composition of fresh milk changes slightly throughout the year due to seasonal change.

Table 5.1-1 Composition of milk.

Parameter	Fresh milk [%]*	Powder-milk (per 100g powder)**
water	86.3-88.0 %	-
dry solids	12.0-13.7 %	-
fat	1.5-3 %	1 g
protein	3.1-3.7 %	36 g
lactose	4.5-5.1 %	51 g
minerals	0.7-0.8 %	1.25 g

*) From: Svenska mejerimjölkens sammansättning (The composition of the Swedish milk), 1973

**) Manufacturer's (Semper) product information

The experiments were conducted at room temperature. The sludge was harvested just before the experiments were initiated and the temperature in the beakers was therefore lower than in the laboratory. The temperature has probably a large impact on the adsorption process (Riffat and Dague, 1995). This was, however, not investigated. Sludge was taken both from the Rya WWTP (from the end of the aeration tank and return sludge) and from the pilot plant. At the Rya WWTP, ferrous sulphate is added to chemically precipitate phosphorus. Simultaneous precipitation is adopted and the dosage is about 1.1 mole Fe^{2+}/mole P. The iron could effect the change in turbidity (discussed later in this section) and therefore tests were also made with activated sludge from the pilot plant which does not contain iron. Small amounts of iron are, however, found in the WWTP influent.

In this study, the amount of adsorption was measured as the amount of turbidity (NTU) removed. Each µl (10^{-6} litre) of milk added to 1 litre of water contributes to about 0.027-0.042 NTU and 0.036-0.087 NTU for milk containing 1.5 and 3 % fat, respectively. When the powder-milk was used a stock solution of 100 g powder diluted to 1 litre was prepared. Each µl of this solution added to 1 litre contributed to about 0.012-0.027 NTU. For each experiment, the turbidity for different dosages of milk was measured. The slope of the regression line could differ from one measurement to an other, probably due to varying composition of the milk (variation in composition due to seasonal change) and of the wastewater. One measurement is illustrated in Figures 5.1-13 and 5.1-14. The corresponding contribution to COD was about: 0.13; 0.17 and 0.10 mg O_2/l per µl of milk added to 1 l sample for milk containing 1.5 % fat, 3 % fat and stock solution of powder milk, respectively.

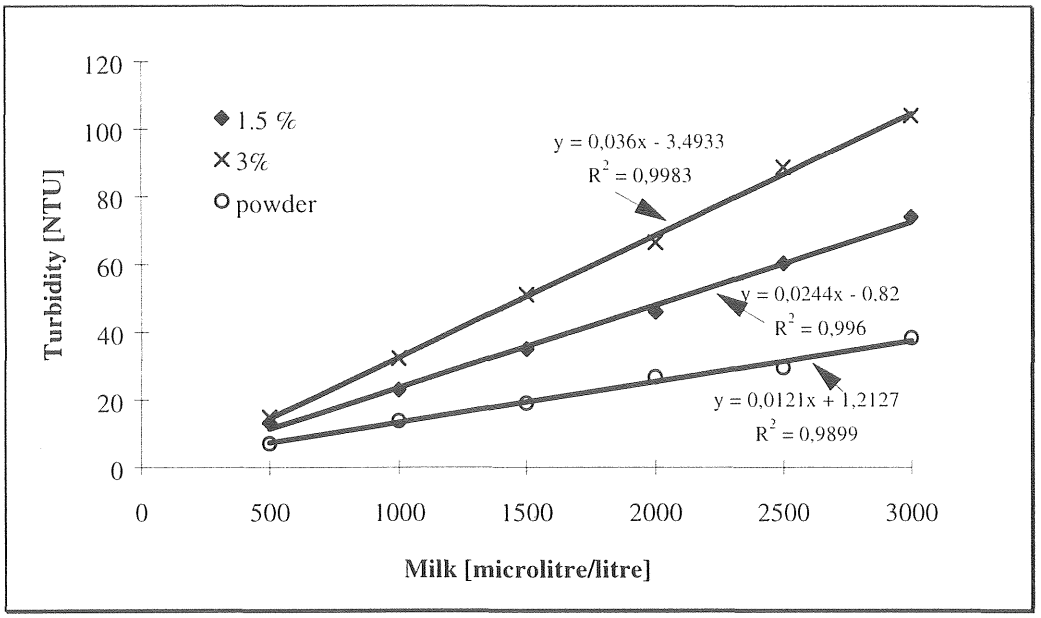


Figure 5.1-13 Relationship between added amount of milk [µl/l] to tapwater and turbidity [NTU].

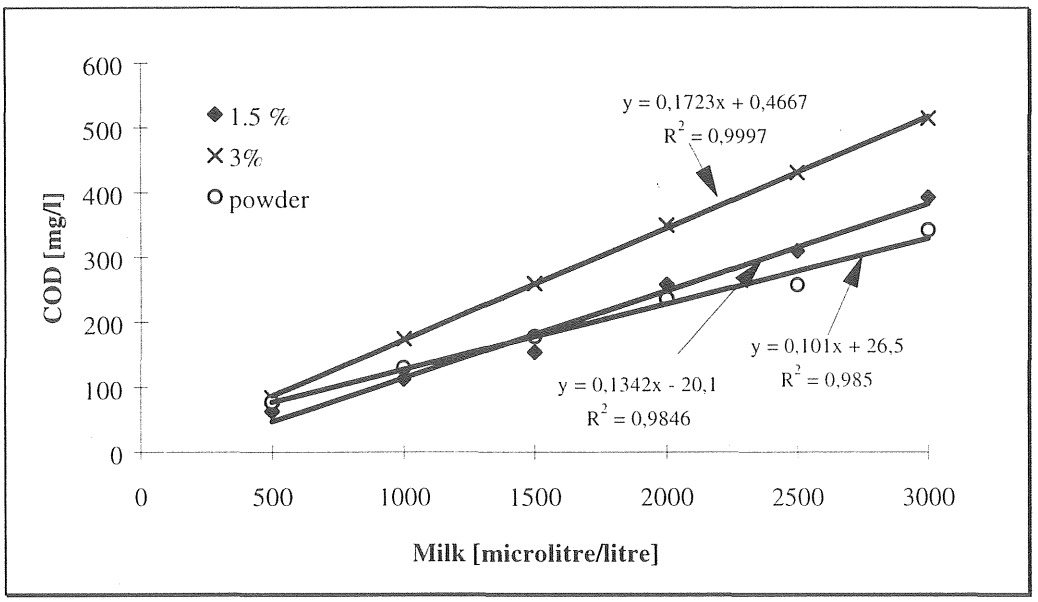


Figure 5.1-14 Relationship between added amount of milk [µl/l] to pre-settled wastewater and COD [mg O_2/l].

During the biosorption process larger molecules are believed to first adsorb physically onto the floc surface and thereafter they are, by means of enzymes, broken down to smaller constituents which can pass the cell wall for oxidation. Therefore, a real adsorption equilibrium will never be reached. However, during the first period of contact, the rate of removal of turbidity (expressed as ΔNTU/g biomass·min) is much higher. Thereafter, the removal rate is much slower: as the larger particles are degraded, new particles can adsorb to the surface. When the turbidity in the supernatant is decreasing slowly, a pseudo-equilibrium is considered to be reached. Generally this was achieved within 15-60 minutes, depending on the load of colloidal particles on the flocs and on the floc characteristics.

Activated sludge from Rya WWTP: In the first experiment, return activated sludge was pre-aerated (to restore the adsorptive capacity and to oxidize possible Fe^{2+} ions to Fe^{3+} ions) for about 30 minutes after which it was mixed with pre-settled wastewater at a ratio of 1:3 in 1 litre beakers. Milk (1.5 % fat) was added to the mixture of return activated sludge and pre-settled wastewater in different amounts (0-3500 µl). The decrease in turbidity for the different colloidal loadings (given as µl added to 1000 ml of sample) is illustrated in Figure 5.1-15 and 5.1-16. In this experiment, the equilibrium was considered as being reached after 60 minutes. Then about 40-50 % of the turbidity was removed in the anaerobic beakers, and about 80-85 % in the aerobic beakers.

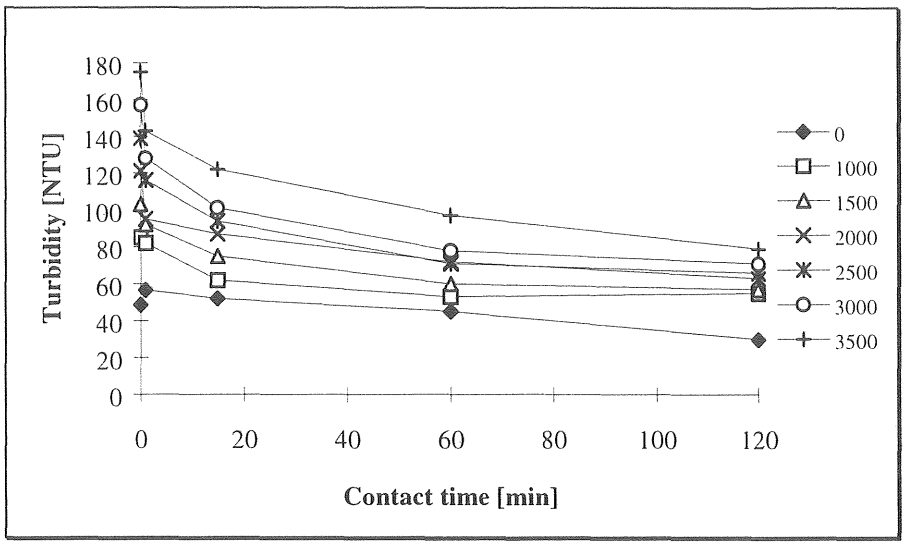


Figure 5.1-15 Decrease in turbidity at different colloidal loadings (test I: anaerobic conditions).

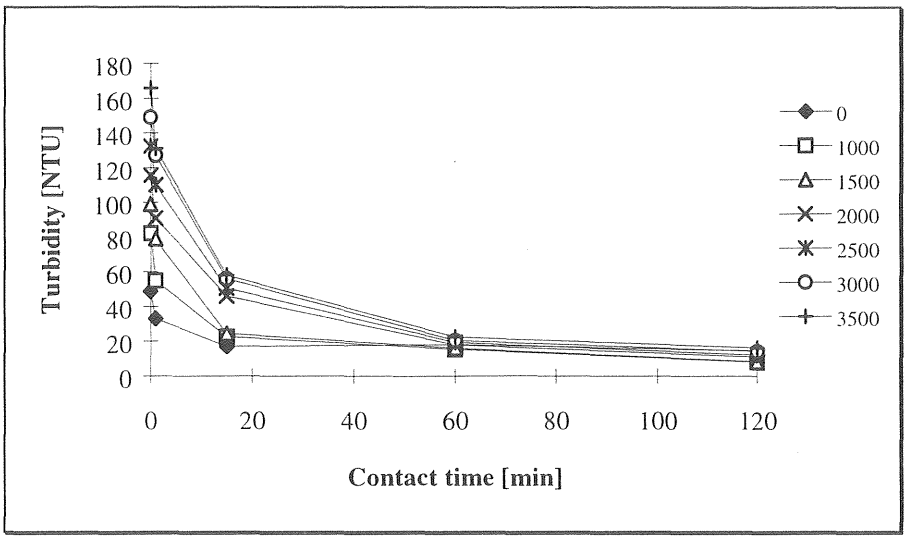


Figure 5.1-16 Decrease in turbidity at different colloidal loadings (test I: aerobic conditions).

The data obtained were fitted to Freundlich and Langmuir adsorption isotherms. The Freundlich isotherm can be written as:

$$Q_e = \frac{(C_o - C_e)}{C_x} = K \cdot C_e^{1/n} \qquad (1)$$

where

Q_e = equilibrium uptake of colloidal material onto biomass [NTU/g biomass·litre];
C_o = initial turbidity [NTU];
C_e = equilibrium turbidity [NTU];
C_x = mixed liquor suspended solids concentration [g/l];
K, 1/n = Freundlich constants.

The constant K is related to the capacity of the adsorbent for the substrate, and $1/n$ is a function of the strength of the adsorption. The Langmuir isotherm can be written as:

$$Q_e = \frac{Q_o \cdot b \cdot C_e}{1 + b \cdot C_e} \qquad (2)$$

where

b, Q_0 = Langmuir constants.

Figure 5.1-17 illustrates the Freundlich and Langmuir isotherms after 60 minutes contact time. The data could be well fitted to both Freundlich and Langmuir isotherms for 60 and 120 minutes contact time (Table 5-2). For the aerobic beakers, the fit was poor for 1 and 15 minutes ($r^2 < 0.5$) while it was better for the anaerobic beakers (Freundlich: $r^2 = 0.63$ and 0.89, respectively; Langmuir: $r^2 = 0.55$ and 0.94, respectively). The reason for this may be that it was difficult to keep exactly the same DO concentration in all beakers. The adsorption at aerobic conditions was considerably higher than at anaerobic conditions.

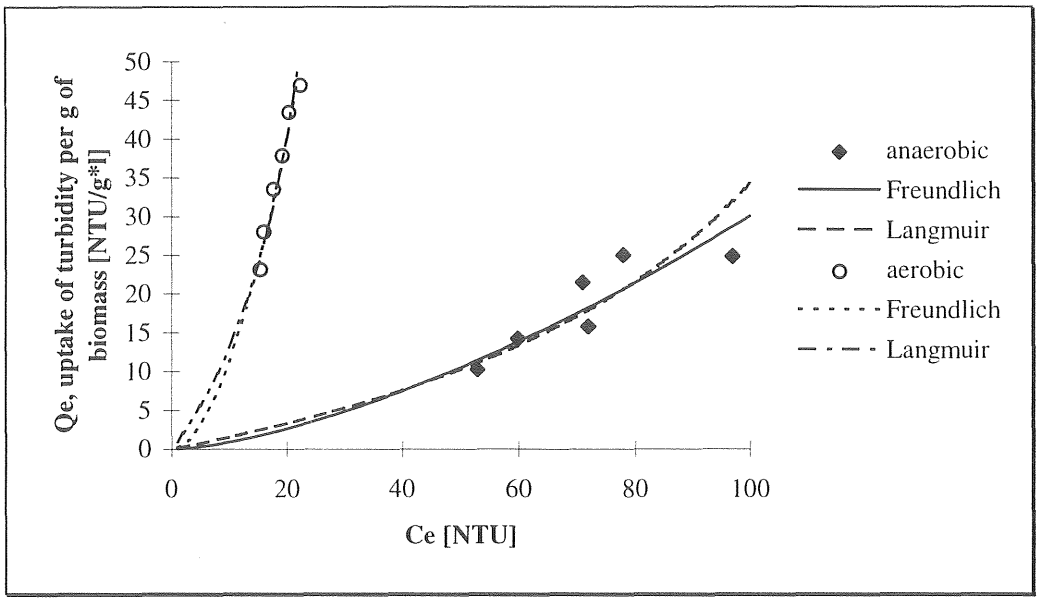


Figure 5.1-17 Adsorption data fitted to Freundlich and Langmuir isotherms after 60 minutes contact time (test I, milk with 1.5 % fat).

In similar experiment activated sludge was taken from the end of the aeration tank at the Rya WWTP. Equilibrium was considered as being reached after 15 minutes contact time. The adsorption was studied at two different DO concentrations: high (pure oxygen gas) > 5 mg/l, low (compressed air) < 0.5 mg/l, as well at anaerobic conditions. The data could be fitted well to both Freundlich and Langmuir isotherms (Figure 5.1-18, 5.1-19). The uptake was much larger at aerobic conditions than at anaerobic conditions but there was a small difference between high and low DO concentration. This indicates that it is sufficient with a low DO concentration to obtain a high degree of adsorption. However, it was difficult to keep the DO concentration at the desired level and it tended to increase as the experiment proceeded. The

adsorption onto activated sludge from the end of the aeration tank was poorer than the adsorption onto return sludge that was pre-aerated (see previous test).

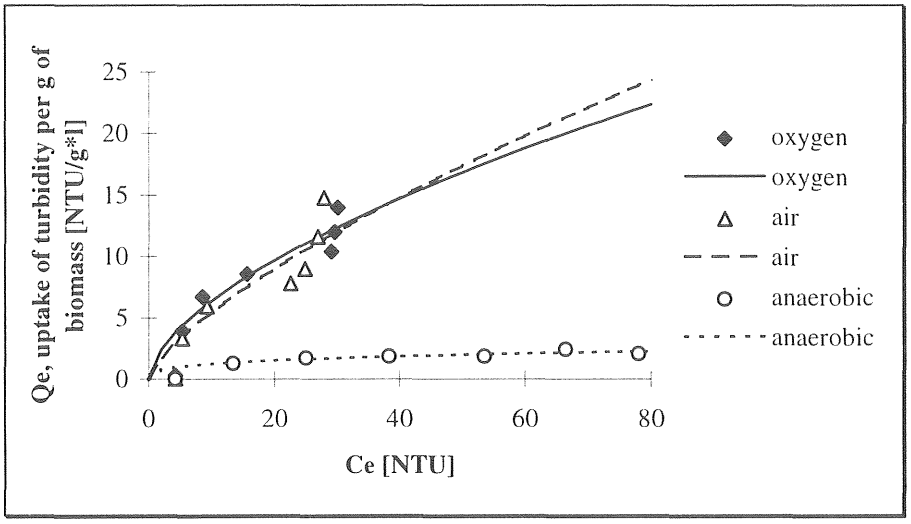


Figure 5.1-18 Data fitted to Freundlich isotherms after 15 minutes contact time (test II, powder milk).

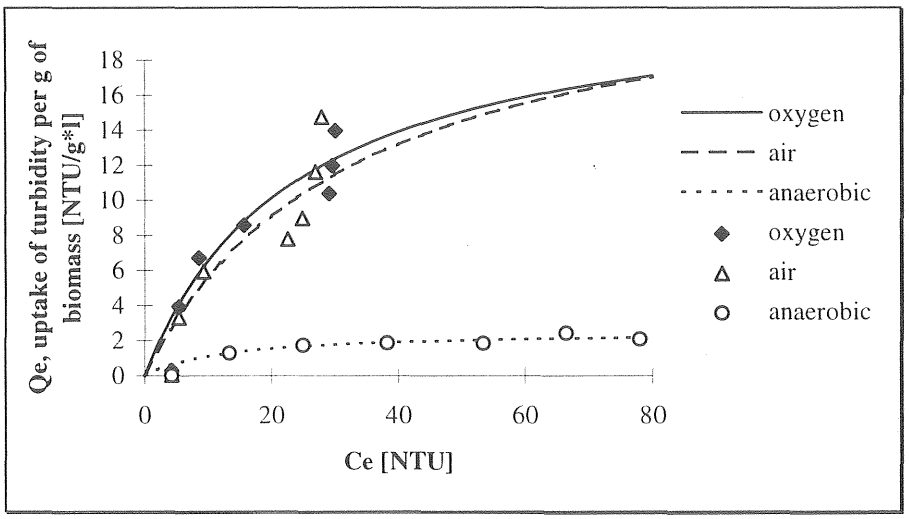


Figure 5.1-19 Data fitted to Langmuir isotherms after 15 minutes contact time (test II, powder milk).

In the next test, the activated sludge was pre-aerated before addition of milk, to assure that all ferrous (Fe^{2+}) iron was oxidized to ferric (Fe^{3+}) iron. Two different pre-aeration times were investigated: I) 1 hour; II) 3 hours. The adsorption was higher at aerobic conditions than at anaerobic conditions but there was no difference in adsorption capacity between the two pre-aeration periods (Figure 5.1-20).

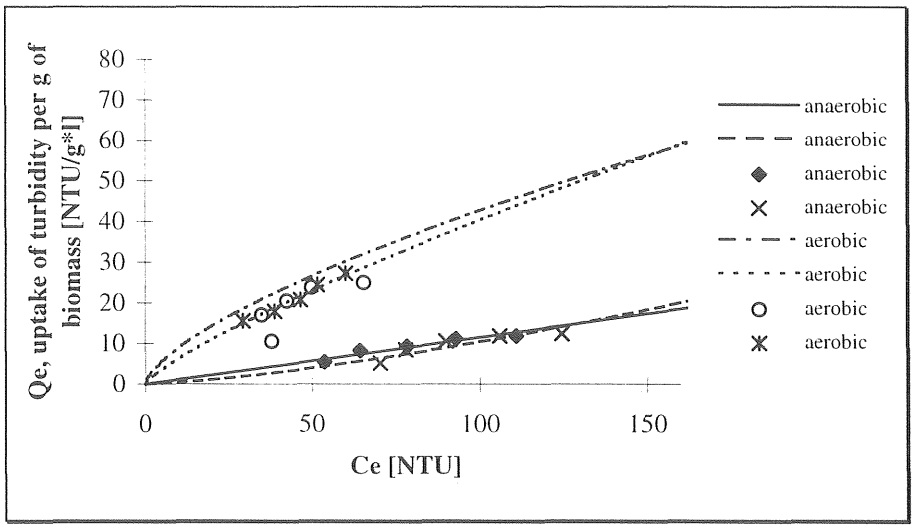


Figure 5.1-20 Data fitted to Freundlich isotherms after 15 minutes contact time (test III, milk with 1.5% fat).

The results from the different tests are summarized in Table 5.1-2 .

Table 5.1-2 Summary of the parameters in the Freundlich and Langmuir isotherms.

		Freundlich			Langmuir			Colloidal loading
Test	min	1/n	K	r^2	Q_o	b	r^2	[NTU_o/g biomass·l]
I*								
aerobic	60	1.82	0.17	0.97	-38.02	-0.03	0.95	30-60
aerobic	120	0.84	4.79	0.96	270.27	0,014	0.94	"
anaerobic	60	1.52	0.03	0.80	-25.25	-0.005	0.84	"
anaerobic	120	2.96	$8.48 \cdot 10^{-5}$	0.89	-8.24	-0.01	0.80	"
II**								
anaerobic	15	0.30	0.63	0.83	2.536	0.08	0.91	2-20
high DO	15	0.60	1.58	0.93	22.222	0.04	0.96	"
low DO	15	0.72	1.04	0.88	23.981	0.03	0.95	"
III**								
(average of 2 tests)								
aerobic	15	0.91±0.1	0.68±0.3	0.93	90.9±5.8	0.009±0.002	0.94	20-50
anaerobic	15	1.20±0.04	0.06±0.04	0.85	-44.8±30.4	-0.003±0.001	0.83	"

*) Return activated sludge plus pre-settled wastewater.
**) Activated sludge from the end of the aeration tank.

Activated sludge from the pilot plant: The activated sludge flocs in the pilot plant were larger, more irregularly shaped and contained more filamentous bacteria. The SVIs were rather high. This could explain that the difference in adsorption capacity between aerobic and anaerobic conditions was smaller compared to the results obtained with sludge from the full scale plant.

In the first test, activated sludge (sludge taken during Part I of the experiment) was mixed with pre-settled wastewater before addition of milk (3% fat). The adsorption isotherms after 15 minutes are illustrated in Figure 5.1-21. The adsorption was higher at aerobic conditions than at anaerobic conditions after 15 minutes contact time. After 60 minutes contact time, there was no difference between aerobic and anaerobic conditions. The SVI was 200 ml/g.

The same experiment was repeated when the SVI was lower in the pilot plant (sludge taken during Part II of the experiment): 100 ml/g. In this experiment, the adsorption capacity was much higher at aerobic conditions than at anaerobic conditions (Figure 5.1-22). Already after one minutes contact time, there was a difference in turbidity removal between the aerobic and the anaerobic beakers (Figure 5.1-23, 5.1-24).

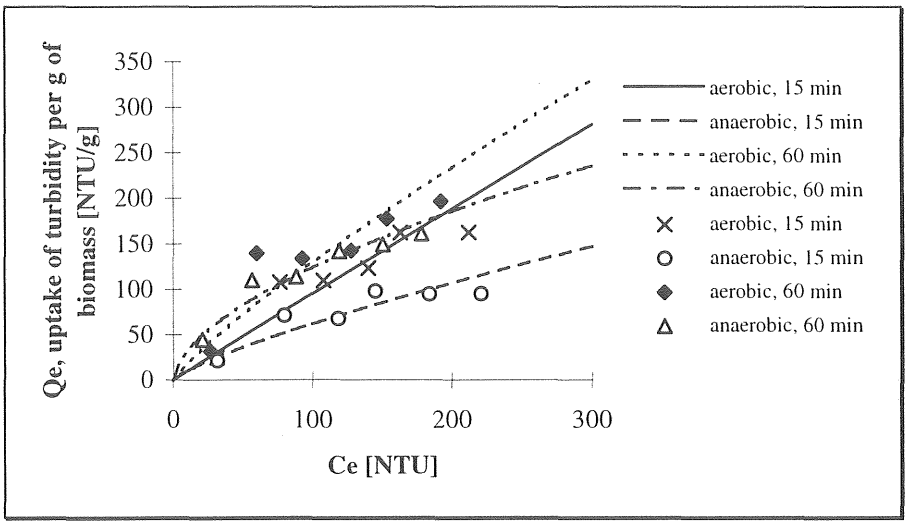


Figure 5.1-21 Data fitted to Freundlich isotherms after 15 and 60 minutes contact time (test I, milk with 3% fat).

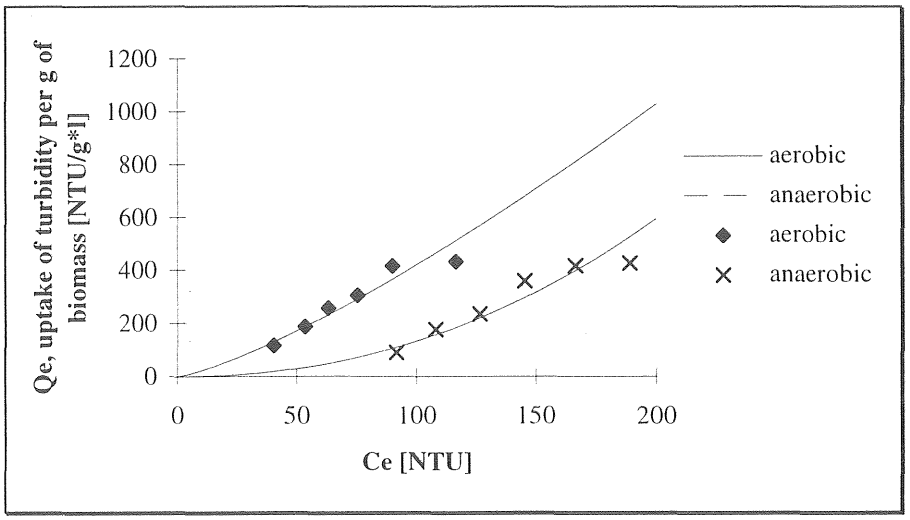


Figure 5.1-22 Data fitted to Freundlich isotherms after 20 minutes contact time (test II, milk with 3 % fat).

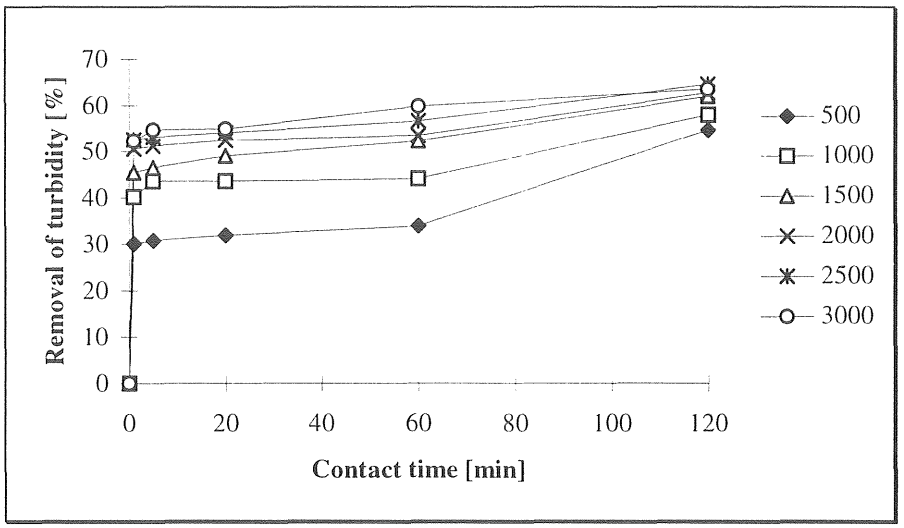


Figure 5.1-23 Percentage removal of turbidity (NTU) versus time at anaerobic conditions (test II).

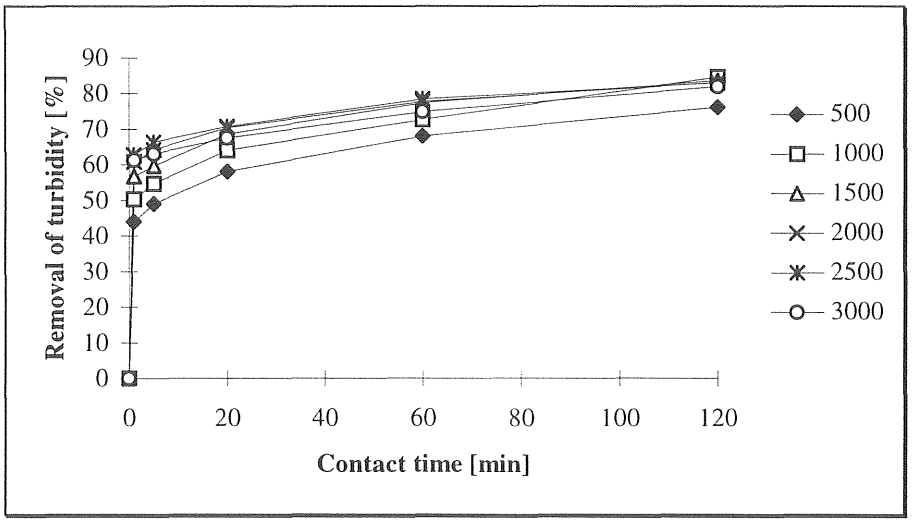


Figure 5.1-24 Percentage removal of turbidity (NTU) versus time at aerobic conditions (test II).

Two adsorption tests were made with sludge from the pilot plant taken during *Part III* of the experiment. The sludge in reactor A and B was acclimatized to a DO concentration of 0.5 and 2.0 mg/l, respectively. The purpose was to see if acclimatization of activated sludge to different DO concentrations affects the adsorption capacity. The settling properties were different in the two reactors and the sludge in reactor A contained more filamentous bacteria than the sludge in reactor B. In the first experiment (960604), the SVI was 190 [ml/g] in reactor A and 90 [ml/g] in reactor B. In the second experiment (960708) the SVIs were 370 and 170 [ml/g] for reactor A and B, respectively. There were filamentous bacteria present in both reactors but the number was larger in reactor A than in reactor B. The contact time was 20 minutes. The results are illustrated in Figures 5.1-25 and 5.1-26. The sludge from reactor A had a higher adsorption capacity than the sludge from reactor B. This may be explained by the higher SVI in reactor A. In test IV, the difference in adsorption capacity was small between reactor A and B at aerobic conditions.

The Freunlich parameters from the tests made by sludge from the pilot plant are summarized in Table 5.1-3.

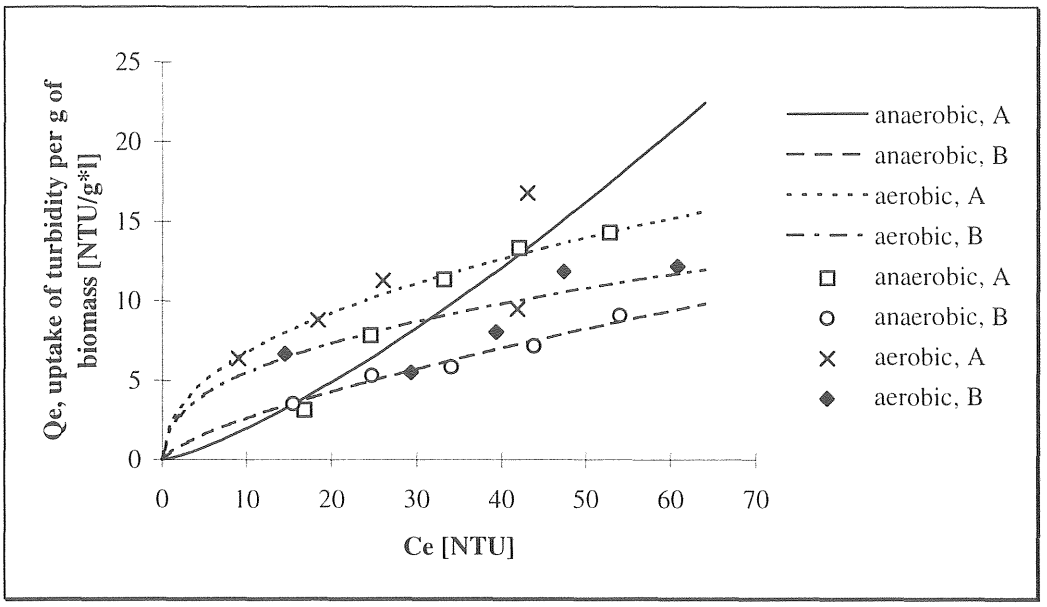


Figure 5.1-25 Data fitted to Freundlich isotherms after 20 minutes contact time (test III, milk with 1.5 % fat).

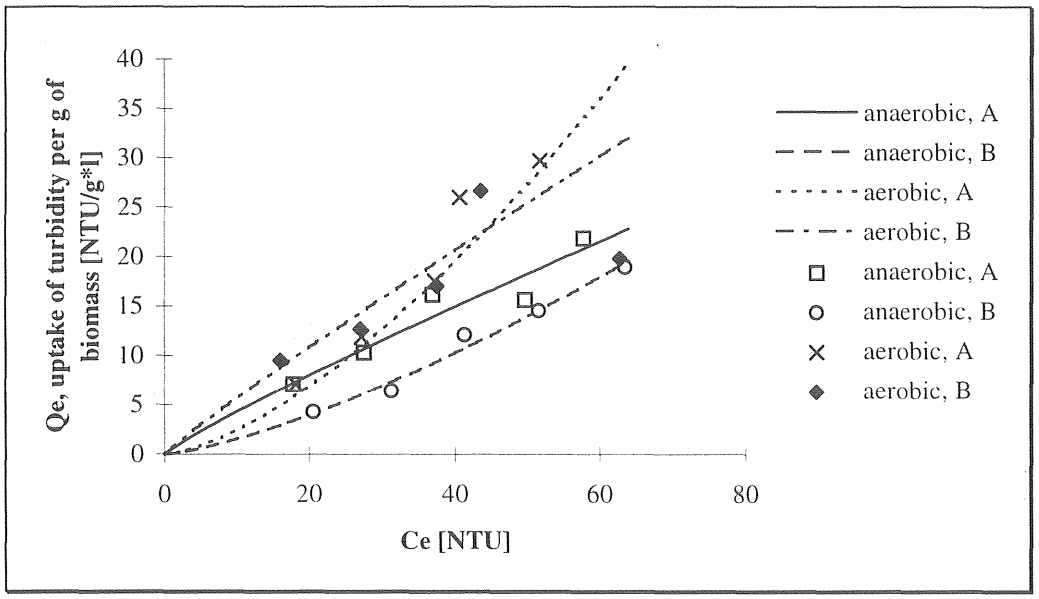


Figure 5.1-26 Data fitted to Freundlich isotherms after 20 minutes contact time(test IV, milk with 1.5 % fat).

Table 5.1-3 Summary of the parameters in the Freundlich isotherms.

	Contact time	Parameters			SVI [ml/g]	Colloidal loading
Test	[min]	1/n	K	r^2		[NTU_o/g biomass·l]
I						
aerobic	15	0.985	1.009	0.90	200	70-530
anaerobic	15	0.788	1.241	0.88	"	88-440
aerobic	60	0.850	2.594	0.83	"	70-530
anaerobic	60	0.587	8.268	0.94	"	88-440
II						
aerobic	20	1.292	1.096	0.94	100	200-640
anaerobic	20	2.167	0.006	0.93	"	280-777
aerobic	60	1.214	2.436	0.91	"	200-640
anaerobic	60	2.423	0.002	0.97	"	280-777
III						
aerobic, reactor A	20	0.450	2.403	0.676		6-50
aerobic, reactor B	20	0.422	2.079	0.80		"
anaerobic, reactor A	20	1.309	0.097	0.90		"
anaerobic, reactor B	20	0.713	0.507	0.98		"
IV						
aerobic, reactor A	20	1.4319	0.109	0.97		10-80
aerobic, reactor B	20	0.935	0.657	0.88		"
anaerobic, reactor A	20	0.902	0.535	0.937		"
anaerobic, reactor B	20	1.378	0.064	0.977		"

The above results show that the turbidity in the supernatant is a function of the DO concentration. The adsorption of particulate and colloidal particles was higher at aerobic than at anaerobic conditions. There was no large difference in adsorption capacity at high and low DO concentrations. Pre-aeration or periods of anaerobic conditions (0.5-2.5 hours) did affect the adsorption capacity, but not significantly. When the SVI was high, the difference in adsorption capacity between aerobic and anaerobic conditions was less.

What is the explanation for this? Adsorption and conversion of colloidal and particulate material is a very complex process which is poorly understood. Factors such as particle size, surface properties of the particles as well as of the activated sludge flocs are important. Recent studies have shown that activated sludge and particles in the wastewater can be described as a colloidal system (Busch and Stumm, 1968; Loosdrecht *et al*, 1989; Zita and Hermanson, 1994). Therefore, parameters like pH and ionic strength also play an important role in the adsorption processes. By changing the environment surrounding the bacteria, floc stability can change quickly. When the mixture of return activated sludge and pre-settled wastewater is oxygenated, the pH will decrease as a consequence of CO_2 production or due to nitrification. Further, the surface of activated sludge flocs has a charge depending on ionization of groups like COO^-, SO_3^-, PO_4^{2-} and NH_4^+. At the pH for wastewater, the sludge flocs acquire a net negative charge. A decrease in pH would therefore reduce the net negative charge of the floc surface and the adsorption of particulates and colloids in the wastewater would improve. This is, however, only a speculation and further studies have to be performed to get more understanding for the processes involved.

An other explanation could be the presence of iron ions in the sludge and/or in the wastewater. Ferrous iron (Fe^{2+}) is often added to WWTPs to precipitate phosphorus. The ferrous iron is

oxidized to ferric (Fe^{3+}) iron which form ferric hydroxide. This is a compound with low solubility and particulate and colloidal matter can attach to the chemical flocs. Ferrous iron is easily oxidized and by the end of the aeration tank most of it should be ferric hydroxide. Rasmussen *et al* (1994) and Rasmussen and Nielsen (1996) have found that Fe^{2+} can be found in anaerobic storage tanks prior to dewatering. Fe^{3+} can be reduced by microorganisms which use Fe^{3+} or H_2S as an electron acceptor. In their study it was found that 70-90 % of the iron was present as Fe^{3+} in fresh activated sludge and almost all Fe^{2+} and Fe^{3+} was associated with the floc matrix. Reduction of Fe^{3+} could occur at anaerobic conditions like in the secondary settler and in the return sludge pipe. The knowledge about the reaction rates for these processes is still limited.

If there are Fe^{2+} ions present in the sludge, they would be oxidized at aerobic conditions and ferric hydroxides would be formed onto which particulate and colloidal material could attach and thereby decreasing the turbidity.

Further, divalent metal ions are assumed to be very important for the flocculation process by binding bridges of exopolymers together (e.g. Eriksson and Axberg, 1981; Kakii *et al*, 1990; Eriksson and Alm, 1991; Bruus *et al*, 1992). Fe^{3+} ions may play a significant role and Fe^{2+} is probably less efficient in the bridging process and can therefore reduce the floc strength (Rasmussen and Nielsen, 1996). This can cause deflocculation and has also been observed in anaerobic storage tanks (Rasmussen *at al*, 1994).

5.2 Long term effects of dissolved oxygen concentration

The pilot plant was fed with domestic wastewater with a fluctuating composition. To be able to distinguish the effect of different DO concentrations, two reactors were operated in parallel: one reactor was operated as a reference and changes were made in the other reactor.

5.2.1 Process conditions

The objective of this study was to investigate how different DO concentrations as well as alternating oxic and anoxic conditions influence the settling and thickening properties of activated sludge. This was studied in pilot plant continuous reactors fed with domestic wastewater. The pilot plant studies were run for a period of about one year. The experiment was divided into four parts:

- Part I: the effect of low DO concentration;
- Part II: the effect of alternating aerobic and anaerobic conditions;
- Part III: the effect of DO concentration at different solids retention times;
- Part IV: the effect of high DO concentrations.

In Table 5.2-1, the durations of the different experiments are summarized. The process operation parameters and the process conditions are summarized in Table 5.2-2 and 5.2-3.

Table 5.2-1 The duration of the different experiments.

Experiment		DO [mg/l]	Days
Part I	*start-up*	2	30
	period I	2	36
	period II	1	22
	period III	0.5	20
Part II	*start-up*	2-4	45
	period I	4 (alternate aerobic/anaerobic period of 1 h)*	15
	period II	4 (alternate aerobic/anaerobic period of 2 h)*	40
	Period III	4 (alternate aerobic/anaerobic period of 4 h)*	35
Part III	*start-up*	-	**
	period I	0.5-2 (SRT = 5 d)***	17
	period II	0.5-2 (SRT = 2.5 d)***	28
	period III	0.5-2 (SRT = 1.25 d)***	3
Part IV	*start-up*	2-5	21
	period I	2-5 (SRT = 5 d)****	33

*) Reactor B was operated at a constant DO concentration of 4 mg/l.
**) Already acclimatized sludge from part II was used.
***) Reactor A was operated at 0.5 mg O_2/l and reactor B at 2.0 mg O_2/l.
****) Reactor A was operated at 2 mg O_2/ and reactor B at 5 mg O_2/.

Table 5.2-2 Process operation parameters.

Experiment	DO [mg/l]	SRT [day]	HRT (aeration tank) [hour]	HRT (settler) [hour]	F/M [gCOD/ g MLSS·d]	C/N [mg COD/ mg NH_4^+]	C/P [mg COD/ mg P_{tot}]
Part I	0.5-2	5	7	4	0.57± 0.2	12± 4	not measured
Part II: reactor A	0-4 (alternating periods: 1 h, 2 h, 4h)	5	7.5	4	0.99± 0.4	12± 2.5	74± 15
Part II: reactor B	4	5	7.5	4	0.97± 0.3	12± 2.5	74± 15
Part III: reactor A	0.5	1.25-5	5	3	0.88± 0.1- 1.92± 0.05	14± 2.5	67± 10
Part III: reactor B	2	1.25-5	5	3	1.0± 0.2- 1.7± 0.2	14± 2.5	67± 10
Part IV: reactor A	2	5	5	3	0.99± 0.3	13± 3	60± 10
Part IV: reactor B	5	5	5	3	0.87± 0.3	13± 3	60± 10

Table 5.2-3 Summary of the process conditions.

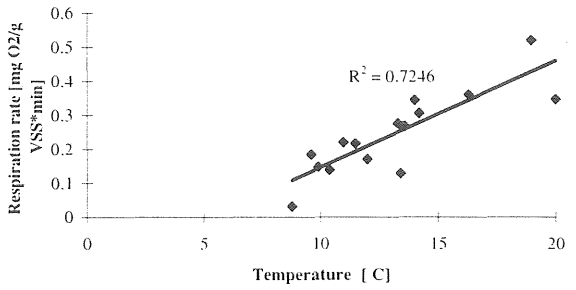
Exp.	pH [-]	Temp. [°C]	COD (infl) [mg/l]	COD (effl) [mg/l]	COD-red [%]	MLSS [g/l]	VSS/ MLSS [-]	HCO_3^- (infl) [mg/l]	P-tot (infl) [mg/l]	NH_4^+ (infl) [mg/l]
Part I	6.9±0.2	15±3	208±53	60±10	70±8	1.6±0.4	0.66± 0.07	-	-	18±5
Part II										
reactor A	7.0± 0.1	15.5±1.5	286±76	68±18	75±7	1.0±0.4	0.75± 0.04	241±40	3.4±0.7	23±5
reactor B	6.7± 0.2	15.5±1.5	286±76	64±18	77±6	1.0±0.3	0.75± 0.04	241±40	3.4±0.7	23±5
Part III										
reactor A	6.9±0.1	18±1.3	292±65	57±14	80±5	1.3±0.3	0.72± 0.018	260±30	4.4±0.9	21±3
reactor B	6.7±0.2	18±1.3	292±65	56±14	80±5	1.3±0.3	0.73± 0.019	260±30	4.4±0.9	21±3
Part IV										
reactor A	6.9±0.2	18±1	296±85	69±21	75±9	1.5±0.3	0.74± 0.04	260±53	5.1±1.2	23±6
reactor B	6.9±0.2	18±1	296±85	65±18	76±9	1.7±0.3	0.73± 0.06	260±53	5.1±1.2	23±6

Although the objective was not to achieve nitrification (short sludge age, 5 days), some ammonium was converted to nitrate, especially in the late spring and in the summer when the temperatures were higher. In Table 5.2-4, the degree of nitrification (calculated as NO_3^- (effluent)/ NH_4^+(influent)*100) is summarized. The concentration of nitrate in the influent was almost zero. In Part I, the degree of nitrification increased gradually during period I to 60-70% within a month. In Part IV the degree of nitrification increased gradually over a period of one moth to about 50% in reactor A and to about 80% in reactor B. A N-balance was made over the systems to evaluate if any denitrification was taking place, and it appeared to be insignificant.

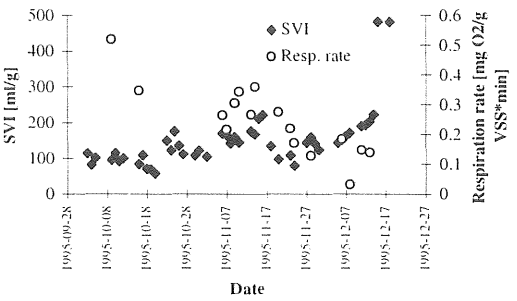
Table 5.2-4 Degree of nitrification during the different parts of the experiment.

Experiment	NH_4^+ (influent) [mg/l]		NO_3^- (effluent) [mg/l]		Nitrification [%]	
	reactor A	reactor B	reactor A	reactor B	reactor A	reactor B
Part I						
period I	18±6	18±6	2±3	2±3	14± 21	
period II	16±6	16±6	1±0.5	1±0.5	7± 9	
period III	17±3	17±3	0.5±0.5	0.5±0.5	4± 4	
Part II						
period I	24±2	24±2	≈ 0	0.5±0.3	≈ 0	2±1
period II	25±2	25±2	0.5±1	2.5±1	2±4	10±6
period III	17±5	17±5	1±1	6±3	6±8	39±23
Part III						
period I	20±3	20±3	3±1.5	12±1.5	15.5±8	66±11
period II	21±4	21±4	2±2.5	5±3	10±11.5	27±16
period III	24±1	24±1	0.4±0.2	3±1	2±1	11±3.5
Part IV	23±6	23±6	20±7.5	17±8	7±12	19±24

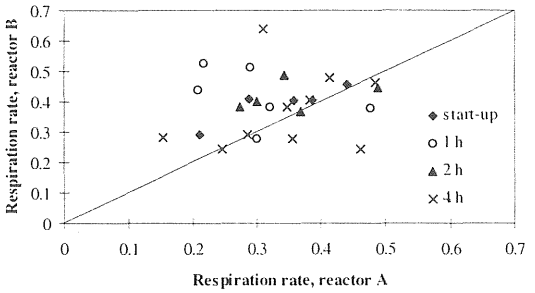
Respiration rates were measured to see if they were affected by changes in the DO concentration. In Part I and III of the experiment, there was a linear relationship between respiration rate (mg O_2/g VSS·min) and temperature (Part I: r^2=0.73; Part III: A) r^2=0.58 B) r^2=0.48) (Figure 5.2-1a,b,d). In Part II, the correlation between respiration rate and temperature was poor (the respiration rate was not measured in Part IV). Generally, the respiration rate was slightly higher in reactor B (Figure5.2-1c). In Part III, there was no large difference in respiration rate between reactor A and B and it could neither be related to SVI nor the turbidity of the supernatant.



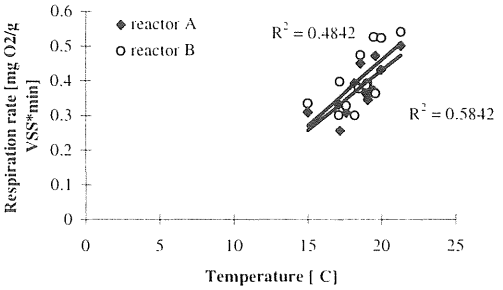
a) Part I



b) Part I



c) Part II



d) Part III

Figure 5.2-1 Respiration rate [mg O_2/g VSS·min].

5.2.2 Change in sludge volume index (SVI)

The SVI is a relative measure of the flocculation and settling characteristics of the activated sludge. The lower the sludge volume index, the better thickening and settling characteristics. High SVI is usually a result of excessive growth of filamentous bacteria. A sludge with a SVI > 150 ml/g is generally considered as bulking. By stirring in the cylinder glass during settling, the stirred sludge volume index (SSVI) can be determined (White, 1976). The SSVI is normally lower than the conventional SVI. The SSVI is measured in a special standard cylinder with a volume of about 3.5 litre. Parameters like suspended solids concentration, cylinder diameter and height and temperature affect the SVI (Dick and Vesilind, 1969). This shows that SVI is a very non-specific and arbitrary measure of the physical characteristics of activated sludge. It is also important to know that the settling characteristics in a settling cylinder are not the same as in a full scale settler and it is difficult to compare SVI for different plants. However, for comparison purposes it is still useful and easy to measure.

In this study, SVI was measured without stirring in a 1 litre cylinder with a diameter of 6 cm. The stirred sludge volume index has to be measured in a 3.5 litre cylinder, and it was in this experiment inconvenient to remove such a large volume at the same time. The changes in SVI for each part of the experiment are described separately. The SVI was measured on undiluted samples (SV: 80-600).

Part I: The DO concentration was decreased from 2 mg/l to 0.5 mg/l over a period of about three months (start-up period not included). At a DO concentration of 2 mg/l, the activated sludge flocs gradually changed from being small, irregularly shaped and fragile to being more compact, regularly shaped and large (see section 5.2.6). There were filaments present, but not in excessive numbers (category 2 = small number). The change in SVI is illustrated in Figure 5.2-2.

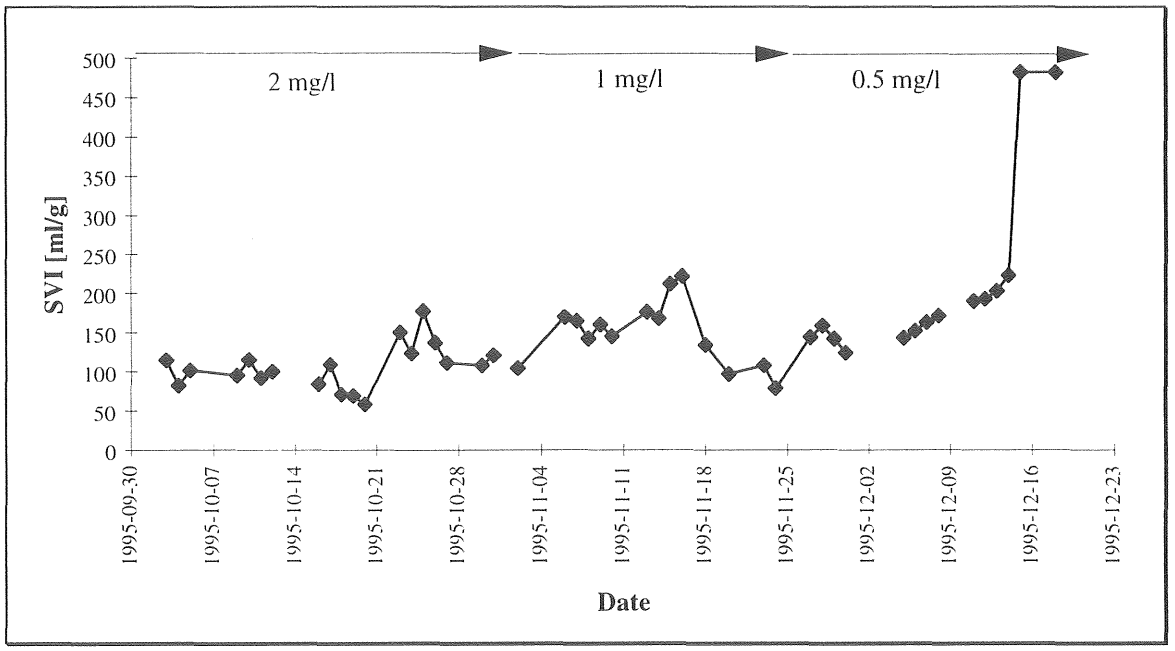


Figure 5.2-2 Change in SVI for Part I of the experiment.

When the DO concentration was decreased from 2 to 1 mg/l, filamentous and Zoogloea bacteria started to grow in larger numbers, from category 2 to 3, and the SVI increased to about 220 ml/g. The dominating type was, by a microscopic investigation, judged to be *Sphaerotilus natans* and the second most common filaments were probably *Thiothrix I-II* (or possibly type 021N). There was a sudden drop in SVI after 10 days of operation (16-18th of Nov.) at 1 mg O_2/l, which was due to a pump failure. This led to an accumulation of activated sludge in the settler for about 20 hours and anaerobic conditions made many of the filamentous and Zoogloea bacteria disappear. After about four days the SVI started to increase again. When the DO concentration was further decreased from 1 to 0.5 mg/l, the number of Zoogloea and filamentous bacteria increased dramatically (category 5). A process failure (27-28th of Nov.), similar to the previous one led to floc dispersion and the SVI increased slightly. Thereafter, the SVI increased to almost 500 ml/g within 10 days.

An interesting notation can be made; the 18th of October, a change was made to the feed tank: from the beginning of the experiment, a large feed tank was used with a HRT of a few hours. This was then exchanged for a smaller tank with a much shorter HRT (1.2 hours). After the change, the number of Zoogloea bacteria and the SVI increased.

Part II: Three different lengths of alternating oxic/anoxic periods were studied: 1 h, 2 h and 4 h (the alteration took place in reactor A and reactor B was operated at a constant DO concentration). During the start-up period, both reactors were run at a constant dissolved oxygen concentration (2 mg/l). After about 10 days of operation, large numbers of filamentous bacteria started to grow (see section 5.2.6) and the SVI increased from about 100 to 800 ml/g. To combat this, a selector in which return sludge and influent wastewater was mixed (without aeration), was installed in front of each reactor (HRT ≈ 6 minutes). This successfully reduced the numbers of filaments within the time of three sludge ages (Figure 5.5-3). To avoid the return of filaments, the DO concentration was increased from 2 to 4 mg/l. The activated sludge flocs grew in size (from an average floc diameter of 100 μm to 300-400 μm) and became more compact. When the SVI had decreased to 50-60 ml/g, the alternating period of 1 hour was initiated (there were small numbers of filaments present; category 2).

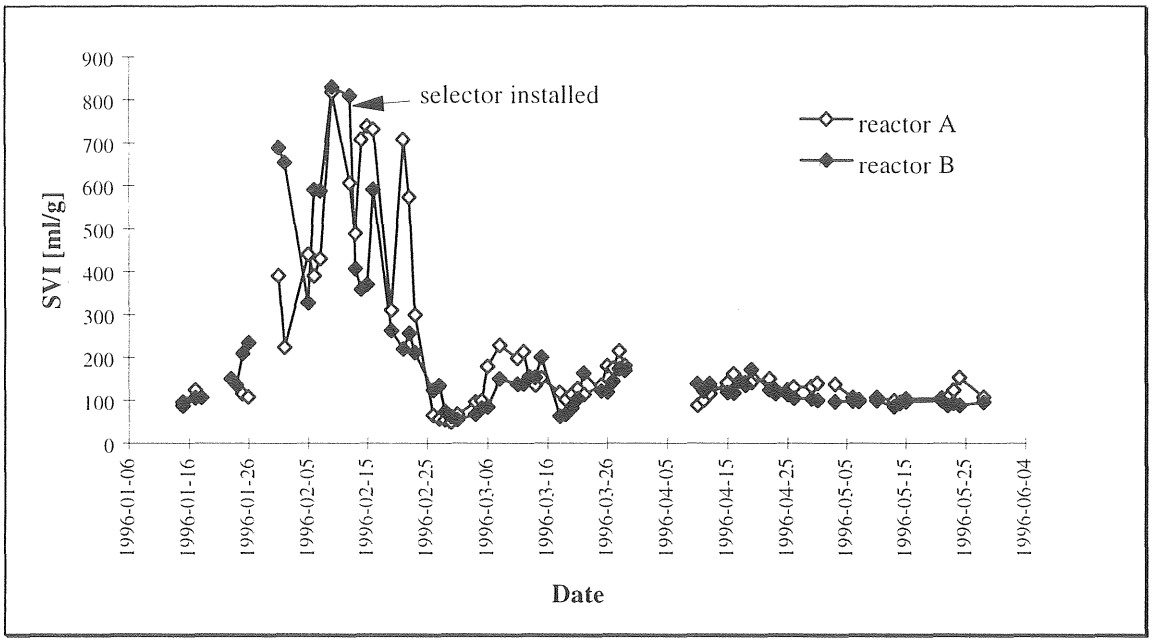


Figure 5.2-3 Change in SVI for Part II of the experiment.

Alternating period of 1 hour: After one week of operation, the settling properties started to deteriorate in both reactors (Figure 5.2-4). The SVI increased as a result of filamentous and Zoogloael growth. The increase in SVI was larger and faster in reactor A than in reactor B. However, the number of filaments was slightly smaller in reactor A than in reactor B (category 2-3 and 3-4, respectively). The lower SVI's for reactor B was probably due to the higher compactness of the flocs compared to the ones in reactor A. Long filaments grew between the sludge flocs (probably type 021N, see section 5.2.6) and caused deteriorated settling properties. After a further week of operation, the settling properties in reactor A improved and the SVI decreased to about 140 ml/g (the sludge in reactor A then seemed to get used to the alternating conditions) in both reactor A and B. When the settling properties were almost the same in reactor A and B, an alternating period of 2 hours was initiated.

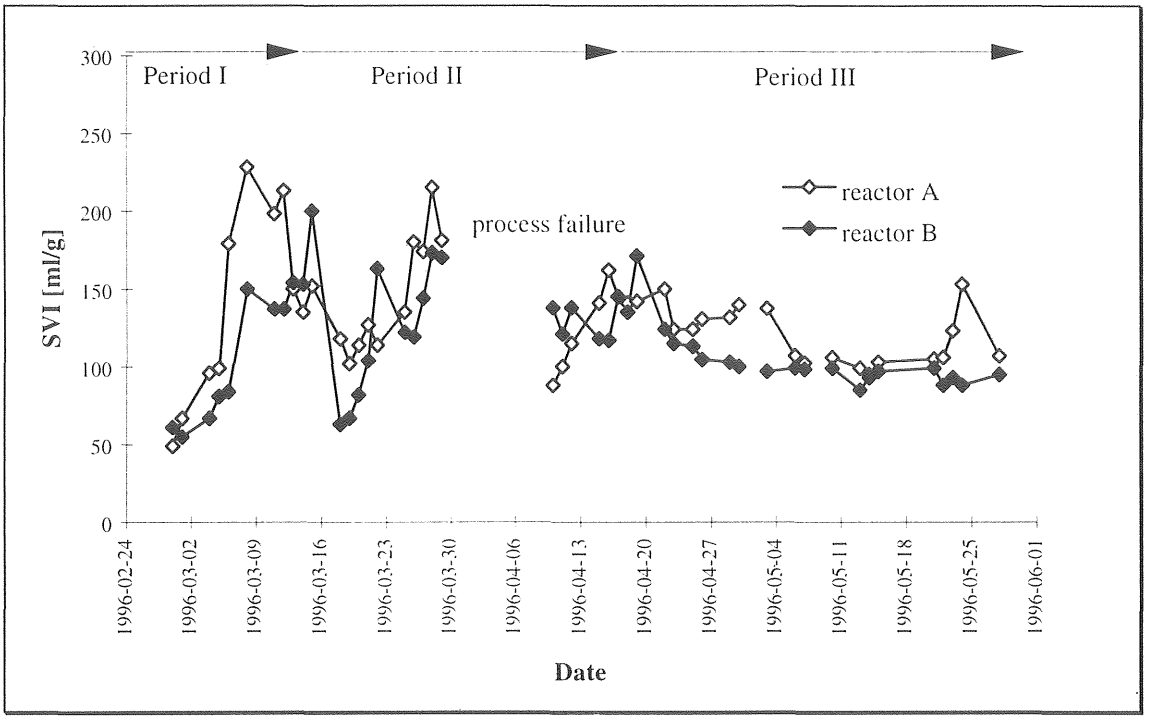


Figure 5.2-4 Change in SVI for Part II of the experiment (B: constant oxic conditions; A: alternating oxic/anoxic conditions; period I=1 h; period II=2 h; period III=4 h).

Alternating period of 2 hours: At the beginning of period II, the filamentous bacteria (see section 5.2.6) almost disappeared and the SVI decreased to about 100 ml/g in reactor A and to 60-70 ml/g in reactor B. Thereafter the SVI increased gradually for both reactor A and B (from 150 ml/g to 220 and 170 ml/g, respectively). The increase was, however, larger for reactor A. On the 30th of March a process failure led to the loss of activated sludge from the reactors and it took some ten days to get the desired concentrations of suspended solids back in the aeration tanks. Thereafter, the SVI fluctuated between 100 and 150 ml/g in both reactors. The number of filaments was slightly smaller in reactor A than in reactor B.

Alternating period of 4 hours: The SVI was slightly higher in reactor A than in reactor B (100-140 and 85-100 ml/g, respectively). The flocs in reactor A had a porous floc structure and they contained large numbers of Zoogloea bacteria (see section 5.2.6).

In summary, it can be concluded that alternating oxic/anoxic periods do not affect the SVI to a large degree. Alternating conditions gave slightly higher SVIs. At alternating periods of 1-2 hours, the number of filaments was often smaller than at a constant DO concentration. Longer alternating periods produced less compact flocs than constant DO concentrations did (see section 5.2.6). The change in SVI followed the same pattern for reactor A and B which indicates that it was something in the wastewater and/or in the reactor design that favoured the growth of filamentous bacteria.

Part III: The effect of DO concentration was studied at three different solids retention times (SRT): 5 d, 2.5 d and 1.25 d. In reactor A the DO concentration was kept at 0.5 mg/l and in reactor B at 2 mg/l.

SRT = 5 days: The SVI increased gradually from about 100 ml/g to 500 ml/g within 5 days for reactor A and within 15 days for reactor B (Figure 5.2-5). There were filamentous bacteria

present in both reactors, but the number was larger in reactor A (see section 5.2.6). Moreover, the flocs in reactor B were compacter and slightly larger than in reactor A. Before the sludge age was decreased to 2.5 days, the activated sludge in reactor B was divided into two and the activated sludge in reactor A was discarded.

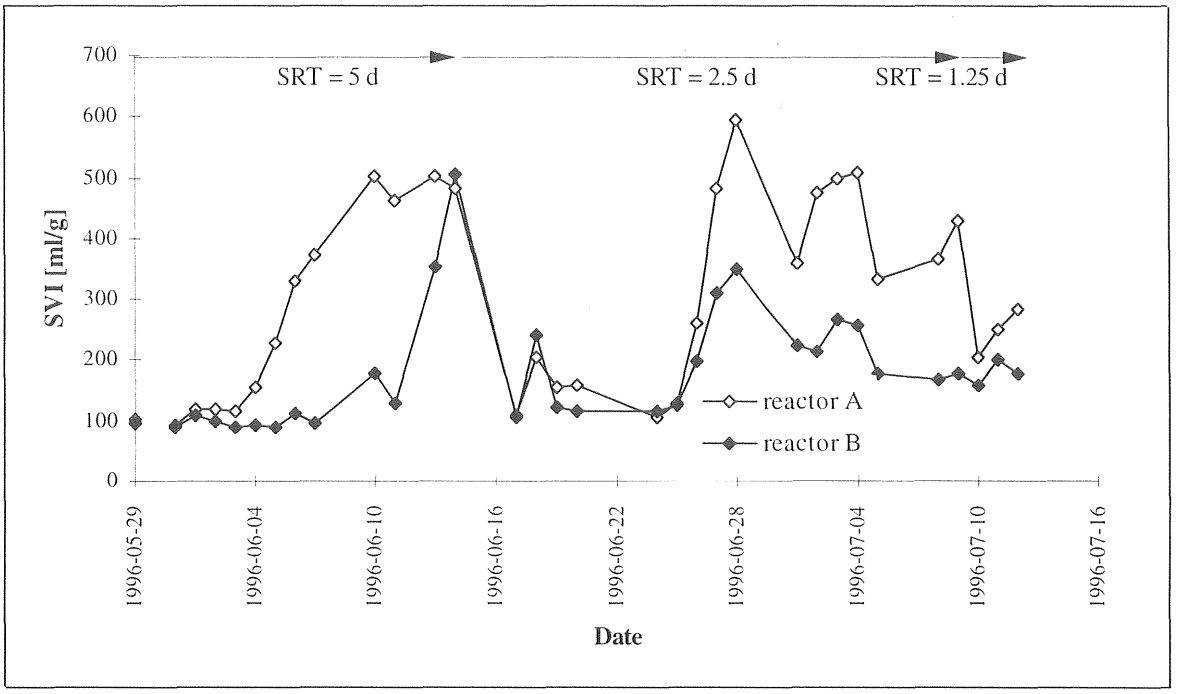


Figure 5.2-5 Change in SVI for Part III of the experiment (A: 0.5 mg O_2/l; B: 2.0 mg/l).

SRT = 2.5 days: The change in sludge age was followed by a sudden drop in SVI and the number of filamentous bacteria was drastically reduced. About three sludge ages later, the SVI started to increase in both reactor A and B (from about 100 ml/g to 600 and 350 ml/g, respectively). Thereafter, the SVI started to decrease gradually in both reactors: to about 400 ml/g in reactor A and to about 180 ml/g in reactor B. There were large numbers of filaments in both reactors (category 4). Before the sludge age was decreased to 1.25 days, the activated sludge in reactor B was divided into two and the activated sludge in reactor A was discarded

SRT = 1.25 days: As in the previous test, the decrease in sludge age caused a sudden drop in SVI. In reactor A it started to increase again after 2 days while it was kept fairly constant in reactor B.

Something in the wastewater or in the design of the pilot plant seemed to favour the proliferation of filamentous bacteria, and the change in SVI followed the same pattern in both reactor A and B. Probably a combination of a completely mixed system and wastewater composition caused the large number of filamentous bacteria. A higher DO concentration seemed to be able to repress the degree of deterioration of the settling properties; there were less filamentous bacteria present and the flocs also settled better because of a higher compactness.

Part IV: One reactor was run at a DO concentration of 2 mg/l, which in general is considered as being enough to avoid oxygen limitation. The other reactor was run at a high DO concentration: 5 mg/l. The reactors were run for about two sludge ages before the actual experiment was initiated. After about ten days the SVI increased, unexpectedly, in reactor B which was run at a DO concentration of 5 mg/l (Figure 5.2-6). Filamentous bacteria started to grow in large numbers. Two sludge ages later, they suddenly disappeared. At the same time, the microscopic investigation showed that there were large numbers of dispersed bacteria between the sludge flocs. The reason to this could be that a toxic compound had entered the pilot-plant with the influent. This is, however, only a speculation. Some ten days later, the SVI increased dramatically in reactor A due to excessive numbers of filamentous bacteria. The activated sludge flocs were very porous and did not settle at all. The SVI in reactor B remained at a lower level (80-180 ml/g). When the DO concentration in reactor A was increased to 5 mg/l, the SVI decreased to 150 ml/g within three days. Thereafter both reactors were run at the same conditions for about three sludge ages to see if this would produce sludge with similar characteristics. The SVI remained low and there were hardly any filamentous bacteria present. The flocs were also very round and compact.

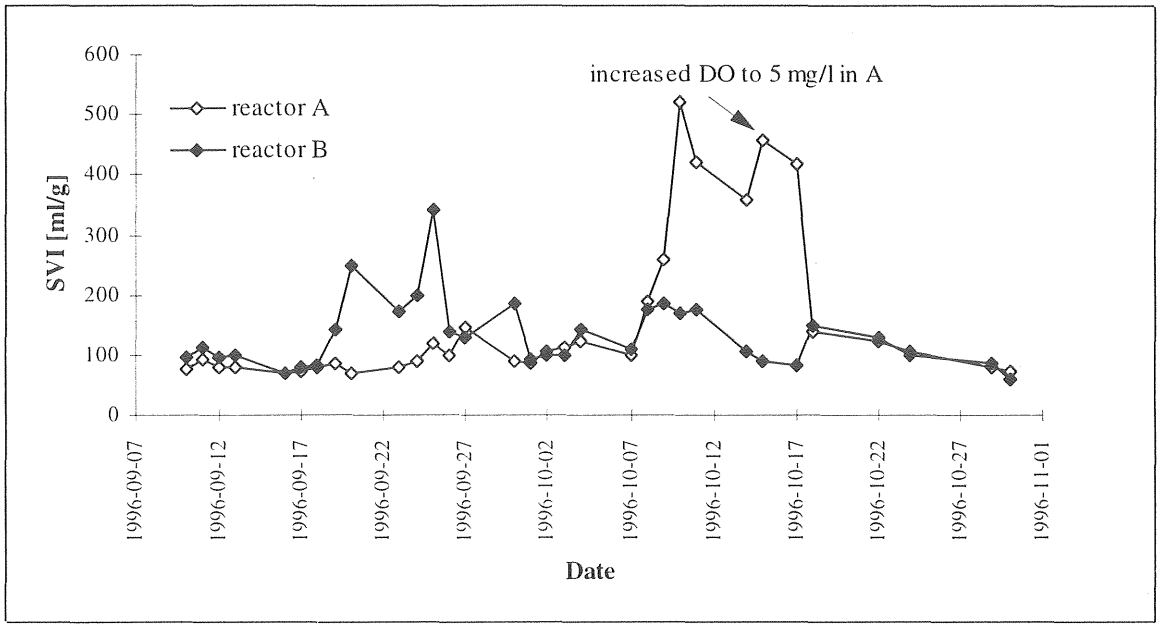


Figure 5.2-6 Change in SVI for Part IV of the experiment (A: 2 mg O_2/l; B: 5 mg O_2/l).

The above results show that it is very difficult to avoid proliferation of filamentous bacteria in small pilot plants with completely mixed reactors. In small plants, the surface-to-volume ratio is very large. This makes it easier for filamentous bacteria to grow in tubings etc. The tubings were therefore cleaned with hypo-chlorite every 2-3 days and the reactor walls were brushed every day to remove wall growth.

In Part I, the number of filaments increased as a result of decreased DO concentrations. However, it is difficult to draw any conclusive findings. Only one reactor was run and the experiment lasted for three months. The temperature decreased gradually due to seasonal changes (from about 20 to 10 °C), which may affect the growth of filamentous bacteria. In Part III, a DO concentration of 2 mg/l could repress the filamentous growth longer than a DO concentration of 0.5 mg/l. When the SRT was decreased to 2.5 days, a smaller difference in filamentous growth could be noticed between a DO concentration of 0.5 and 2 mg/l. In Part II,

a rather high DO concentration was kept in the aeration tanks: 4 mg/l, which seemed to repress excessive proliferation of filamentous bacteria to a certain extent. A DO concentration of 5 mg/l (Part IV) produced a sludge with better settling properties than a DO concentration of 2 mg/l, except for a period at the beginning of the experiment (which was very surprising). At a DO concentration of 2 mg/l the settling properties deteriorated considerably after about one month of operation. When the DO concentration was increased to 5 mg/l the filamentous bacteria disappeared within a few days.

The conclusion which can be drawn from all this is that higher DO concentrations than 2 mg/l are necessary to repress bulking sludge. The DO concentration needed to avoid excessive numbers of filamentous bacteria is probably dependent on the organic loading. Palm and Jenkins (1980) studied the relationship between organic loading, DO concentration and the formation of bulking sludge. They found a linear relationship between substrate (COD) removal rate and the formation of bulking sludge at different DO concentrations in the aeration basin. Further they found that the DO uptake rate is a linear function of the COD removal rate. This should make it possible to decide the minimum DO concentration necessary in the aeration tank to avoid bulking sludge by measuring the respiration rate. In this study no such relationship could be found; even at low organic loadings, bulking sludge could appear at rather high DO concentrations. The relationship between substrate removal rate and DO uptake rate was not very linear probably depending on the difference in temperature between the different measurements.

The installation of a selector seemed to reduce the number of filaments considerably. However, in other experiments it did not seem to help. This was, however, not investigated further since this was not the aim of the project. It was also noticed that changes in the process like SRT could change the settling properties drastically (sometimes it improved the settling properties). This has been discussed by Ericsson and Eriksson, 1988. They suggested that by introducing considerable changes in certain operational parameters, within certain time scales, sludge properties could be controlled.

5.2.3 Turbidity

Part I: No clear correlation could be found between the DO concentration and the turbidity in the supernatant after settling. However, only one reactor was run and the experiment extended over about three months. To be able to see effects of DO concentration on the turbidity, in spite of changing wastewater composition, two reactors were run in parallel in the subsequent experiments. After a decrease in DO concentration, the turbidity increased slightly (Figure 5.2-7) and the microscopic investigation showed that the number of dispersed bacteria increased as well.

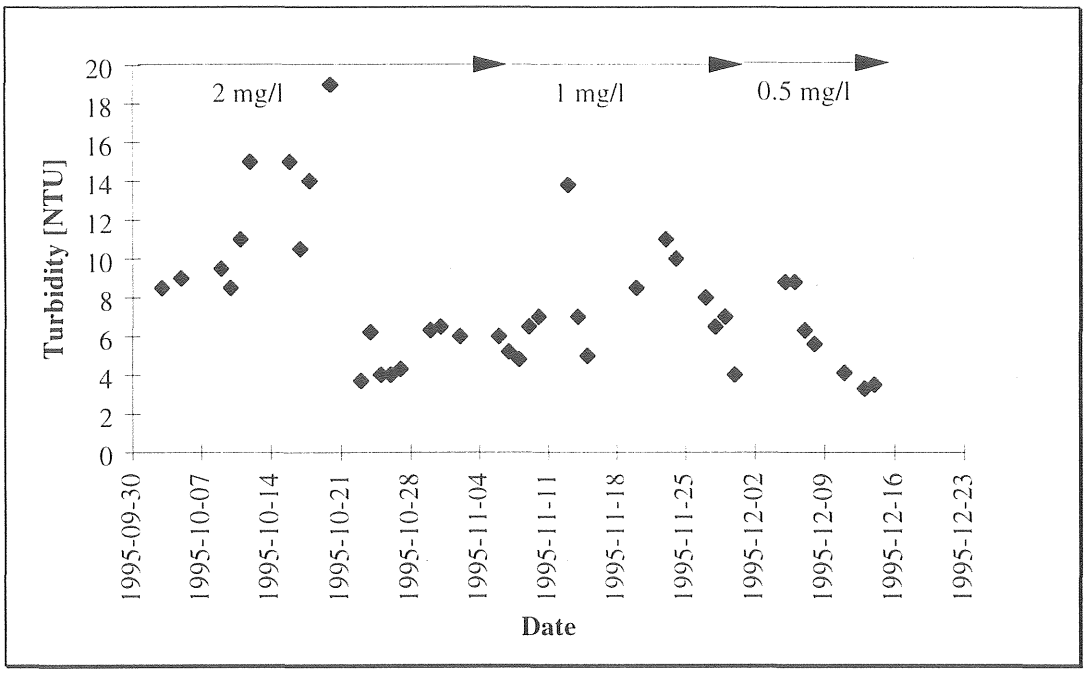


Figure 5.2-7 Turbidity in the supernatant after 20 minutes settling (Part I).

The turbidity seemed to be more affected by the structure of the activated sludge flocs; excessive growth of filamentous and Zoogloea bacteria causes a sweep effect owing to the enmeshment of small flocs and dispersed bacteria in the network of filaments and Zoogloea bacteria. During the first part of the period with a DO concentration of 2 mg/l, the flocs were very round and compact and the turbidity in the supernatant after settling was high. Around the 20th of October, the structure of the flocs suddenly changed to be more irregularly shaped and Zoogloea bacteria started to grow in large numbers. This led to a very clear supernatant. In Figure 5.2-8, the turbidity is plotted against the SVI and this shows that there is a trend towards lower turbidities at higher SVI.

Part II: During the alternating period of 1 hour (period I), the turbidity in reactor A (sample for turbidity measurement taken at the end of the oxic period) was slightly higher than in reactor B. The alternating period of 2 hour (period II), did not give any difference in turbidity. That the difference in turbidity was so small in period I and II could probably be explained by the large number of filamentous bacteria present in both reactors (see section 5.2.6), which seems to produce clear supernatants. Another hypothesis is that a DO concentration of 4 mg/l is high enough to produce a clear supernatant even at alternating oxic/anoxic periods of 1-2 hours. During the alternating period of 4 hours (period III), there was no difference when the DO concentration was 4 mg/l. However, when the DO was decreased to 2 mg/l in both reactors, a clear difference could be noticed (Figure 5.2-9). A comparison of the turbidity for reactor A and B during the different alternating periods is illustrated in Figure 5.2-10

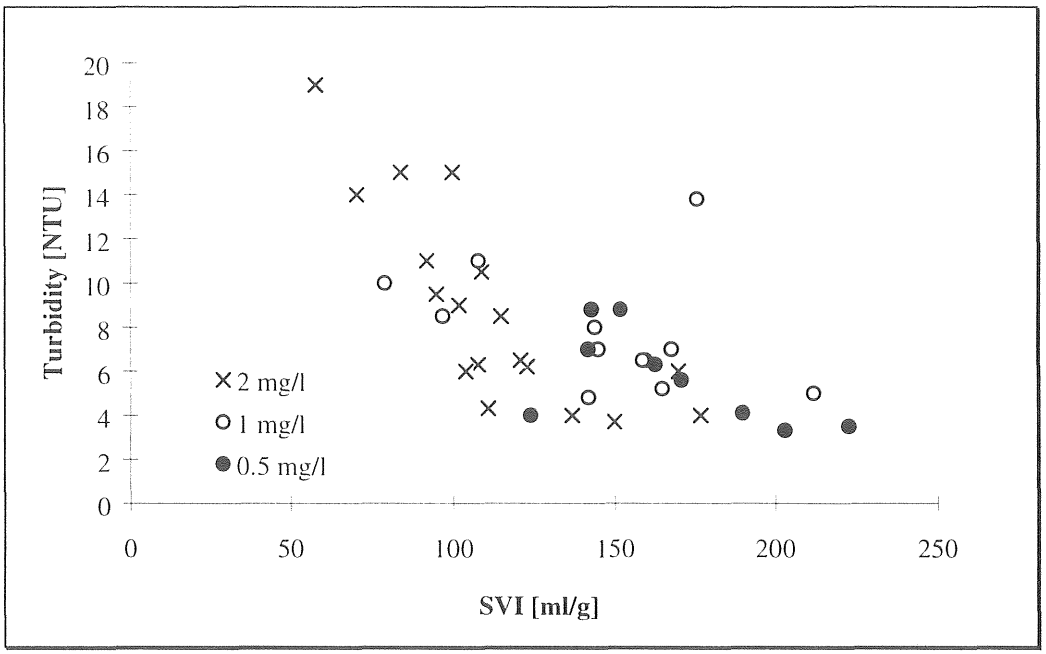


Figure 5.2-8 Turbidity as a function of SVI (Part I).

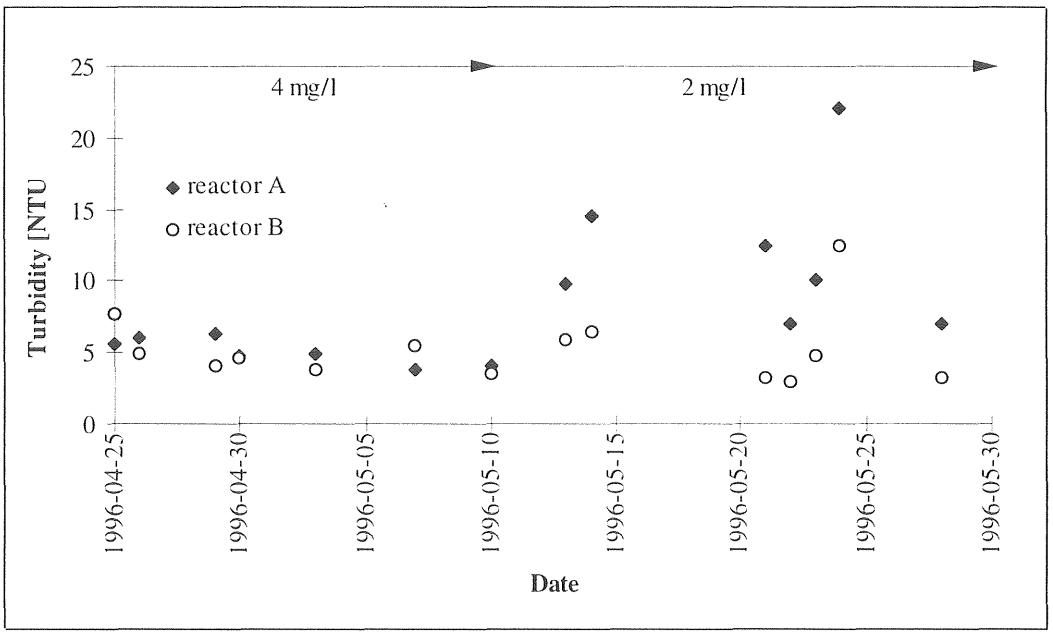


Figure 5.2-9 Turbidity after 20 minutes settling at an alternating period of 4 hours (Part II).

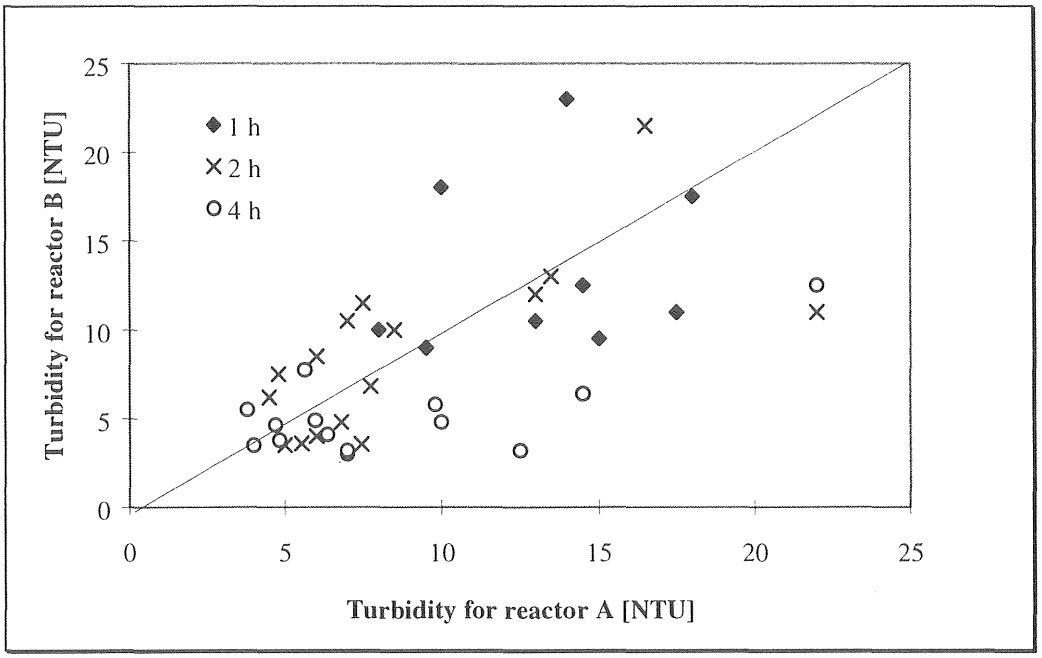


Figure 5.2-10 Comparison of the turbidity in reactor A and B (Part II).

During the alternating oxic/anoxic periods, the change in turbidity was followed over the different cycles. In Figure 5.2-11, a 4-hour-cycle is depicted (2 hours at anoxic and 2 hours at oxic conditions). The turbidity in reactor A increased gradually during the anoxic period while the turbidity in reactor B remained fairly low (the DO concentration in reactor B was held at a constant value of 4 mg/l). Directly after that the oxygen supply was turned on again in reactor A, the turbidity decreased and after about two hours the turbidity was almost back to the initial value. The turbidity was by the end of the oxic period, the same for reactor A as for reactor B. The turbidity in the pre-settled wastewater varied but was not monitored. However, the turbidity increased slightly in reactor B during the cycle of 4 hours which indicates that the turbidity in the influent wastewater increased as well.

The pH was followed over the cycle and it increased directly upon a decrease in DO concentration. The pH followed the same pattern as the change in turbidity. This may change the surface charge of the activated sludge flocs and thus affecting the flocculation. This is further discussed in section 4.2.3.

Another test was made in which the influent was turned off during a 4-hour-cycle. The purpose was to see whether the turbidity increases as a result of floc dispersion and/or desorption of adsorbed wastewater. In Figure 5.2-12, it can be seen that the turbidity decreased in both reactors when the influent was turned off. This is expected since the organic material which is adsorbed onto the activated sludge flocs will be broken down gradually in the presence of oxygen. Immediately when the oxygen was turned off in reactor A, the turbidity started to increase. Whether this was due to floc dispersion or desorption of organic material which has not yet been broken down is difficult to say. The pH changed in the same way as the turbidity: the pH decreased gradually in reactor B as a result of biological degradation which produces CO_2 (the CO_2 reacts with the water to form H_2CO_3 which is an acid) and no dilution with wastewater which has a higher pH than the activated sludge suspension. The increase in pH is probably due to an decreased biological activity and a

reduced production of CO_2 and/or due to denitrification. The stirring also strips the CO_2 to the atmosphere which further increases the pH.

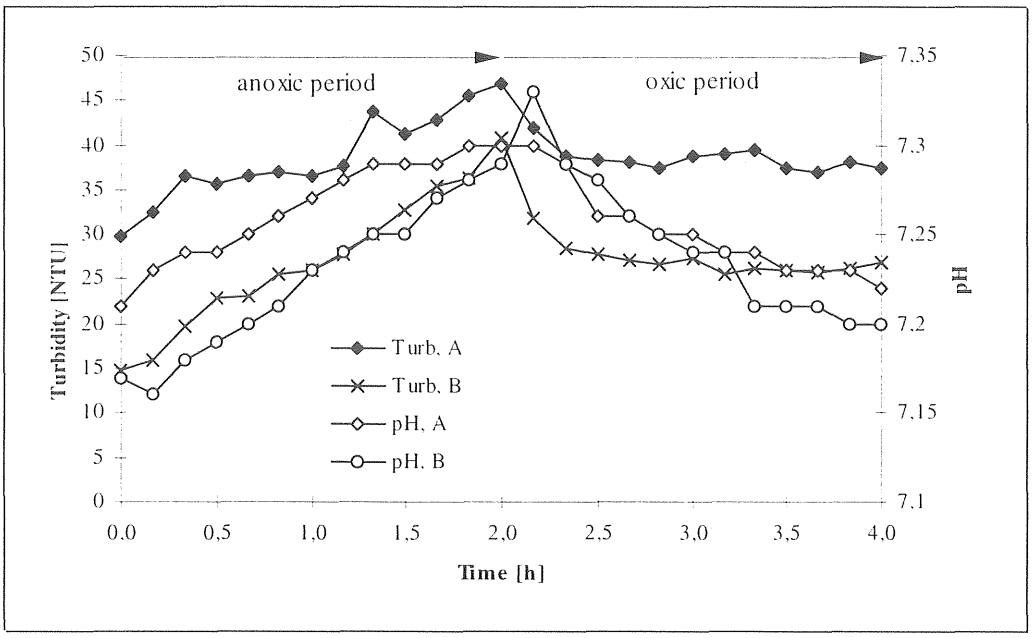


Figure 5.2-11 The change in turbidity and pH during a 4-hour-cycle, Part II (960415).

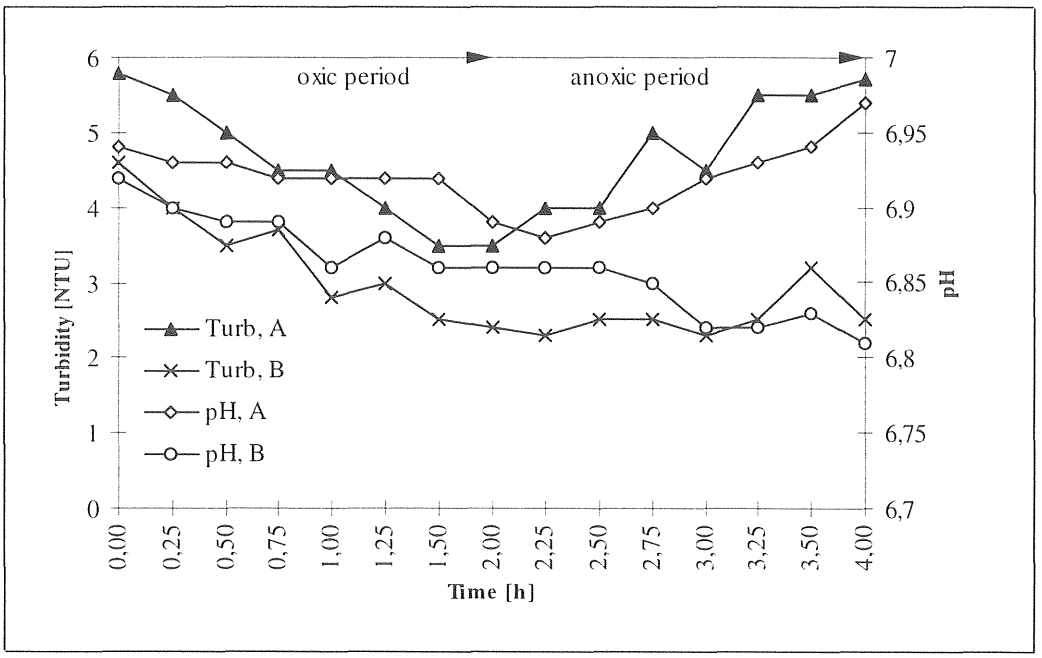


Figure 5.2-12 The change in turbidity and pH during a 4-hour-cycle without influent, Part II (960417).

A measurement over an 8-hour-cycle is depicted in Figure 5.2-13 (turbidity after 20 minutes settling). The measurements started in the middle of the oxic period. The turbidity of the influent wastewater was also measured (Figure 5.2-14) as a comparison. In spite of the large

fluctuations in the turbidity of the pre-settled wastewater, the turbidity in reactor B remained low; only a slight increase could be noticed.

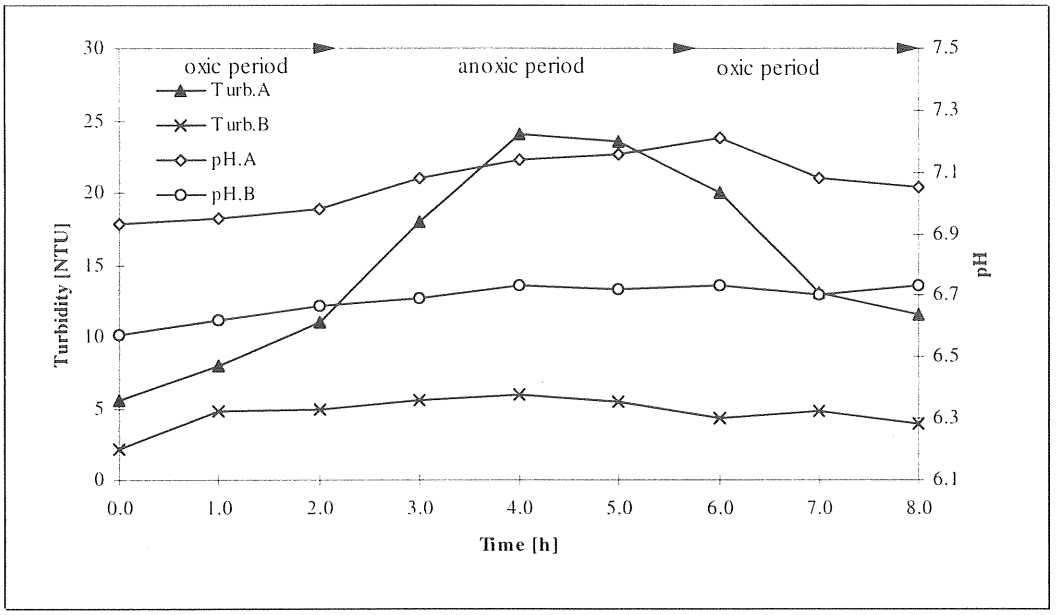


Figure 5.2-13 Change in turbidity and pH during an 8-hour-cycle, Part II (960515).

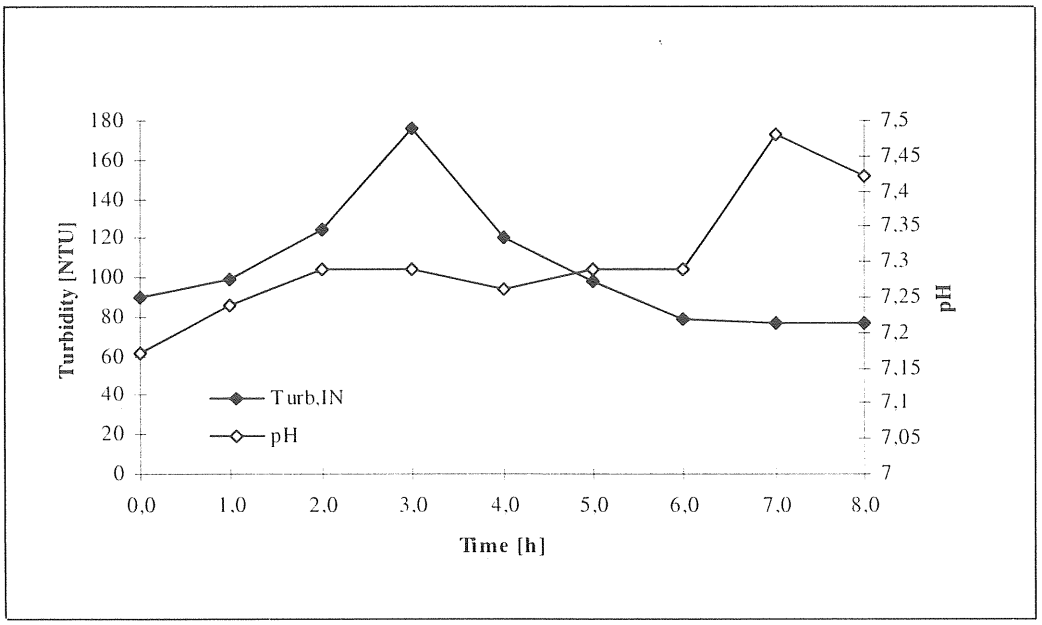


Figure 5.2-14 Turbidity and pH of the pre-settled wastewater, Part II (960515).

In Figure 5.2-15, a part of an 8-hour-cycle without influent is depicted. The same results as for the 4-hour-cycle were obtained.

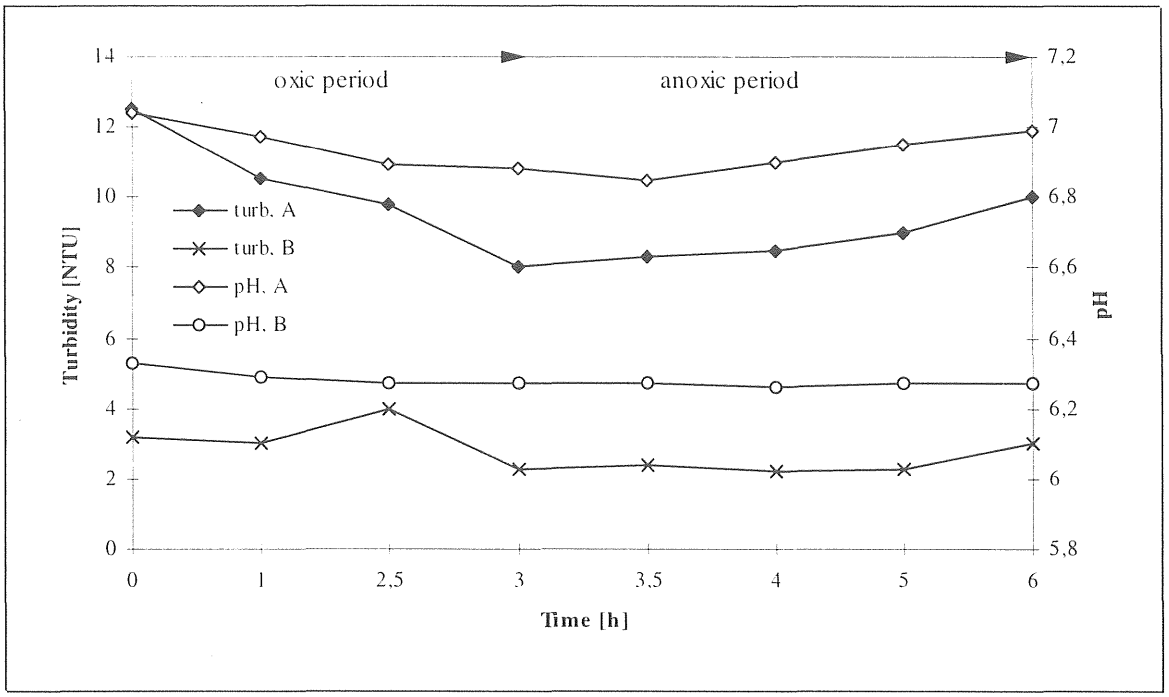


Figure 5.2-15 Change in turbidity and pH during an 8-hour-cycle without influent, Part II (960521).

Part III: The turbidity increased as the SRT decreased (Figure 5.2-16). Between May 29 and June 11, the turbidity was most of the time, lower for reactor B (DO = 2 mg/l) than for reactor A (DO = 0.5 mg/l). Thereafter, the DO concentration was held at 2 mg/l in both reactors until the 20th of June (to create similar conditions in both reactors). Between June 21-28, the DO concentration in reactor A was decreased to 0.5 mg/l. This led to a higher turbidity in reactor A. By then, large numbers of filamentous bacteria started to proliferate and, as an attempt to combat this, the DO concentration was increased to 5 mg/l for a few days. This immediately led to decreased turbidity but it did not remove the filamentous bacteria (the period with a high DO concentration was probably too short). From the 4th of July and to the end of the experiment, the DO concentration was kept at 0.5 mg/l in reactor A. No difference in turbidity could, however, be seen, probably because of the relatively large number of filamentous bacteria present. To be able to see the difference more clearly, the turbidities for reactor A and B are plotted against each other (only the points for which the DO concentration in reactor A was 0.5 mg/l are included) in Figure 5.2-17. From this it can be seen that the turbidity was lower in reactor B most of the time. That the difference was small, probably has to do with the large number of filaments present.

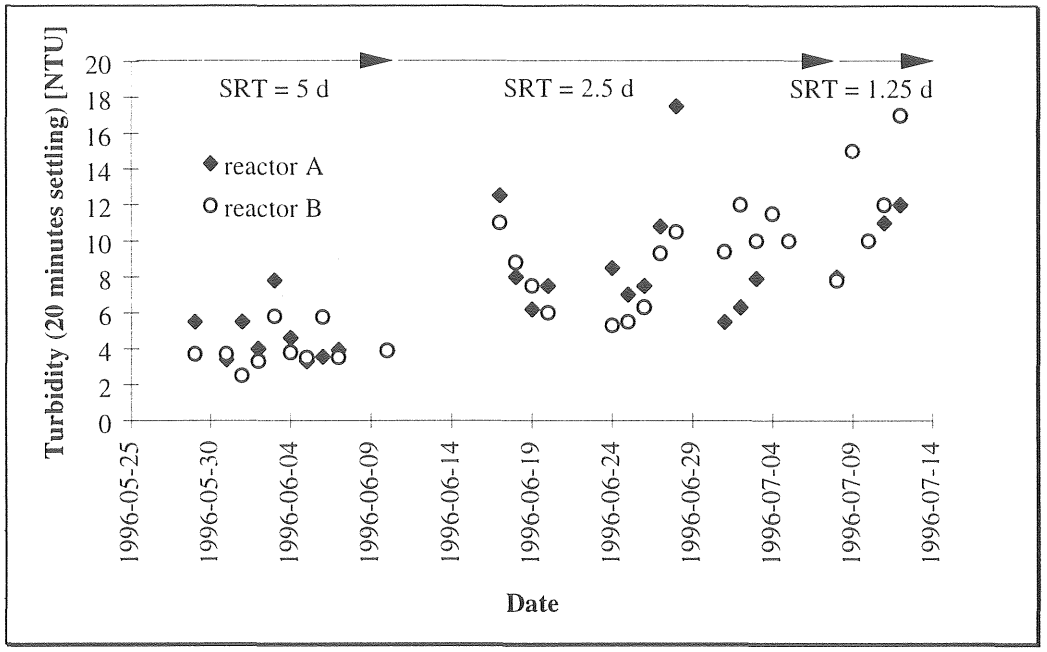


Figure 5.2-16 Turbidity in the supernatant after 20 minutes settling (Part III).

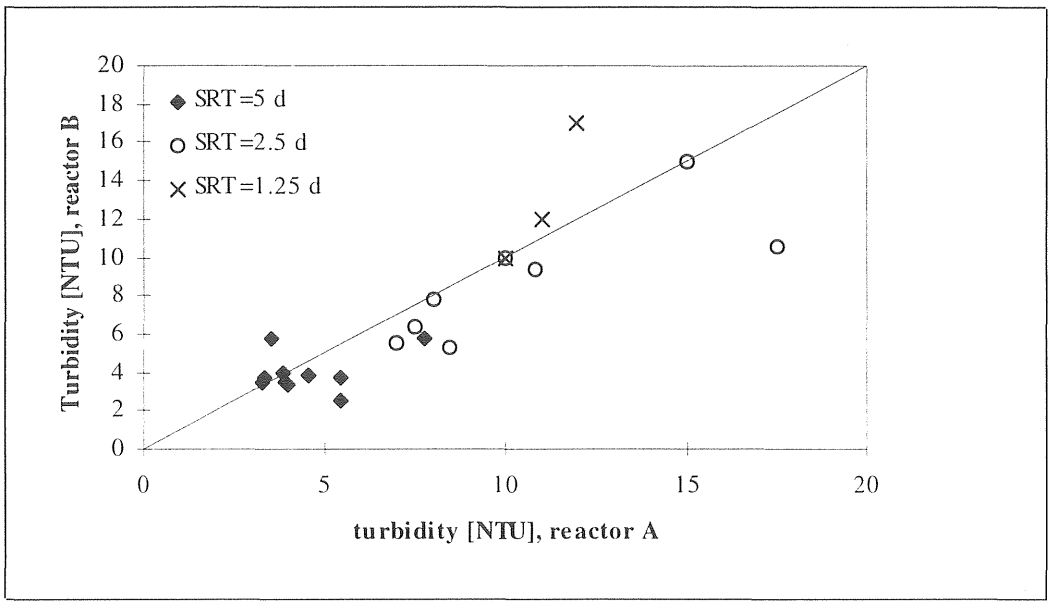


Figure 5.2-17 Comparison of the turbidity of reactor A and B (Part III).

Part IV: The turbidity was higher in reactor A (DO concentration of 2 mg/l) than in reactor B (DO concentration of 5 mg/l) during the major part of the experiment (Figure 5.2-18). The difference between the two reactors was largest at higher turbidities (Figure 5.2-19).

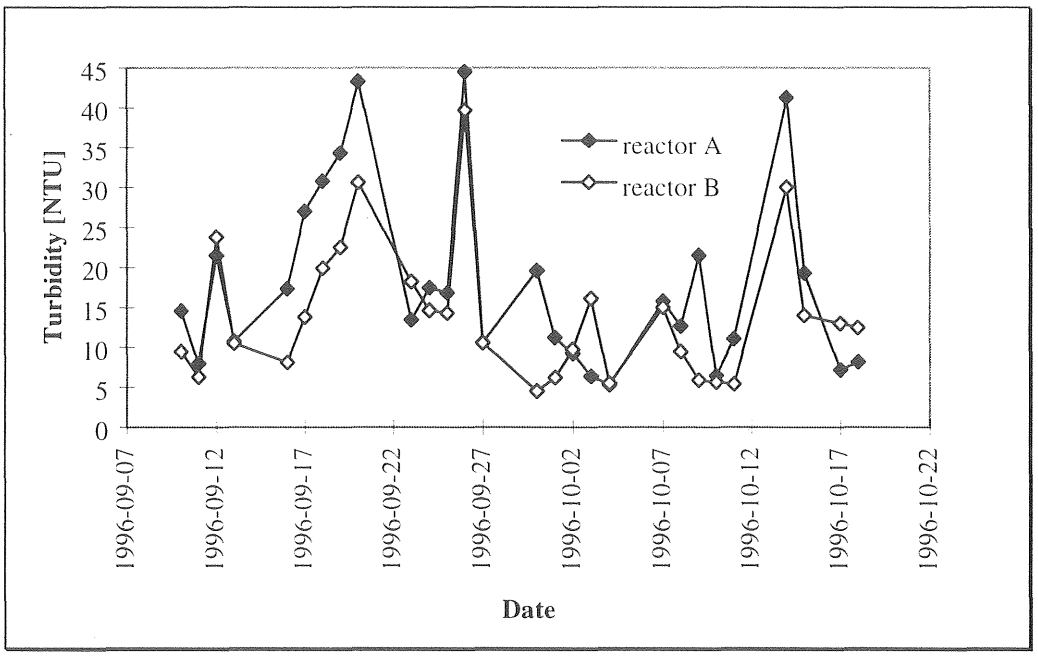


Figure 5.2-18 Change in turbidity (Part IV).

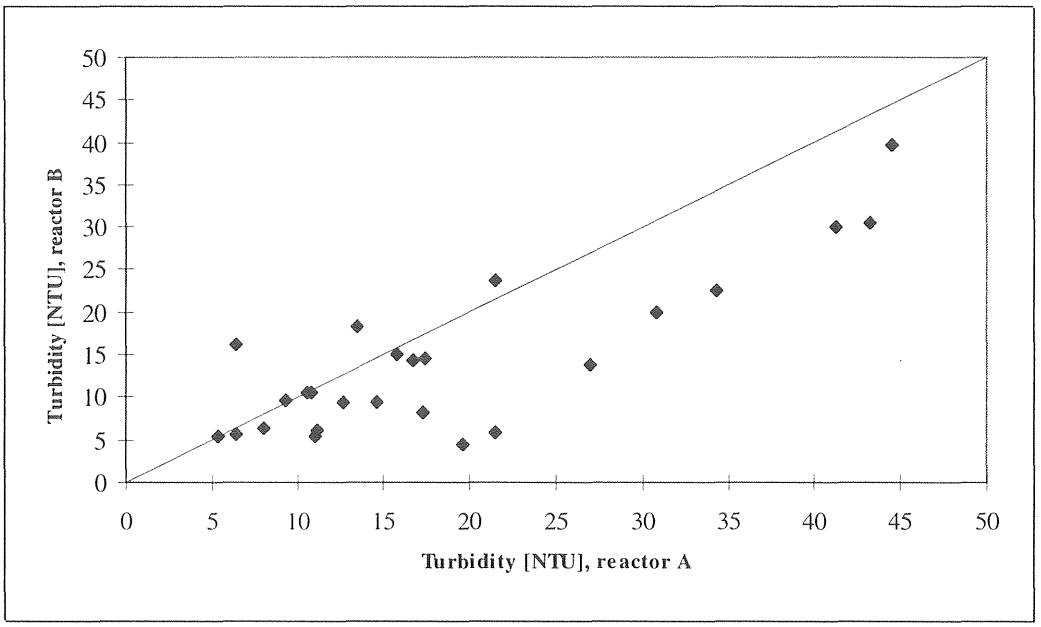


Figure 5.2-19 Comparison of the turbidity for reactor A and B (Part IV).

To test whether activated sludge acclimatized to high or low DO concentrations show different sensitivity to anoxic conditions, the oxygen supply was turned off and the turbidity in the supernatant after 30 minutes settling was monitored over a 4-hour-cycle (two hours with oxygen and two hours without). In the experiments, the turbidity of the influent was fairly high (about 120 NTU) and the turbidity increased considerably during the anoxic period (Figure 5.2-20). The background turbidity was about 27 NTU for reactor A and 10 NTU for reactor B. The turbidity increased by about 13-15 NTU/g MLSS in both reactors (the MLSS concentration and the SVI were about the same in reactor A and B: 1.465 g/l and 74 and 81 ml/g, respectively). Immediately after the oxygen supply was turned on again, the turbidity started to decrease. Two hours after that the oxygen supply was turned on, the turbidity in

reactor A was back to the initial value. On the other hand, in reactor B the turbidity decreased to a level which was higher than the initial value. This could mean that sludge acclimatized to high DO concentrations are more sensitive to low oxygen levels. The pH followed the same pattern as the turbidity.

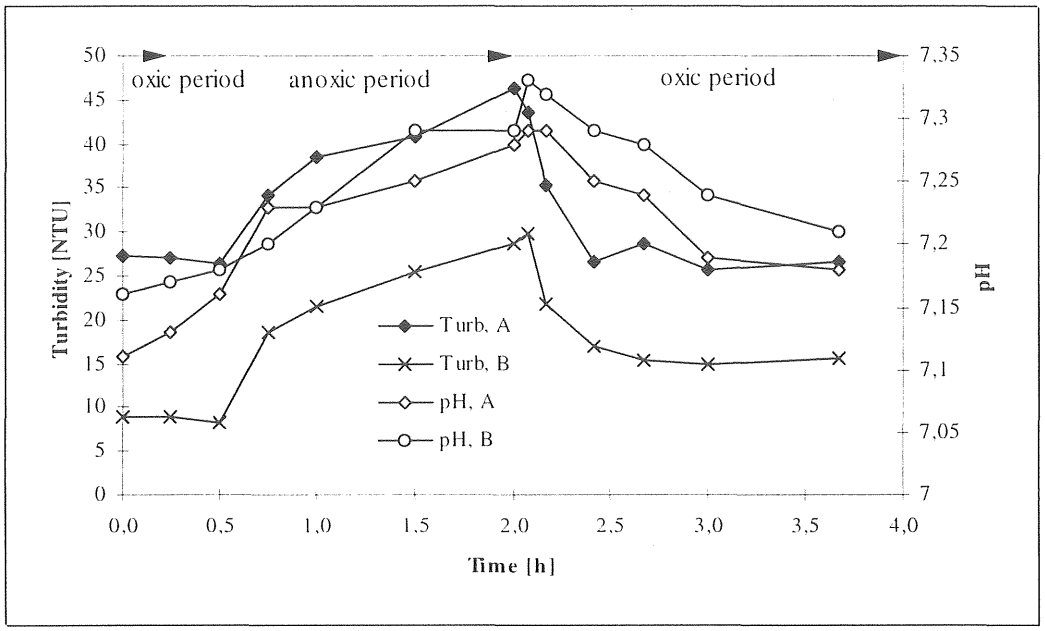


Figure 5.2-20 Change in turbidity and pH during a 4-hour-cycle, Part IV (960917).

The same experiment was repeated (Figure 5.2-21) the following day. Similar results were obtained. The turbidity increased more in reactor B (17 NTU/g biomass) compared to in reactor A (11 NTU/g biomass). By the end of the aerobic period, the turbidity in reactor A remained at a level which was 7 NTU-unit higher than the initial value. The corresponding value for reactor B was 12 NTU units higher, which confirms the previous test.

If the DO concentration was decreased to zero and the influent was turned off, the turbidity increased (Figure 5.2-22). The increase was about the same for reactor A and B: 5.4 and 6.3 NTU/g biomass, respectively. The increase in turbidity could depend on deflocculation and/or desorption of organic material. During the experiment, the floc characteristics were the same as in the previous experiment.

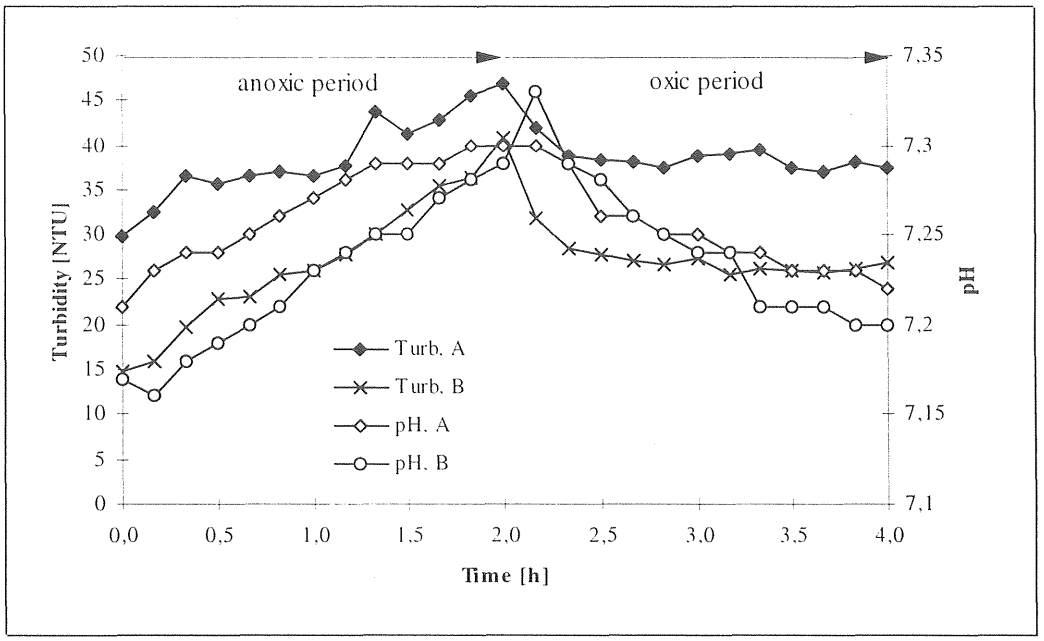


Figure 5.2-21 Change in turbidity and pH during a 4-hour-cycle, Part IV (960918).

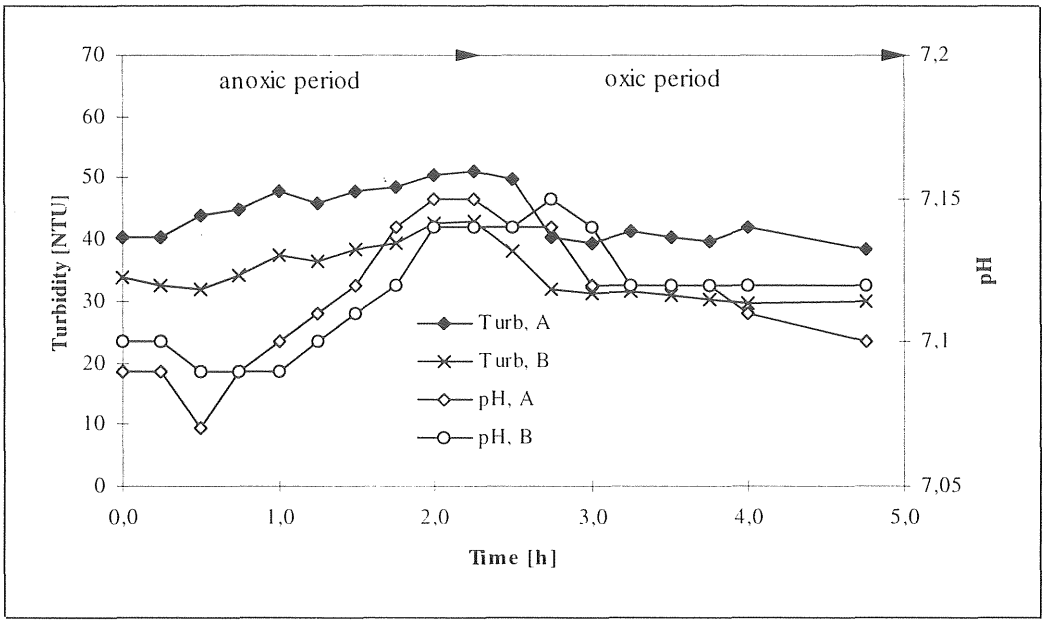


Figure 5.2-22 Change in turbidity and pH during a 4-hour-cycle, without influent, Part IV (960926).

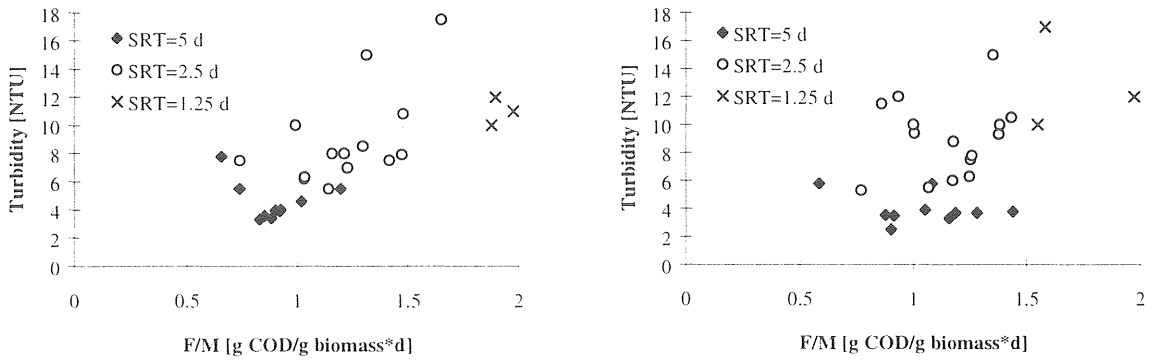
The results show that the DO concentration has a direct influence on the turbidity of the supernatant. The increase in turbidity is probably a combination of loss of adsorptive capacity and floc dispersion. The microscopic investigation did not show any large differences in the number of dispersed bacteria, but it appeared to increase after the anoxic periods. Starkey and Karr (1984) made similar studies, but they used a synthetic wastewater instead. When the DO concentration was decreased from 5 to 0.4 mg/l, the turbidity in the supernatant started to increase after 10 hours which is much longer than what has been found in this study. The number of eucaryotes was also monitored and a decreased activity could be found first 32 hours after the decrease in DO concentration. The amount of exocellular polymers also

decreased when the DO concentration was kept low. They also made tests with different colloidal loadings by adding kaolin. It was found that at higher colloidal loadings, the turbidity increased faster at low DO concentrations. This is in accordance with these results.

The loss in adsorptive capacity could depend on a reduction in exocellular polymer production. This could also reduce the cohesive properties of the flocs. Other possible reasons to increased turbidities could be cell lysis due to oxygen depletion. The question is how fast these processes occur.

During microscopic studies at the Rya WWTP it has been found that activated sludge flocs can suddenly fall apart and many bacteria leave the flocs to swim freely in the bulk solution (Robinsson, 1996). The probable reason to this is oxygen depletion.

Relationship between F/M ratio and turbidity: The organic loading probably has a large impact on the turbidity of the effluent. A fairly constant organic load (about 0.6-1.0 g COD/g MLSS·d) was applied in part I, II and IV of the experiment and no relationship between turbidity and F/M ratio could be found. In part III of the experiment, the pilot plant was operated at a broader range of organic loadings (F/M: 0.88-1.90 g COD/g MLSS·d; SRT: 1.25-5 d). An increase in turbidity with organic load could be found (Figure 5.2-23). No great difference between reactor A and B was, however, observed.



a) Reactor A b) Reactor B

Figure 5.2-23 Turbidity vs organic loading (20 minutes settling), Part III.

The above results show that the DO concentration has an impact on the turbidity. It can be difficult, however, to compare the values from the two reactors since the sludge morphology differed considerably. Flocs containing filaments and Zoogloea bacteria seem to produce a clear effluent.

5.2.4 Initial settling velocity

The initial settling velocity depends on the diameter of the settling cylinder, the concentration of mixed liquid suspended solids (MLSS), the sludge volume (SV), the floc size, the floc shape and the temperature of the water (Daigger and Roper, 1985; Göhle and Björlenius,

1996). Possible temperature effects were not taken into consideration in this study. All measurements were made in the same cylinder and the purpose was simply to be able to compare the results from different measurements and to follow changes in the settling properties. The settling tests were performed on undiluted as well as on diluted sludge samples (to obtain a SV of about 300 ml or lower). The SV seemed to be the parameter which influences the initial settling velocity the most. Therefore, it was found interesting to correlate the initial settling velocity to the SV to be able to compare the results from reactor A and B. A higher floc density and/or a larger floc diameter should give a higher settling velocity at the same SV, if the number of filaments was roughly the same. Filamentous bacteria reduce the settling velocities considerable, even if the flocs are very compact and large.

In Table 5.2-5, the SV and SVI are correlated to the initial settling velocity. The initial settling velocity could best be correlated to the SV in all experiments (Table 5.2-5). In most cases, an exponential function gave the best correlation, however, the difference between the regression coefficients (r^2) for exponential and power functions was not very large. The correlation between SVI and initial settling velocity was poorer. In Part IV of the experiment the correlation was better, probably because of a smaller deviation in suspended solids concentration throughout the experiment.

The initial settling velocities as a function of sludge volume for the different tests are illustrated in Figure 5.2-24 and 5.2-25. Similar results were obtained in the various experiments. The difference in initial settling velocity between reactor A and B was largest in Part III and IV of the experiment. This is in agreement with the fact that the flocs in reactor B were, on an average, larger and more compact than in reactor A in those experiments. In part II of the experiment, there was only a difference in initial settling velocity between reactor A and B at an alternating oxic/anoxic period of 4 hours (Figure 5.2-26). The microscopic investigation (section 5.2.6) showed that the sludge flocs were more compact in reactor B than in reactor A. If the periods with different sludge ages are considered separately in Part III of the experiment, the initial settling velocity in reactor B (DO = 2 mg/l), was larger at a sludge age of 5 and 2.5 days, than in reactor A (DO = 0.5 mg/l). In Part IV of the experiment, there was hardly any difference in settling velocity (slightly higher in reactor B). However, the flocs were larger and compacter in reactor B than in reactor A. The flocs in both reactors were on the average smaller in this experiment compared to the other experiments and there were also filamentous bacteria present which could have minimized the difference in settling velocity between the two reactors.

Table 5.2-5 Correlation between initial settling velocity and SV/SVI for diluted (SV≤300 ml) as well as for undiluted samples.

Experiment	Exponential function	R^2	Power-function	R^2
Part I:	$7.3951e^{-0.0045*SV}$ (SV≤300 ml)	0.65	$187.67*SV^{-0.7861}$ (SV≤300ml)	0.64
	$9.6904e^{-0.0057*SV}$	0.85	$1192.9*SV^{-1.1409}$	0.77
	$7.1289e^{-0.0054*SVI}$	0.48	$97.753*SVI^{-0.6875}$	0.45
Part II:	A: $8.5014e^{-0.0045*SV}$ (SV≤300 ml)	0.73	A: $104.93*SV^{-0.6543}$ (SV≤300ml)	0.77
	B: $9.5064e^{-0.0052*SV}$ (SV≤300 ml)	0.58	B: $95.292*SV^{-0.6242}$ (SV≤300ml)	0.51
	A: $12.769e^{-0.0081*SVI}$	0.62	A: $385.74*SVI^{-0.9253}$	0.56
	B: $10.321e^{-0.0061*SVI}$	0.39	B: $107.21*SVI^{-0.6473}$	0.32
Part III:	A: $10.236e^{-0.0068*SV}$ (SV≤300 ml)	0.54	A: $979.47*SV^{-1.1222}$ (SV≤300 ml)	0.54
	B: $15.368e^{-0.0077*SV}$ (SV≤300 ml)	0.82	B: $1211.2*SV^{-1.1157}$ (SV≤300ml)	0.86
	A: $10.507e^{-0.0058*SV}$	0.81	A: $67610*SV^{-1.9161}$	0.76
	B: $16.22e^{-0.0079*SV}$	0.55	B: $5728*SV^{-1.4338}$	0.53
	A: $15.001e^{-0.0088*SVI}$	0.69	A: $162004*SVI^{-2.1457}$	0.66
	B: $19.787e^{-0.011*SVI}$	0.55	B: $18005*SVI^{-1.7131}$	0.55
Part IV:	A: $10.505e^{-0.0055*SV}$ (SV≤300 ml)	0.83	A: $421.61*SV^{-0.917}$ (SV≤300 ml)	0.81
	B: $11.09e^{-0.0054*SV}$ (SV≤300 ml)	0.58	B: $953.74*SV^{-1.0589}$ (SV≤300 ml)	0.55
	A: $8.795e^{-0.004*SV}$	0.70	A: $1686*SV^{-1.2011}$	0.69
	B: $13.017e^{-0.0062*SV}$	0.82	B: $8604.5*SV^{-1.4818}$	0.73
	A: $10.509e^{-0.0078*SVI}$	0.72	A: $8144.8*SVI^{-1.6314}$	0.71
	B: $13.805e^{-0.0109*SVI}$	0.76	B: $6723*SVI^{-1.5847}$	0.69

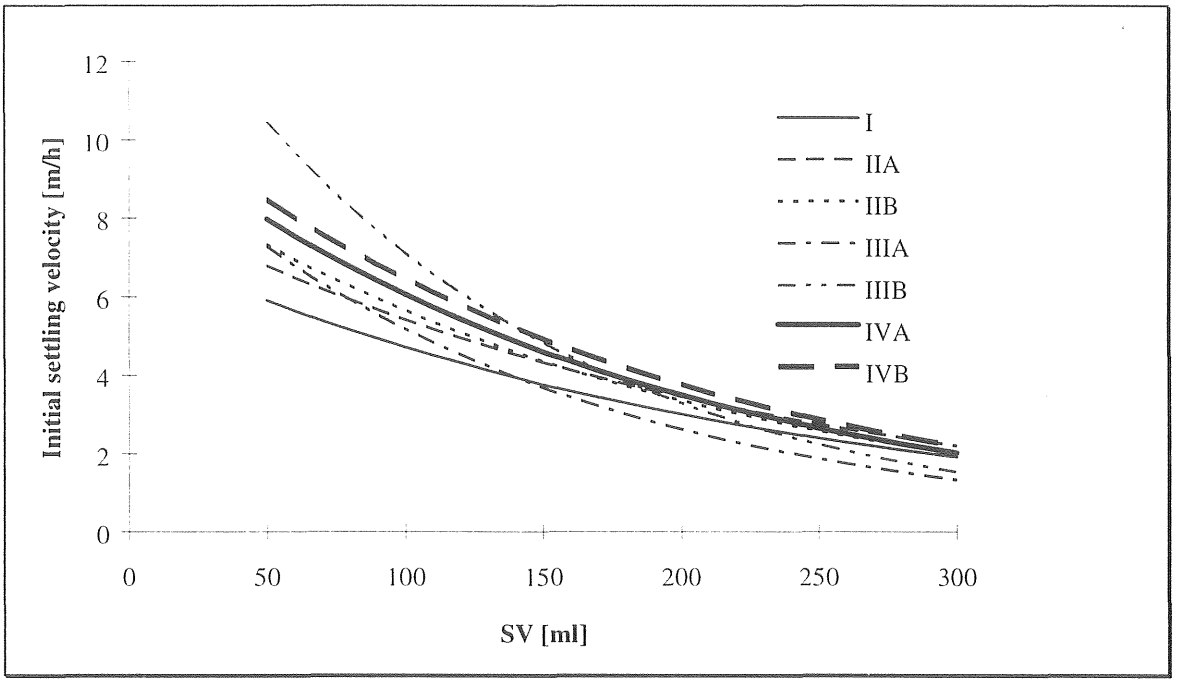


Figure 5.2-24 Relationship between SV and initial settling velocity (exponential functions).

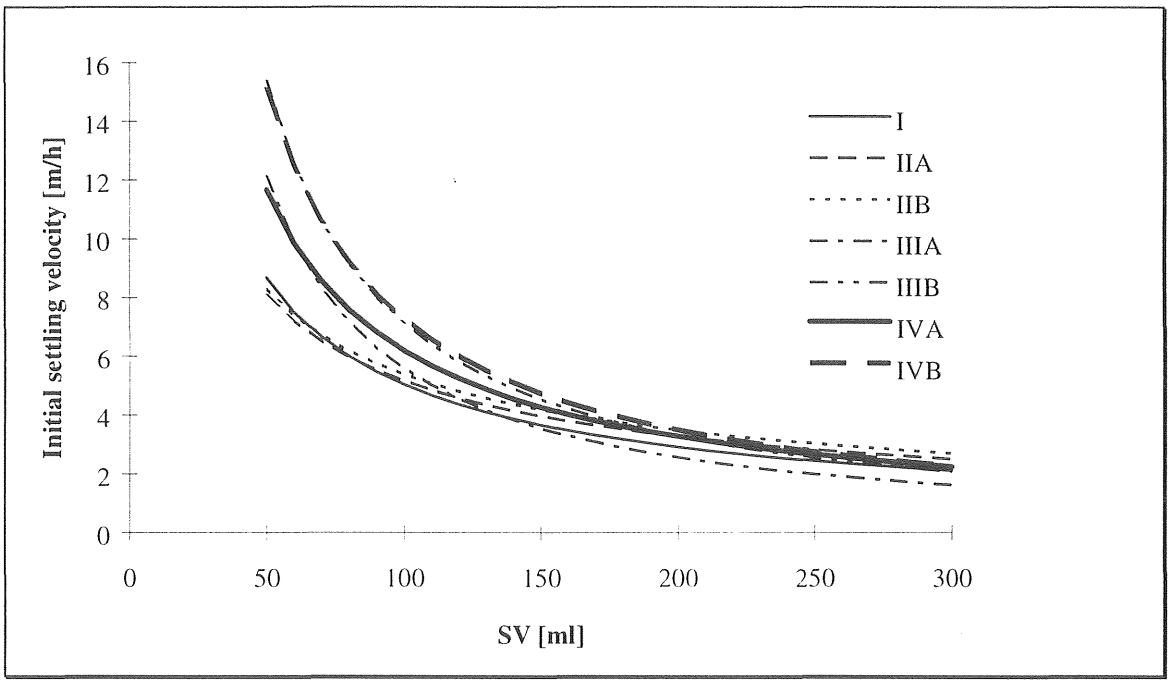


Figure 5.2-25 Relationship between SV and initial settling velocity (power functions).

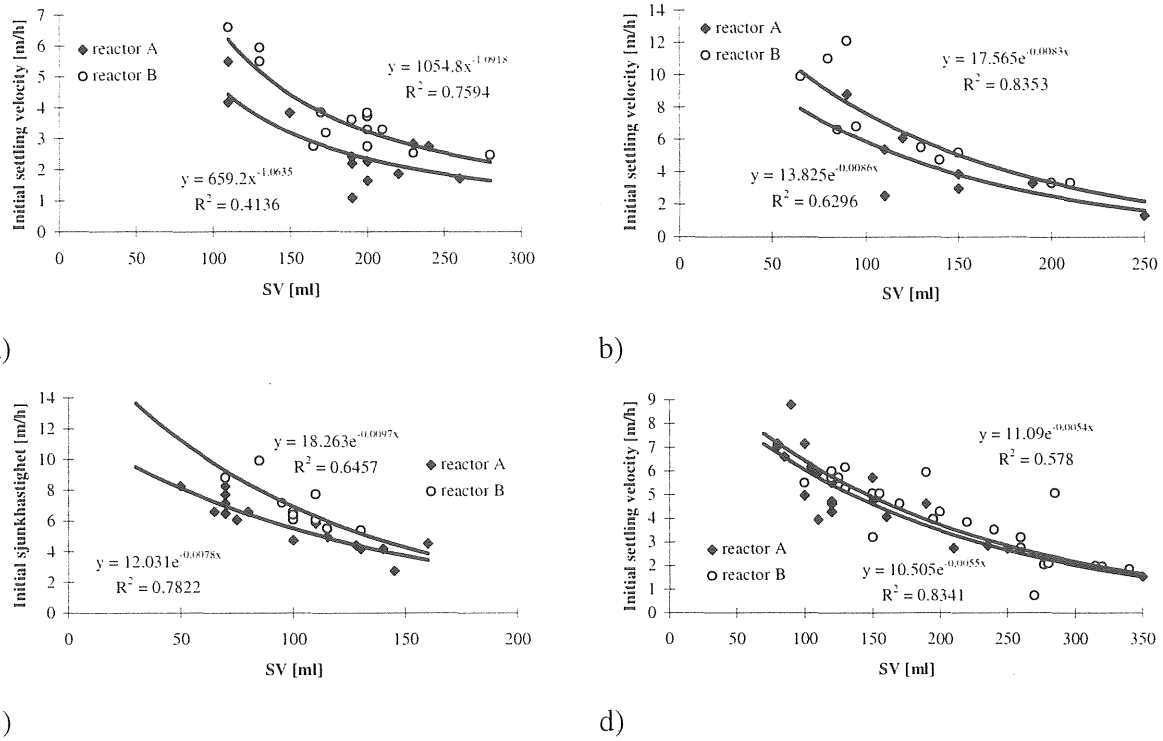


Figure 5.2-26 Relationship between SV and initial settling velocity at: a) a SRT of 2.5 d (Part III); b) a SRT of 5 d (Part III); c) an alternating period of 4 hours (Part II); d) a DO concentration of 5 mg/l (reactor B) and 2 mg/l (reactor A), respectively (Part IV).

The average floc diameter was plotted against the initial settling velocity. As expected, the correlation was poor due to filamentous bacteria which reduce the settling velocity even when the flocs are very large. Further, the settling velocities measured are zone settling velocities and not settling velocities for individual flocs. The correlation was not improved if measurements made when the SV exceeded 200 ml were removed (Figure 5.2-27).

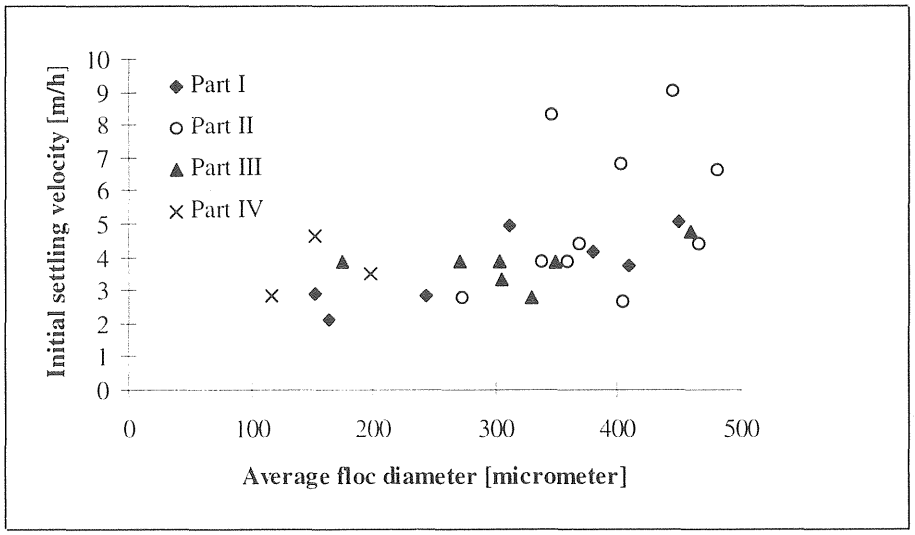
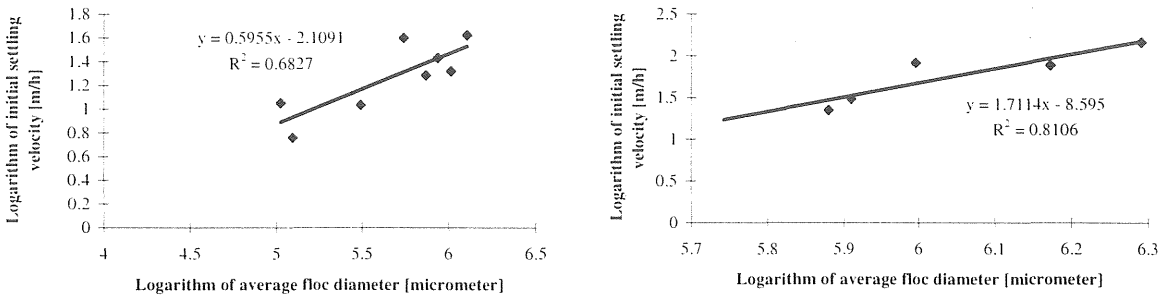


Figure 5.2-27 Correlation between average floc diameter and initial settling velocity (Part I-IV).

Only in Part I and Part II (reactor B) could a reasonable correlation be obtained (values for which SV exceeded 200 ml are removed). The best correlation obtained was: *Part I:* initial settling velocity = $a \cdot d^b$, where a = 0.1213 ; b = 0.5995; r^2 = 0.6827 and *Part II:* a = $1.8503 \cdot 10^{-4}$; b = 1.7114 ; r^2=0.81 (Figure 5.2-28). To be able to get good correlations between settling velocity and floc size, individual flocs have to be studied (Ganczarczyk, 1994). Not only the size affects the settling velocity, but also density, shape and permeability. Large flocs tends to be more irregularly shaped than smaller ones. Therefore, Stokes' law is not valid for activated sludge flocs. Instead it has been proposed that linear or 0.55 power relationships give better correlations (Li and Ganczarczyk, 1987; Patry and Takacs, 1992).



a) b)

Figure 5.2-28 Relationship between average floc diameter and initial settling velocity (logarithmic values) for Part I and Part II (reactor B) of the experiment.

5.2.5 Floc size and size distribution

Flocs in the activated sludge suspension

The activated sludge flocs were large in all experiments. That flocs tend to become larger in small pilot plants than in full scale plants probably has to do with another type of turbulence in such systems compared to the one in full scale plants. This is just a speculation and no estimations of the degree of turbulence were made. This phenomenon has, however, been mentioned in the literature (e.g. Knocke *et al*, 1986; Palm *et al*, 1980). Further, there were, during the major part of the experiment, filamentous bacteria present which seems to produce large flocs. The change in average floc diameter is described in more detail below.

Part I: The activated sludge flocs were large (about 300 µm) and irregularly shaped during the experiment, except for a period (27-28th of November) when a process failure led to floc dispersion due to DO depletion. The flocs became less compact when the DO concentration was decreased, but after a few days they became more compact again. No relationship between floc size and DO concentration could be found. One reason for this could be that Zoogloeal and filamentous microorganisms produce large flocs. It was also unfortunate that the process failure coincided with the decrease in DO concentration. It was interesting, however, that the floc structure and size could change so fast after a process disturbance (see section 5.2.6). The change in floc size is shown in Figure 5.2-29. The average floc diameters measured with the Malvern instrument coincided well with the ones measured with the microscope.

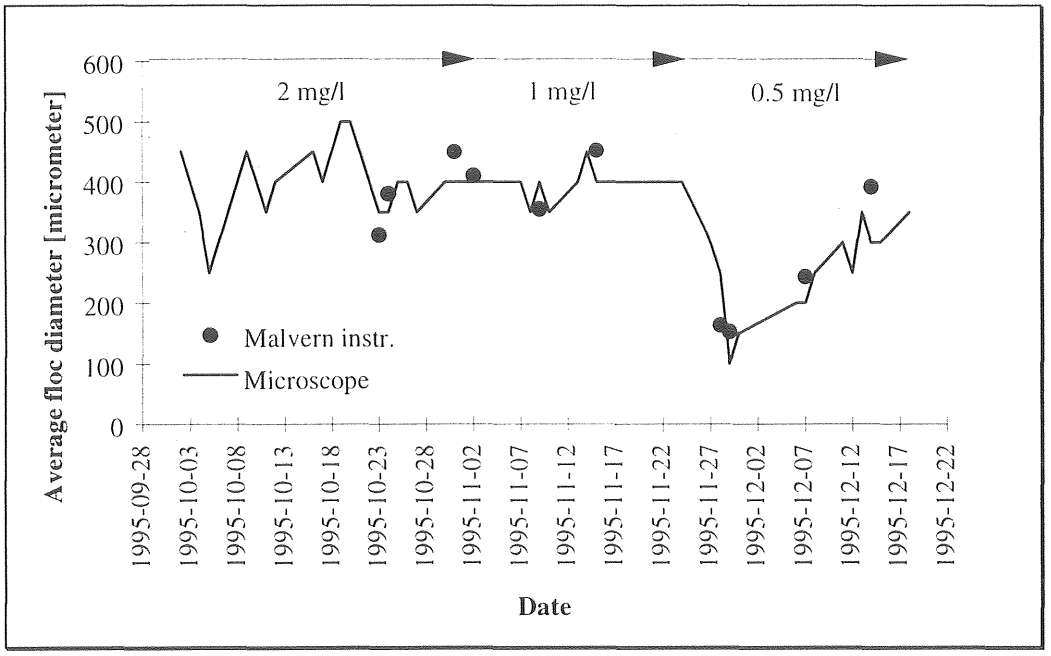


Figure 5.2-29 Average floc diameter, Part I (line = microscope; points = Malvern instrument).

Part II: The average floc size was large in both reactors: 300-500 µm. Alternating oxic and anoxic periods did not seem to affect the floc size to a large degree: they were generally slightly larger in reactor B (Figure 5.2-30), in which the DO concentration was kept constant (4 mg/l).

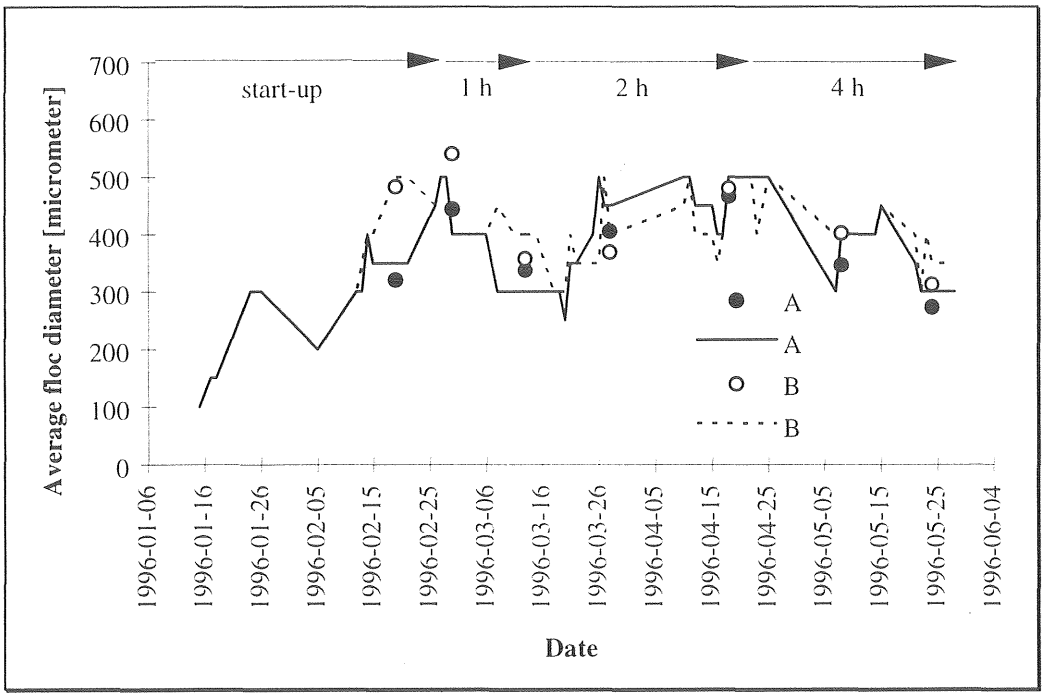


Figure 5.2-30 Average floc diameter, Part II (lines = microscope; points = Malvern instrument).

Part III: The change in floc size followed the same pattern for both reactors. A DO concentration of 2 mg/l produced somewhat larger (6-50%) flocs than a DO concentration of 0.5 mg/l (Figure 5.2-31). As the SRT decreased from 5 to 2.5 days, the average floc diameter decreased. A further reduction of the SRT to 1.25 days did not, however, cause even smaller flocs. The reactors were operated at a short sludge age for only three days which was probably too short to be able to draw any conclusions. As the sludge age decreases, the food-to-microorganism ratio (F/M) increases. This should, according to Barbusiñski and Koscielniak, 1995, produce larger flocs. It has also been found that short sludge ages produce smaller flocs (Bisogni and Lawrence, 1971; Pipes, 1979). These two phenomenon might counteract each other. In this study, the average F/M ratios were 0.94, 1.20 and 1.8 (g COD/g MLSS·d) for the SRT 5, 2.5 and 1.25 days, respectively. The difference in loading between a sludge age of 5 and 2.5 days was probably too small to be able to see large differences in average floc diameter. A shorter sludge age than 1.25 days may be necessary to produce floc dispersion.

Part III of the experiment was performed during the summer. This led to, for Swedish conditions, higher wastewater temperatures (18-20 °C) which could have minimized the effect of DO concentration at low SRT. The turbidities of the supernatant were also low throughout the experiment.

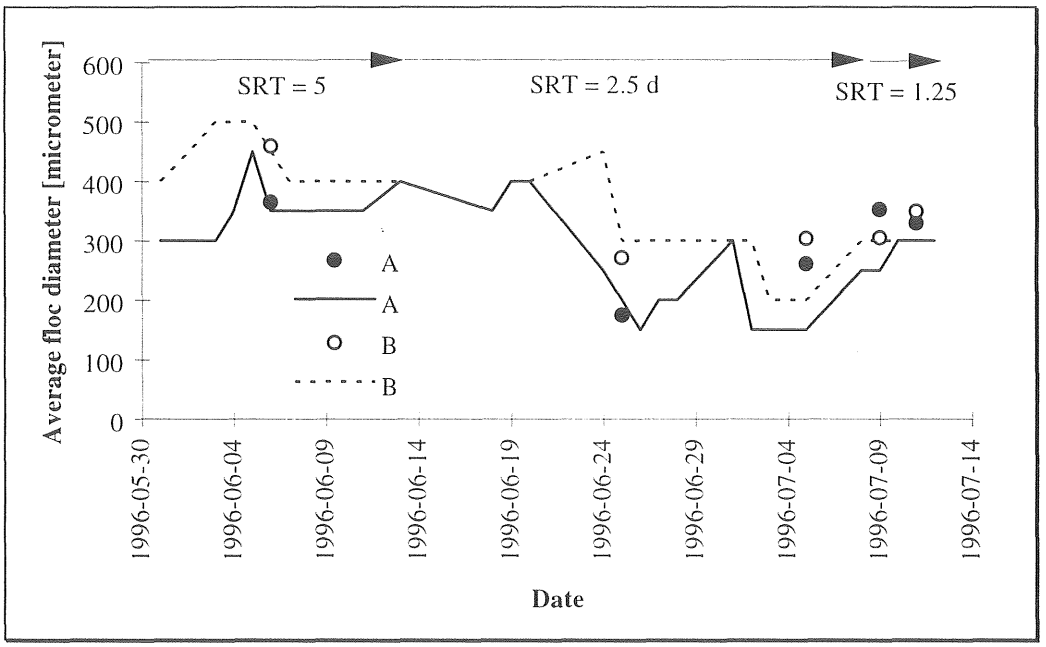


Figure 5.2-31 Average floc diameter, Part III (lines = microscope; points = Malvern instrument).

Part IV: The flocs were larger in reactor B, which was run at a DO concentration of 5 mg/l than in reactor A which was operated at a DO concentration of 2 mg/l (Figure 5.2-32). The measurements made with the particle analyser the 4th and 11th of October coincided well with the microscopic measurement, but on the 27th of October there was a large deviation. The explanation could be that the flocs were then very porous and might fell apart during handling. When the DO concentration in reactor A was increased to 5 mg/l, the average floc diameter increased to the same level as in reactor B.

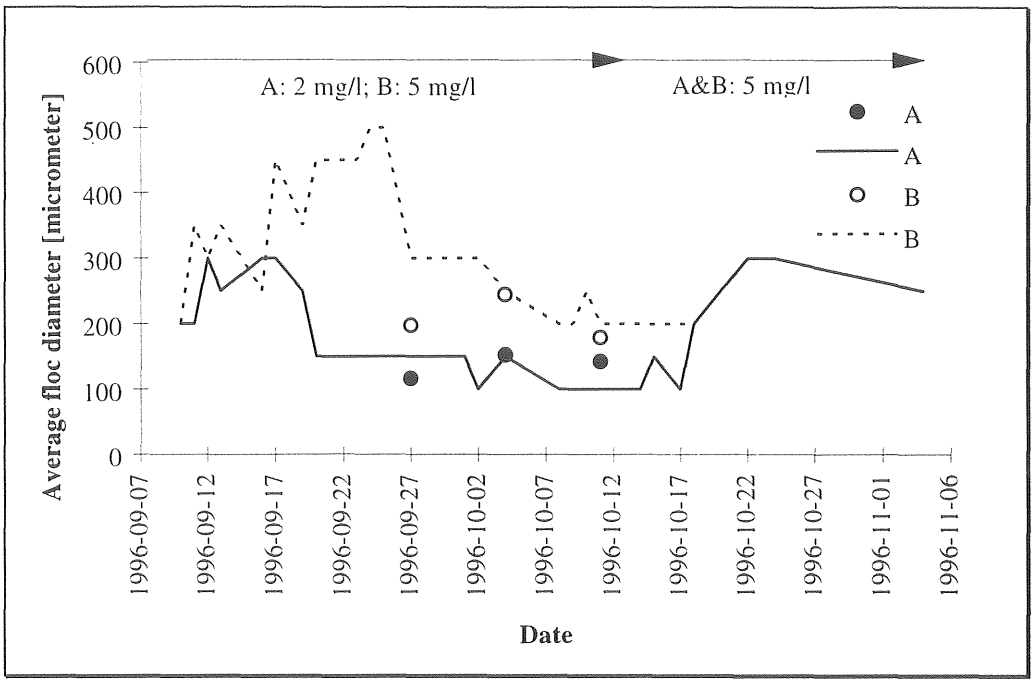
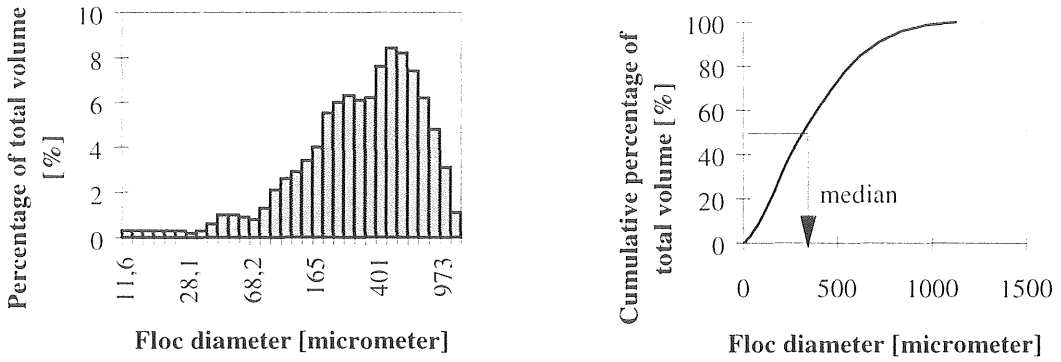


Figure 5.2-32 Average floc diameter, Part IV (lines = microscope; points = Malvern instrument).

No correlation between F/M-ratio and average floc diameter could be found in any of the experiments. This was not expected since the variation in F/M ratio was quite small during the experiment. To be able to study the effect of organic loading, several reactors have to be run in parallel at different sludge ages.

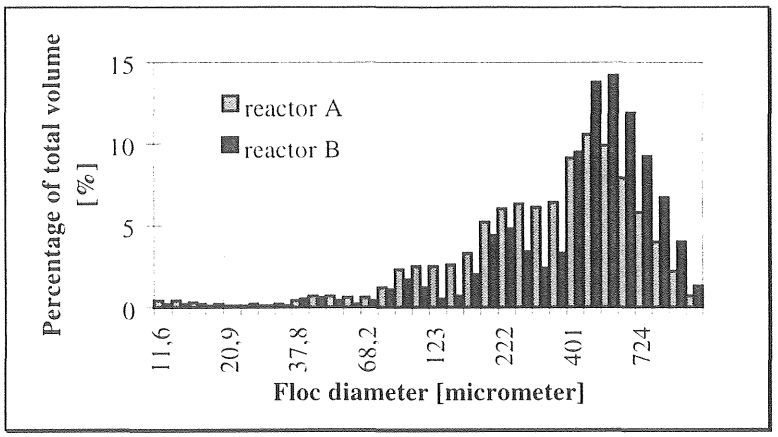
Floc size distribution: The shape of the size distributions (based on floc volume) for flocs larger than about 10 μm had a similar form in all measurements except that they were shifted more or less to the right (ie shifted towards larger flocs). A typical example of a size distribution of activated sludge flocs is depicted in Figure 5.2-33. The size distributions can be illustrated as a *frequency by volume* or as a *cumulative frequency by volume*. The Malvern instrument can measure flocs within a certain size range depending on the lens used. In this experiment, a 600 mm lens was used which can measure flocs within the size range 11.6-1128 μm. Based on microscopic investigations of the flocs, this range was found to be the most suitable. Occasionally, very large flocs were present (> 1000 μm) which, thus, could not be measured correctly. Each sample was measured three times and the deviation between the measurements was very small (less than 5%). The Malvern instrument can measure particles down to 1.2 μm by using the 63 mm lens (can measure the interval 1.2-118 μm). The problem was that the small particles would be incorrectly measured if the larger ones were present, and if the particles in the supernatant were measured, the concentration of particles was below what was necessary for a correct measurement.



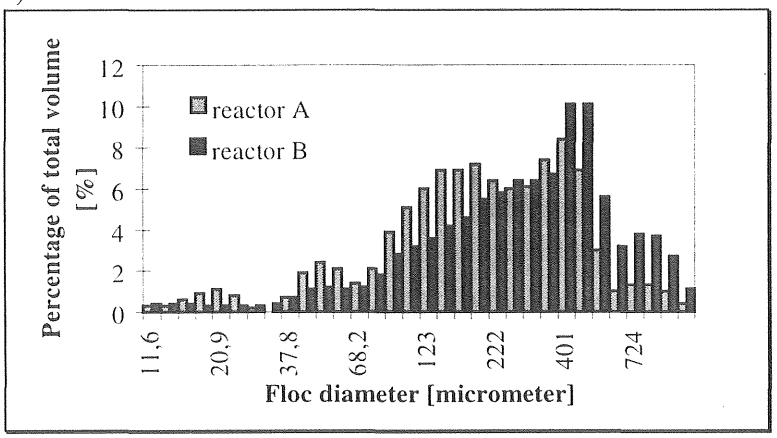
a) b)

Figure 5.2-33 Size distribution of activated sludge flocs: a) frequency by volume; b) cumulative frequency by volume.

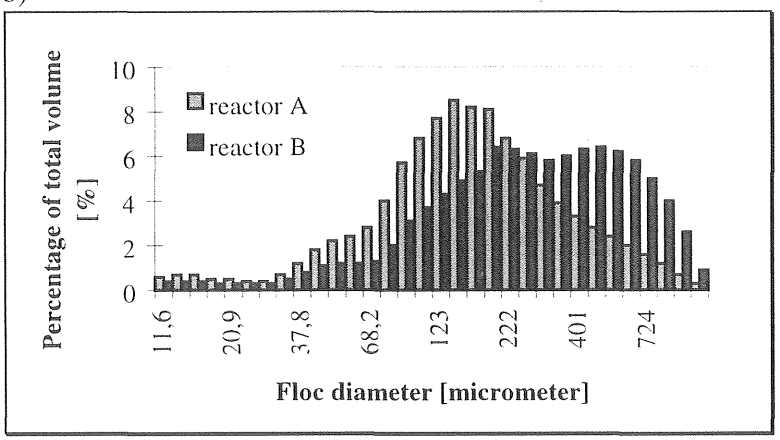
Table 5.2-6 summarizes the percentage of floc volume in the different size intervals. In Part I and II of the experiment, no large difference in distribution into different size intervals could be found between the different test periods. In Part III of the experiment, a slight shift towards larger flocs could be found at a DO concentration of 2 mg/l (reactor B) compared to at a DO concentration of 0.5 mg/l (reactor A). In Part IV, the flocs were, on the average, smaller than in the previous experiments and a DO concentration of 5 mg/l (reactor B) produced larger flocs (30-70%) than a DO concentration of 2 mg/l (reactor A). Examples of size distributions from Part III and IV are illustrated in Figure.5.2-34. Activated sludge flocs with an average floc diameter >50 μm contributed to most of the volume.



a)



b)



c)

Figure 5.2-34 Floc size distributions: a) Part III, SRT = 5 days; b) Part III, SRT = 2.5 days; c) Part IV

Table 5.2-6 Summary of distribution by volume of flocs into six size intervals (Part I-IV).

Size interval [µm]	Percentage of total volume [%]				Average
	I	II	III	IV	I-IV
11.6-50.8	3-11	1-5	3-9	8-11	5
50.8-123	2-29	6-13	6-23	21-33	13
123-222	12-57	10-24	12-33	27-39	23
222-346	16-42	12-28	14-26	21-23	22
346-539	47-75	25-40	21-41	12-23	34
>539	25-55	20-59	5-47	7-19	34

To be able to compare the results from different measurements, it was checked whether the distributions could be fitted to any distribution functions. The size distribution of activated sludge flocs (based on volume) larger than about 10 µm could best be fitted to log-normal distribution functions:

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

where
x = floc size
µ = average of ln x
σ = standard deviation of ln x

The standard deviation (σ) and the average of the natural logarithm of x (µ) were calculated according to:

$$\mu = \frac{\sum \ln x \cdot d\Phi}{\sum d\Phi} \quad (2)$$

where
dϕ = the percentage of flocs of size x

$$\sigma = \sqrt{\frac{\sum (\ln x - \mu)^2 \cdot d\Phi}{\sum d\Phi}} \quad (3)$$

Further, the frequency function f(x) is equal to dϕ/d(ln x), i.e. the percentage of flocs within a certain size interval. To get an estimation of how well the data can be fitted to a log-normal function, the measured frequency function (f(x) = dϕ/d(ln x)) is plotted against the calculated frequency function (log-normal function). A linear regression then gives the r^2-value for the fit of the data to a log-normal function (Figure 5.2-35).

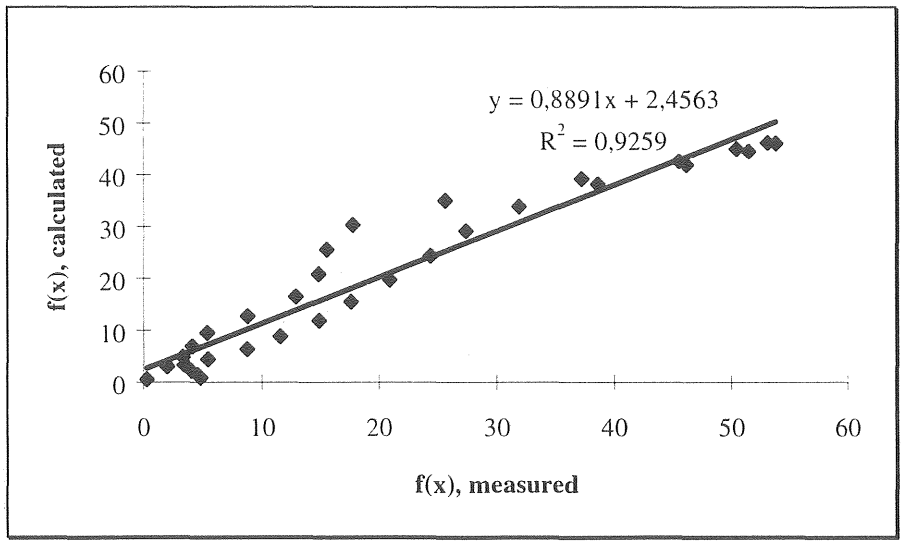


Figure 5.2-35 Linear regression for the fit of the data to a log-normal function.

The parameters in the log-normal distribution functions obtained are summarized in Table 5.2-7. An example of a log-normal fit to a size distribution is illustrated in Figure 5.2-36. Li and Ganczarczyk (1991) and Barbusinski and Koscielniak (1995) showed that the frequency by *number* of flocs > 10 μm fitted well to log-normal distribution functions while Jorand *et al* (1995) found that the *frequency as percentage of total particle volume* fitted well to log-normal distribution functions. The difference in results may depend on the different methods used to measure the activated sludge flocs. Microscopic methods probably underestimate the number of smaller flocs.

Table 5.2-7 Summary of the parameters in the log-normal distribution function.

Parameter	Range	Average	Average regression coefficient (R^2)
Average (μ)	4.813-6.117	5.635	0.783 (0.643-0.938)
Standard deviation (σ)	0.672-1.126	0.828	

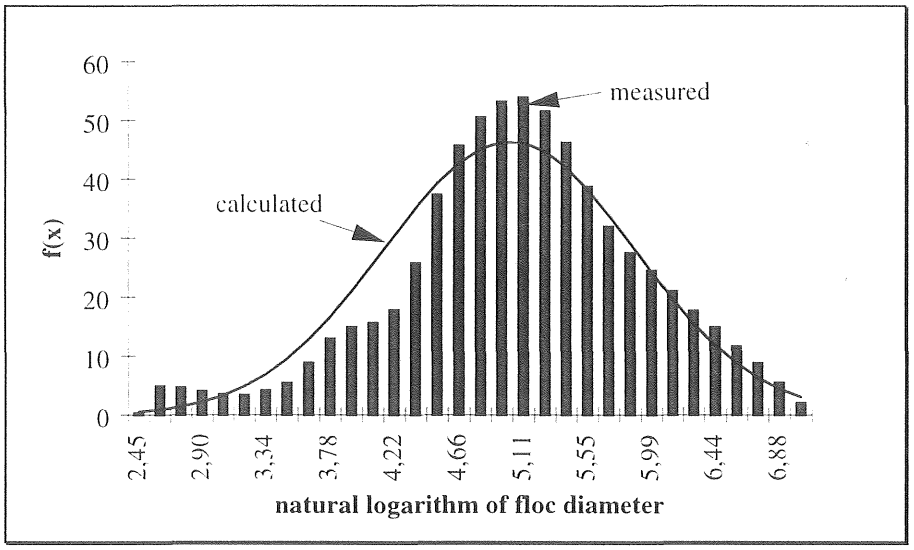
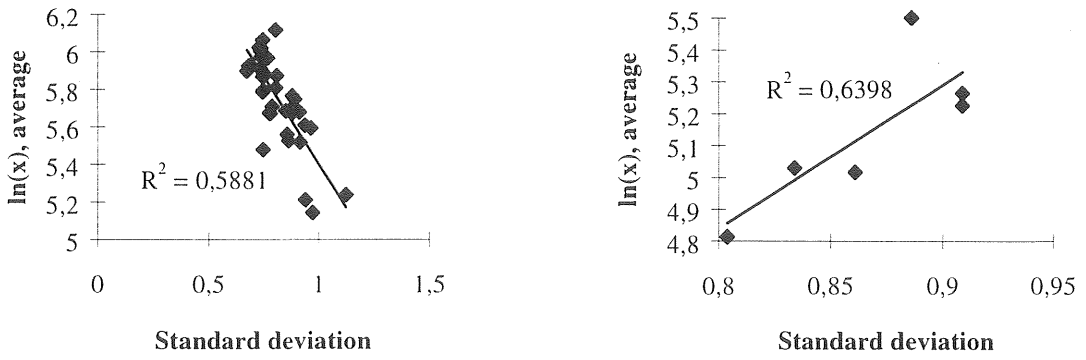


Figure 5.2-36 A size distribution fitted to a log-normal distribution function (r^2=0.9).

In Part I-III of the experiment, there was a trend towards larger standard deviations at smaller average values of ln(x), while it was the other way round for Part IV of the experiment (Figure 5.2-37). The average floc size was generally smaller in Part IV compared to in Part III.

No correlation between the parameters μ and σ and the process parameters temperature, DO, F/M-ratio, pH and MLSS concentration could be found.



a) b)

Figure 5.2-37 Average of ln(x) versus the standard deviation: a) Part I-III; b) Part IV.

The distribution by *surface area* , *volume* and *number* are correlated to each other according to (Allen, 1981):

$$dA_i = \pi x_i^{\ 2} dP_i \qquad (4)$$

$$dV_i = \frac{\pi}{6} x_i^{\ 3} dP_i \qquad (5)$$

where x_i is the average size of the flocs in interval i and $dP_i = dN_i/N$ is the frequency of occurrence (N_i is the number of flocs in interval i and N is the total number of flocs in the

sample). To illustrate this volume distribution data obtained with the Malvern instrument has been converted into area and number distributions. In Figure 5.2-38, it can be seen that the larger flocs contribute to much more volume than the smaller ones even though they are much fewer. Therefore, the larger flocs are more important individually with respect to volume and surface area. It can also be noticed that the smallest flocs contribute to a relatively large proportion of the surface area. In this approximation it is assumed that the flocs are spherical. This is of course a simplification. The surface area of activated sludge flocs is probably much larger due to their irregular shape. Andreadakis (1993) suggested that the surface area is up to two orders of magnitude higher than the geometric surface area. The volume distribution can also be related to the mass distribution. As a first approximation the volume of the particles is proportional to the mass. To be able to calculate the mass of flocs, the density of the activated sludge flocs has to be known. The density is, however, probably not the same for all flocs. Large flocs seem to be less compact than smaller ones and they contain a lesser amount of bacteria. Andreadakis (1993) measured the floc density by means of interference microscopy and suggested that for flocs in the range 10-70 μm, the density was 1.015-1.034 g cm^{-3}. He also found that there was a strong correlation between floc density and size and suggested that the floc density could be expressed as: $\rho_f = 1 + 0.3d^{-0.82}$, where d is the floc diameter. This means that the smallest flocs can contribute to a significant proportion of the biomass, and therefore it is important to remove the small flocs as well.

As a comparison, a typical distribution has been divided into different size intervals and the percentage of volume, area and number has been calculated. The results are summarized in Table 5.2-8. It is important to note that in these calculations only flocs larger than about 10 μm are included. If the whole size interval is included the number of particles ≤ 2 μm will constitute more than 80% of the total number (this is discussed later in this chapter). In this particular case, flocs larger than 50 μm contribute to more than 90% of the volume. This can be compared with data from the literature: Knocke and Zentkovich (1986), Barber and Veenstra (1986) and Sadalgekar *et al* (1988) showed that about 90% of the flocs were smaller than 75 μm.

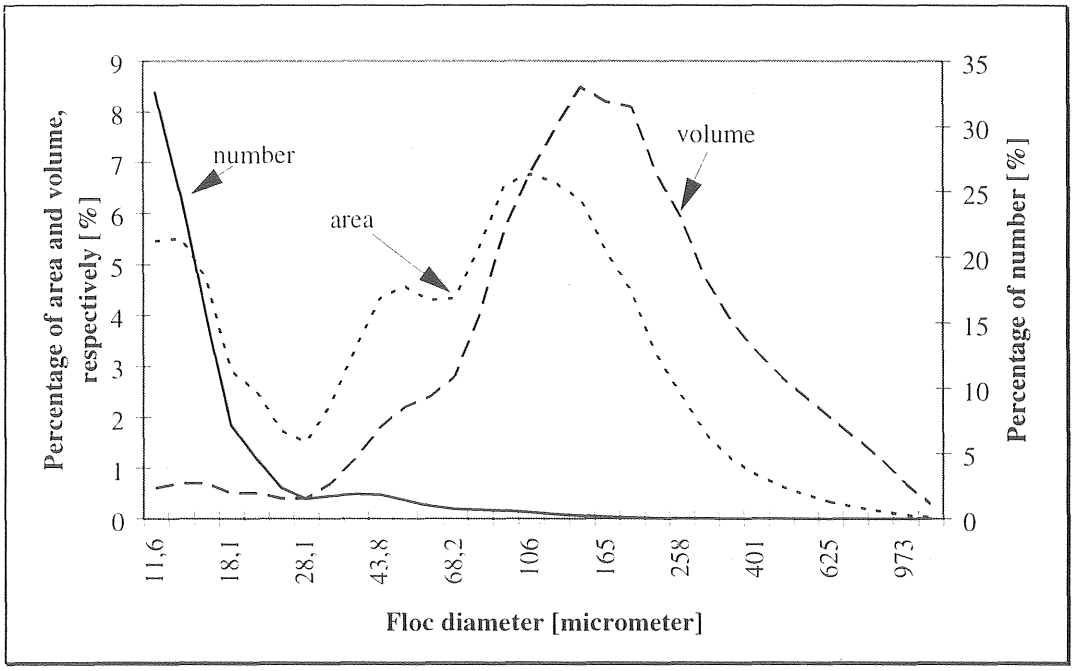


Figure 5.2-38 Example of a distribution by number, volume and area.

Table 5.2-8 Comparison between distribution by number, volume and area.

	Distribution [%] by:		
Floc diameter [µm]	Number	Area	Volume
11.6-50.8	> 90	39	9
50.8-123	5	39	32
123-222	< 1	26	39
222-346	negligible	9	21
346-539	negligible	3	12
≥539	negligible	1	8

Flocs in the supernatant

In Part III and IV of the experiment, samples of the supernatant after 20 minutes settling were analysed several times per week with the particle analyser (Met One). Depending on the number of particles present in the supernatant, the samples had to be diluted 20-100 times with distilled water. This changes the ionic strength of the liquid which might cause a change in the size distribution (Zita and Hermansson, 1994). Since the particle analyser was very sensitive to the number of particles present in the sample, it was inconvenient to dilute the sample in filtrated wastewater (difficult to get particle free water). Another possible risk is that the sample is pumped through a tubing before the particles pass the sensor (the pump is situated after the sensor). This may cause breakage of larger particles. It was also noticed that if the number of particles per ml was high (12,000-16,000), the small particles could not be measured correctly. A dilution of two times did not give a number of particles which was 50% of the original one. It was, however, not desirable to dilute the sample more than 100 times since this increases the error in particle number per volume as well. A test was made were the samples were diluted in electrolytes with different ionic strengths (addition of KCl to distilled water): 0, 2, 20 and 200 mM. No difference in shape of the distributions and total number of counts could be observed.

There were flocs present in the supernatant (after 20 minutes settling) up to a diameter of about 50-60 μm. In most of the measurements, more than 80% of the flocs were smaller than 2 μm. The distribution *by number* could be converted to distribution *by volume* and *area* (assuming spherical flocs). The distribution by number highlights the smaller flocs and the distribution by volume highlights the larger flocs. In Figure 5.2-39 a typical example of a size distribution by volume is illustrated. A comparison of distribution by number, area and volume can be found in Figure 5.2-40. In Table 5.2-9 the distributions are divided into size intervals corresponding to dispersed bacteria, floc fragments, small flocs and medium size flocs. It can be seen that when considering the floc volume, the larger flocs are much more important individually than the smaller ones. The flocs ≤ 2 μm contribute to a relatively large proportion of the surface area which is important when considering the adsorption of compounds onto the flocs.

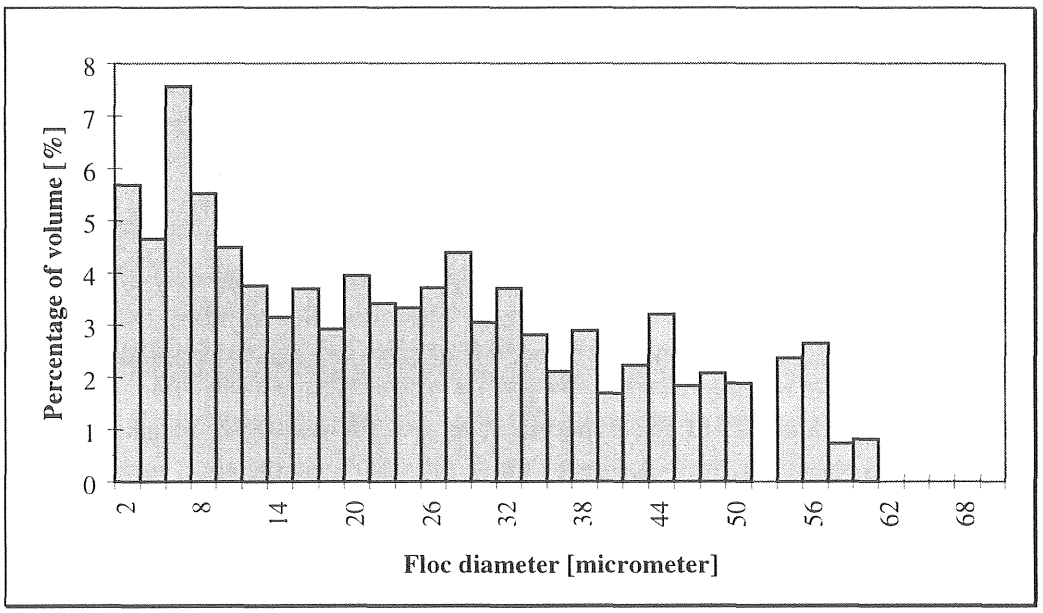


Figure 5.2-39 Example of a a size distribution by volume in the supernatant (20 minutes settling).

Table 5.2-9 Summary of the distribution of particles in the supernatant into different size intervals (20 minutes settling).

	Distribution [%] by:		
Size interval [μm]	Number	Area	Volume
≤ 2	84	31	6
>2-16	15	48	33
16-50	< 1	21	53
50-100	negligible	< 2	8

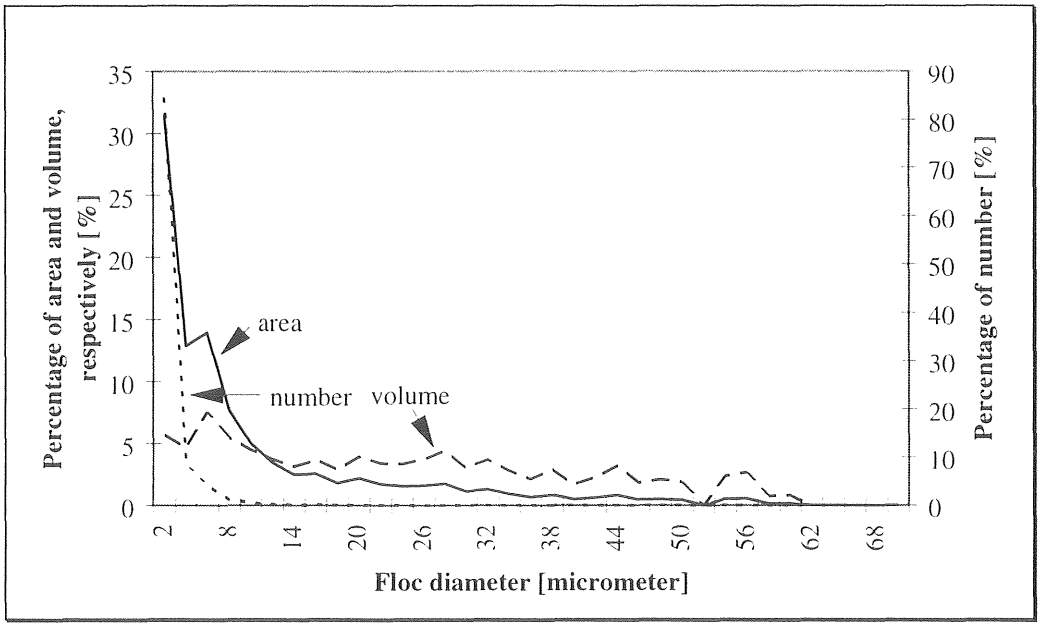


Figure 5.2-40 Example of distribution by number, volume and area.

Part III-IV: In Part III and IV of the experiment, the particles in the supernatant were analysed several times per week. At the same time as the sample was analysed with the particle analyser, the turbidity was measured. The particle number, volume and area (calculated according to equation 4 and 5) per ml of sample, was correlated to the turbidity. The correlation between number of particles per ml and turbidity was better in Part IV than in Part III (Figure 5.2-41and 42) of the experiment (r^2 = 0.81 and 0.52, respectively). Particles smaller or equal to about 2 µm, make up to more than 80% of the total number of particles. Small particles within the size range of the wave length of light (< 1 µm), probably contribute to more turbidity than larger ones. If the number of particles ≤ 2 µm was plotted against turbidity regression coefficients of 0.78 and 0.53 were obtained for Part IV and III, respectively. Particles > 2 µm gave a poorer correlation (r^2 = 0.74 in Part IV and no correlation in Part III). The correlation between turbidity and particle area was better (Part III: r^2 = 0.60; Part IV: r^2 = 0.79) than the correlation to particle volume (Part III: r^2 = 0.45; Part IV: r^2 = 0.62).

As mentioned earlier, small particles (< 1 µm), which cannot be measured with the particle counter, contribute to more turbidity than larger ones and therefore a better linear correlation between turbidity and particle number cannot be expected. The more the sample is diluted the more difficult is it to make a representative measurement and therefore the errors can be quite large when measuring the particles.

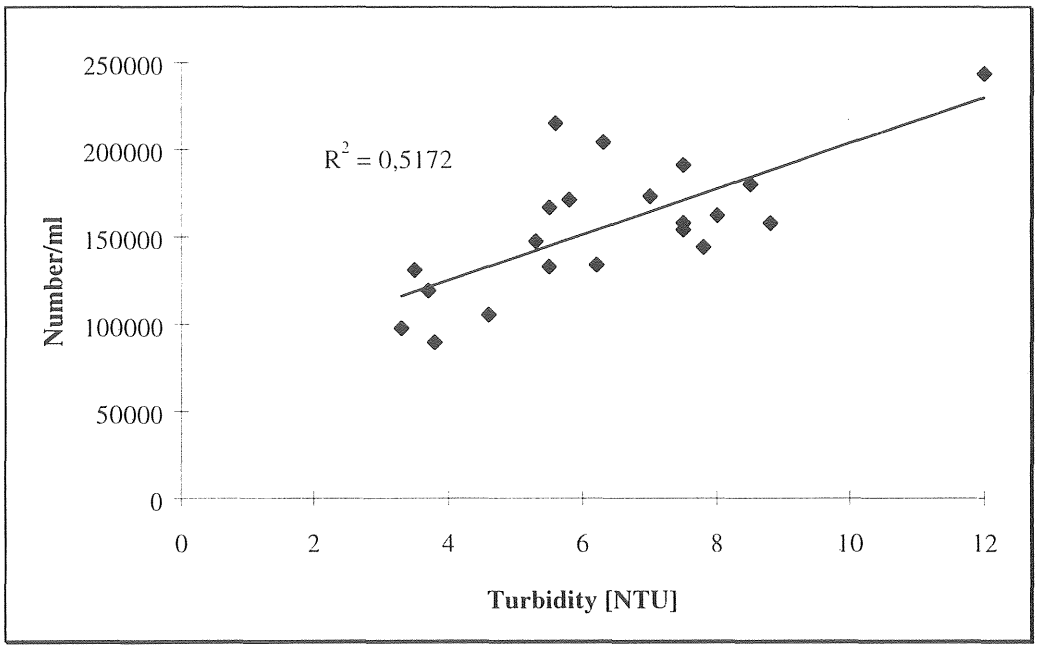


Figure 5.2-41 Correlation between number of particles per ml and turbidity (Part III).

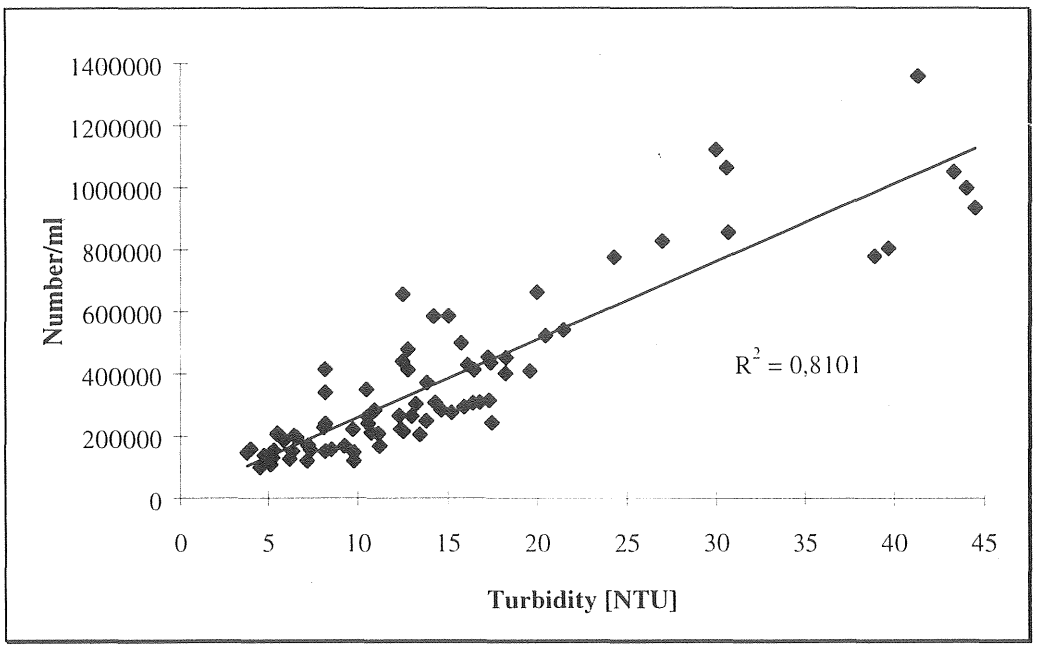
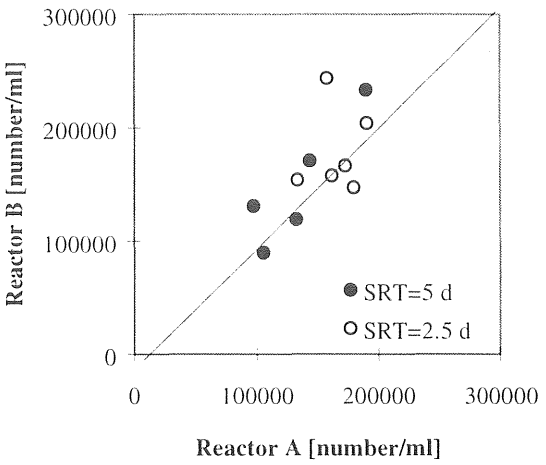


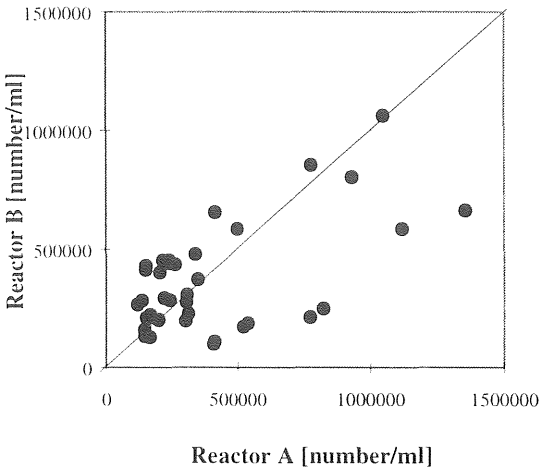
Figure 5.2-42 Correlation between number of particles per ml and turbidity (Part IV).

The total numbers of counts (20 minutes settling) for reactor A and reactor B were compared (Figure 5.2-43 a-b). In Part III of the experiment, there was no large difference in total counts between reactor A and B. This is in accordance with the fact that there was no large difference in turbidity between the two reactors. There were more particles present in the supernatant at a SRT of 2.5 days compared to at 5 days. On the other hand, in Part IV, there was a large difference in total number of particles between reactor A and B. The difference was larger at higher turbidities. To highlight the difference in number of larger particles, the total volume of particles for reactor A and B were compared (Figure 5.2-43 c-d). There were relatively more larger particles at a DO concentration of 0.5 mg/l than at a DO concentration of 2 mg/l in Part

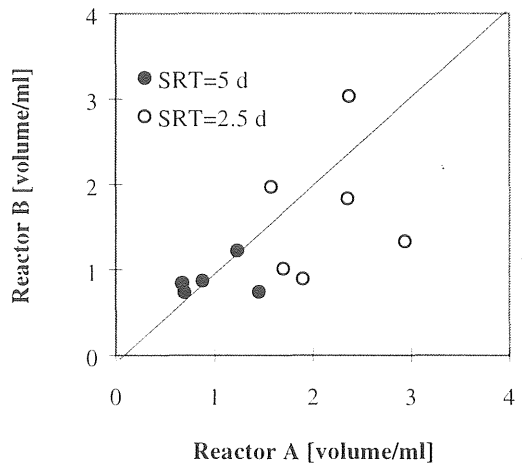
III of the experiment. The difference was larger at a shorter SRT. In Part IV, a greater number of larger flocs were present at a DO concentration of 2 mg/l than at a DO concentration of 5 mg/l. In Part III, there were more particles present at a SRT of 2.5 d than at 5 d and the total number of counts increased with the F/M ratio at a SRT of 2.5 d (Figure 5.2-43 e-f).



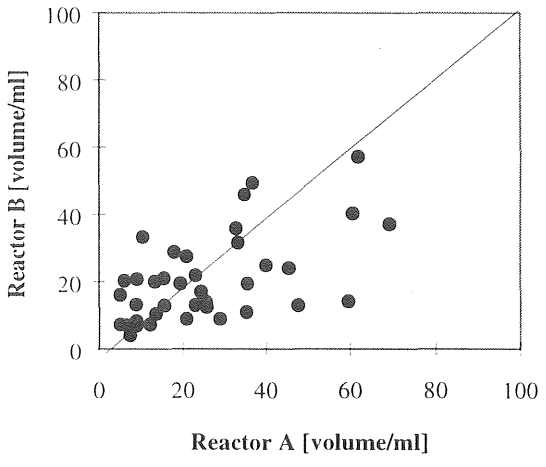
a) Total number of particles/ml: Part III



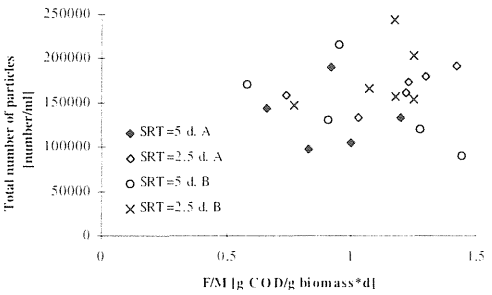
b) Total number of particles/ml: Part IV



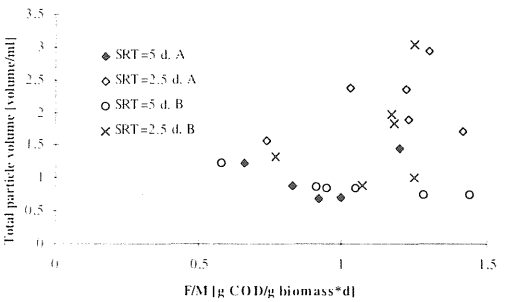
c) Total particle volume/ml [10^7 μm^3]: Part III



d) Total particle volume/ml [10^{10} μm^3]: Part IV



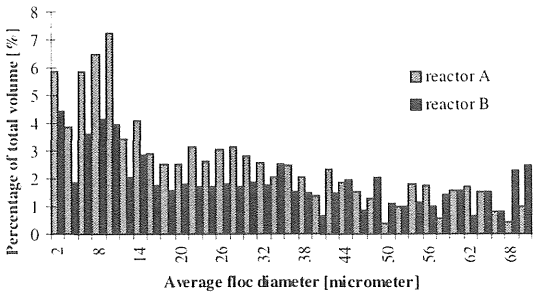
e) Total number of particles vs F/M ratio.

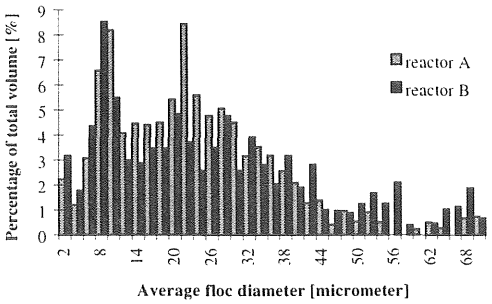


f) Total particle volume vs F/M ratio.

Figure 5.2-43 Comparison between reactor A and B.

The shape of the distributions by volume were largely similar for reactor A and B. At higher turbidities, the size distribution was shifted towards smaller particles. Two examples of size distributions by volume are illustrated in Figure.5.2-44





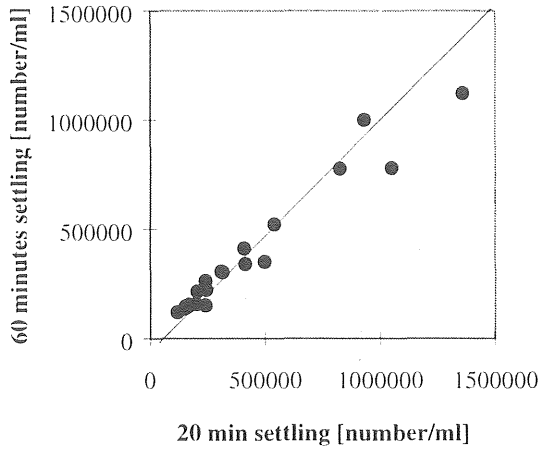
a) experimental date: 960916
A: 17 NTU, 20×15000 counts/ml
B: 8 NTU, 20×11300 counts/ml

b) experimental date: 961001
A: 11 NTU, 20×8403 counts/ml
B: 6 NTU, 20×6339 counts/ml

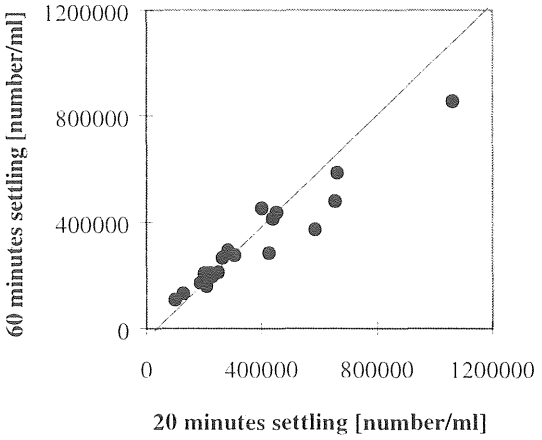
Figure 5.2-44 Distribution by volume after 20 minutes settling (Part IV).

The supernatant was analysed with the particle analyser after 20 and 60 minutes settling in Part IV. In Figure 5.2-45, it can be seen that the difference in total number of flocs is not very large between 20 and 60 minutes settling. However, if only the particles > 2 μm are considered, the difference is larger. This is logical since small particles cannot settle gravimetrically. This can be compared with the differences in turbidity after 20 and 60 minutes settling (Figure 5.2-46). Turbidity and total number of counts were on an average 10-15% lower after 60 than after 20 minutes settling. At times it was observed that the turbidity was higher after 60 minutes settling than after 20 minutes settling. This could be due floc dispersion or desorption of adsorbed material from the flocs at anaerobic conditions.

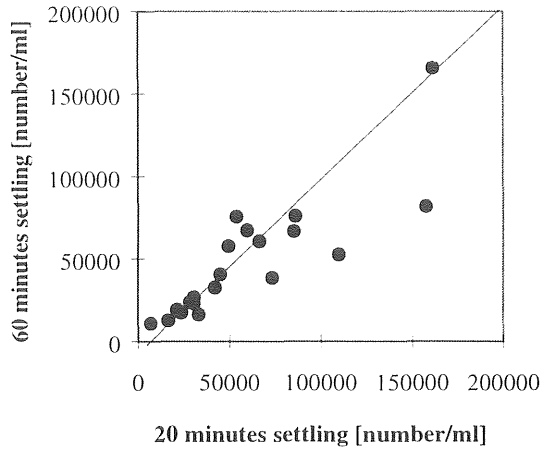
Turbidity as well as total counts of particles were on an average 10-15% lower after 60 minutes than after 20 minutes settling.



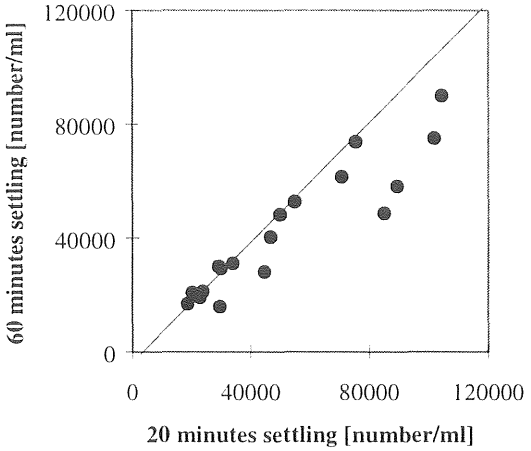
a) reactor A



b) reactor B

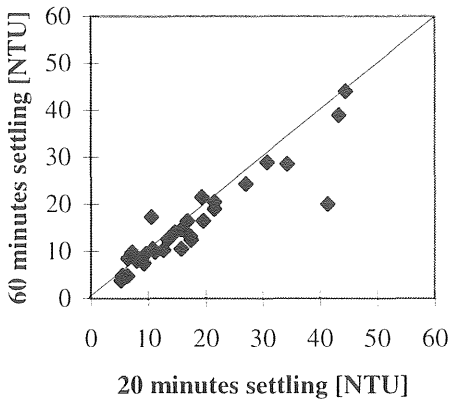


c) reactor A (particles > 2 μm)

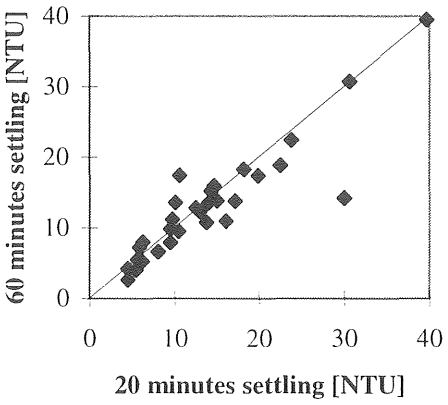


d) reactor B (particles > 2 μm)

Figure 5.2-45 Comparison between number of particles present after 20 and 60 minutes settling (Part IV): a-b) total number of flocs; c-d) number of flocs > 2 μm).



a)



b)

Figure 5.2-46 Comparison between turbidity after 20 and 60 minutes settling (Part IV): a) reactor A; b) reactor B.

Part II: In Part II of the experiment, the supernatant was analysed with the Met One particle analyser to find out which particles were present in the both reactors as well as in the pre-settled wastewater during an 8 hour cycle, see Figure 5.2-13 (4 hours at oxic conditions and 4 hours at anoxic conditions; date of experiment: 960515). The number of particles within the different size intervals by the end of the first oxic period, the anoxic period and the second oxic period are illustrated in Figure 5.2-47. The samples taken from reactor A and B were diluted 20 times and the samples of the pre-settled wastewater were diluted 100 times prior to analysis. This has not been compensated for in the figures below (i.e. add a factor ln20 and ln100 to get the total number of particles in Figure 5.2-47 and 48, respectively). In reactor B, which was operated at a constant DO concentration of 4 mg/l there was no large difference in types of particles present in the supernatant during the 8 hours of experiment. On the other hand, in reactor A, there was a relatively larger number of particles between 2 and 20 μm present by the end of the anoxic period than by the end of the oxic periods. These particles are probably particles in the wastewater which have not been adsorbed onto the activated sludge flocs. There were also more larger particles in the supernatant of reactor A than in the supernatant of reactor B. These particles may be small (pin-point) activated sludge flocs. The particles present in the pre-settled wastewater had a diameter up to about 100 μm. This is illustrated in Figure 5.2-48. These results are comparable with Boller (1993) who fractionated pre-settled wastewater and found that the organic content could be 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).

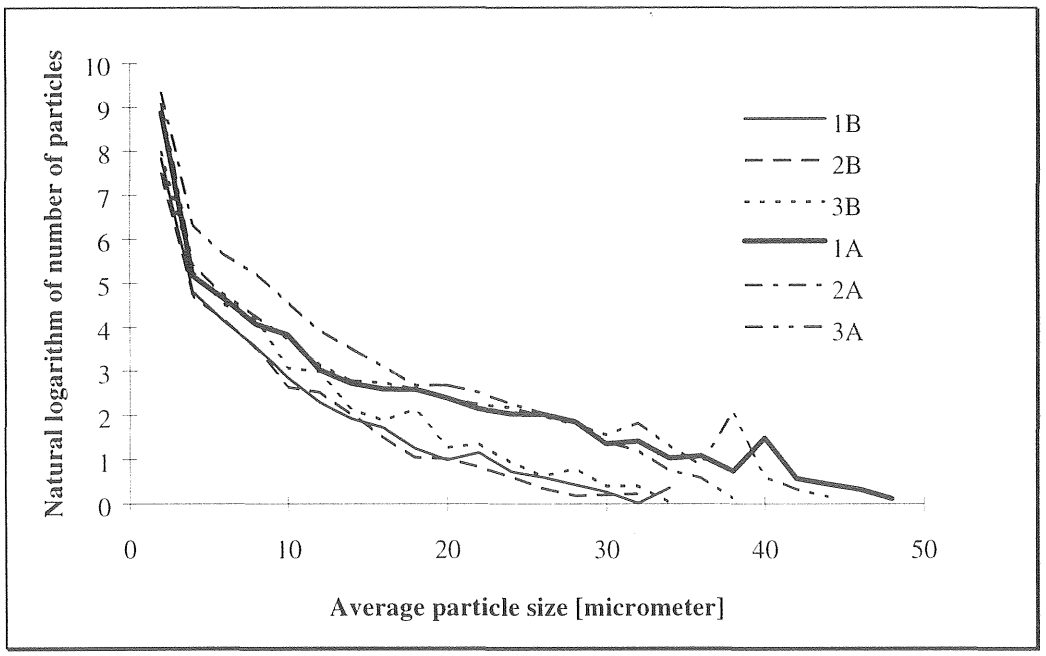


Figure 5.2-47 The natural logarithm of the number of particles within the different size intervals 1) end 1st oxic period; 2) end anoxic period; 3) end 2nd oxic period.

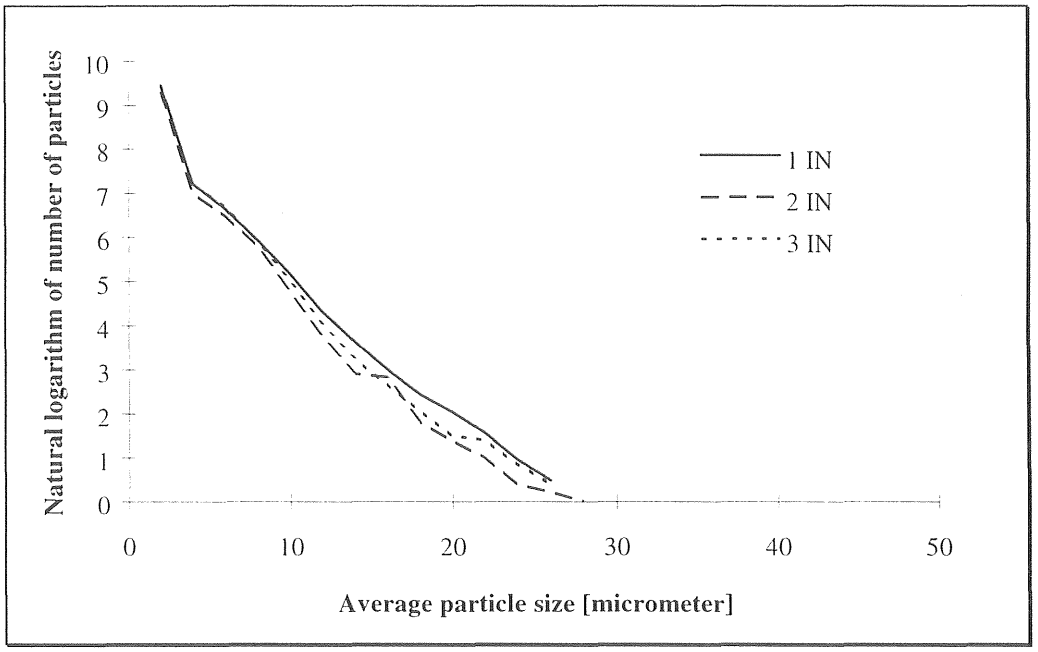


Figure 5.2-48 The natural logarithm of the number of particles in the pre-settled wastewater by the time of 1) end 1st oxic period; 2) end anoxic period; 3) end 2nd oxic period.

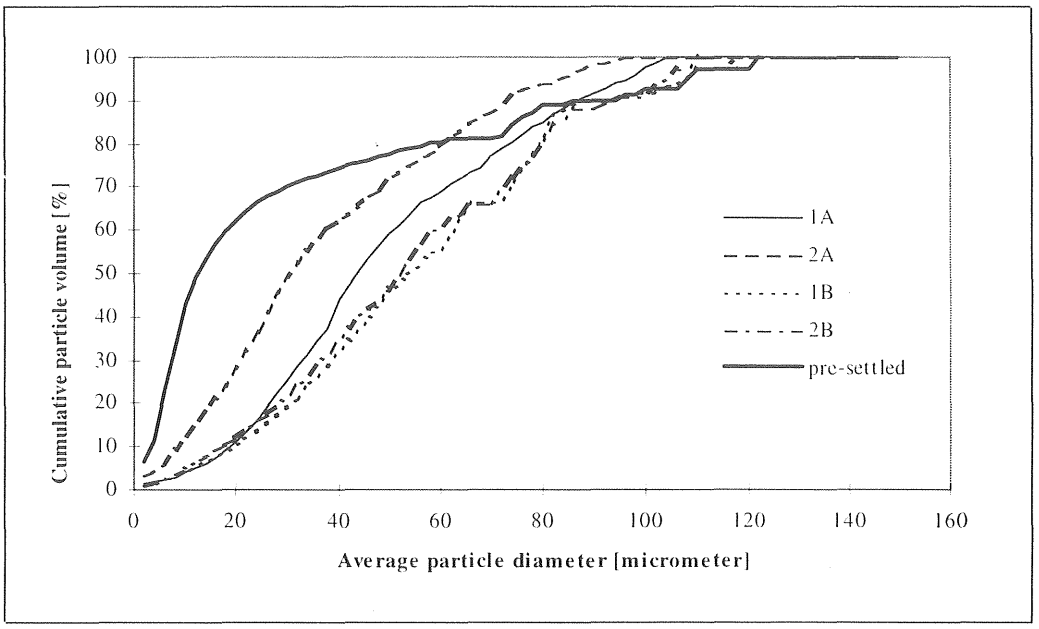


Figure 5.2-49 Particle size distributions in pre-settled wastewater, after the 1st oxic period (1) and after the anoxic period (2).

The distributions by number were recalculated to distributions by volume (equation 4 and 5). In Figure 5.2-50, the volume distributions at the end of the anoxic period is illustrated. The volume distribution was shifted more to the left for reactor A than for reactor B, indicating that there was a comparatively larger number of small particles (1-30 μm) present in the supernatant of reactor A. As a comparison, the volume distribution by the end of the first aerobic period is illustrated in Figure 5.2-51. This shows that there is no large difference in volume distribution during the oxic period (slightly shifted towards larger flocs in reactor B). The volume distribution for the pre-settled wastewater is illustrated in Figure 5.2-52. From

this it is evident that the distribution for the pre-settled wastewater was considerably more shifted towards smaller particles than the distributions for reactor A and B. The cumulative particle size distributions at the end of the first oxic period, at the end of the anoxic period and in pre-settled wastewater is illustrated in Figure 5.2-49.

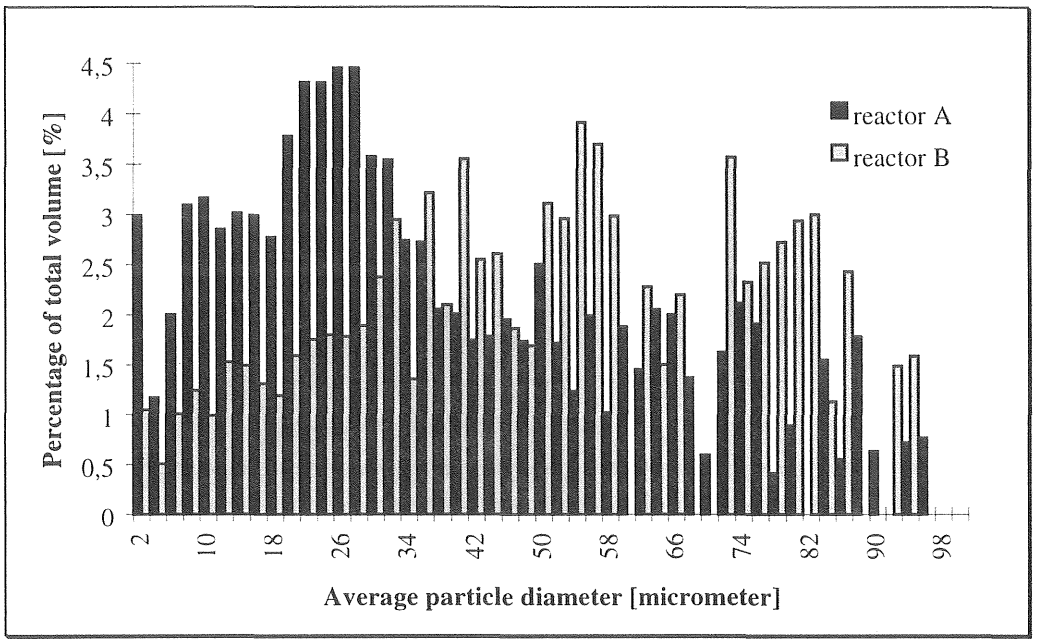


Figure 5.2-50 Distribution by volume by the end of the anaerobic period (Part II).

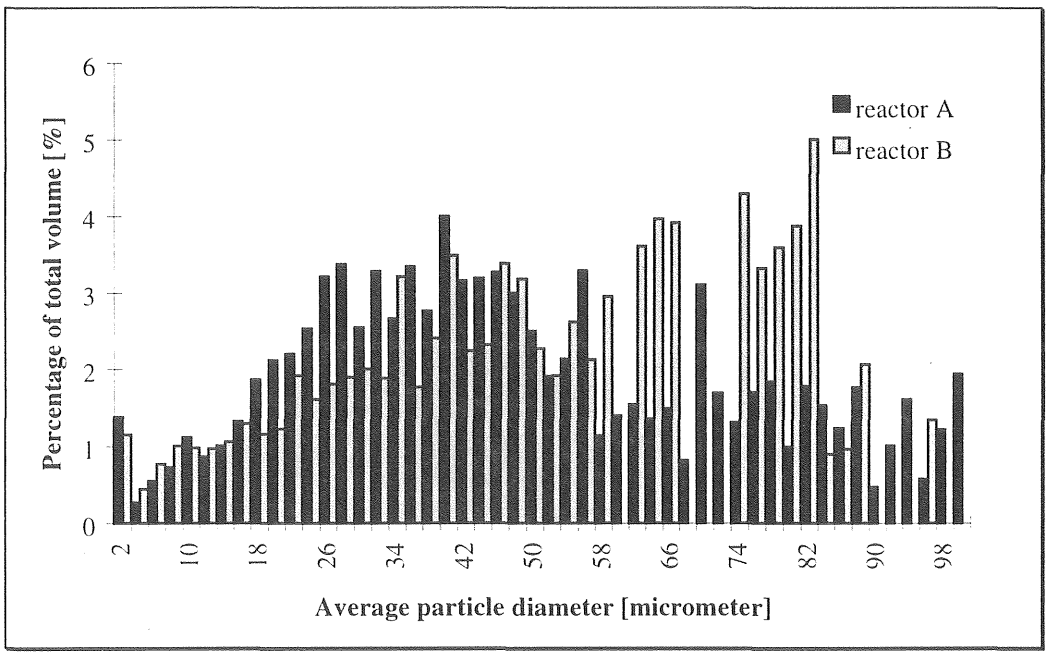


Figure 5.2-51 Distribution by volume by the end of the first aerobic period (Part II).

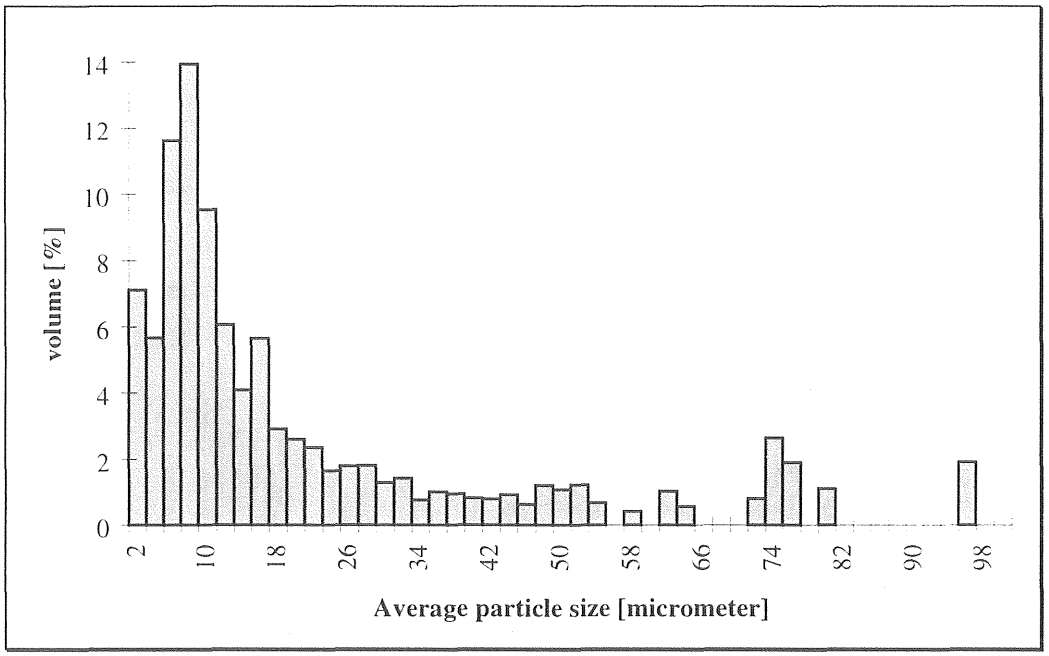


Figure 5.2-52 Distribution by volume for the pre-settled wastewater.

Floc size distribution: The size distribution of small flocs (based on number) in the supernatant after settling could best be fitted to power functions according to:

$$f(x) = ax_i^{-b} \qquad (6)$$

where a and b are constants. The frequency function is defined as: $f(x) = dN_i/dx_i$, where dN_i is the number of flocs of size i and dx_i is the size interval i. The constant a can be related to the total number of flocs within a certain volume (Kavanaugh *et al*, 1980) and b can be related to the number of flocs within each size class. An increase in b leads to a shift of the size distribution from large to small flocs. This means that as b increases, the number of flocs within the small size intervals increases. The parameters for Part III and IV are summarized in Table 5.2-10.

Table 5.2-10 Parameters in the power-function.

Parameter	Range		Average		Regression coefficient (r^2)
experiment	III	IV	III	IV	>0.95
a	$33 \cdot 10^4$-$63 \cdot 10^5$	$85 \cdot 10^4$-$28 \cdot 10^6$	$16 \cdot 10^5$	$43 \cdot 10^5$	
b	3.0-3.8	3.1-4.3	3.4	3.8	

It was also investigated whether the constants in the distribution function, a and b, could be correlated to the turbidity. In Part III, there was no correlation between the parameters a and b and the turbidity could be found. This may be explained by the small differences in turbidity between the measurements. Further, the turbidity was very low (< 12 NTU) throughout the experiment. In Part IV, the correlation between a and turbidity was somewhat better: $r^2 = 0.52$ (Figure 5.2-53). No correlation between turbidity and b could be found. When a was plotted against b, it was found that there was a trend towards higher b at higher a (Figure 5.2-54 and 55). This means that when the concentration of particles was high in the supernatant, the size

distribution was shifted towards smaller flocs. Hardly any difference in *a* and *b* could be found between the two reactors in Part III and IV of the experiment.

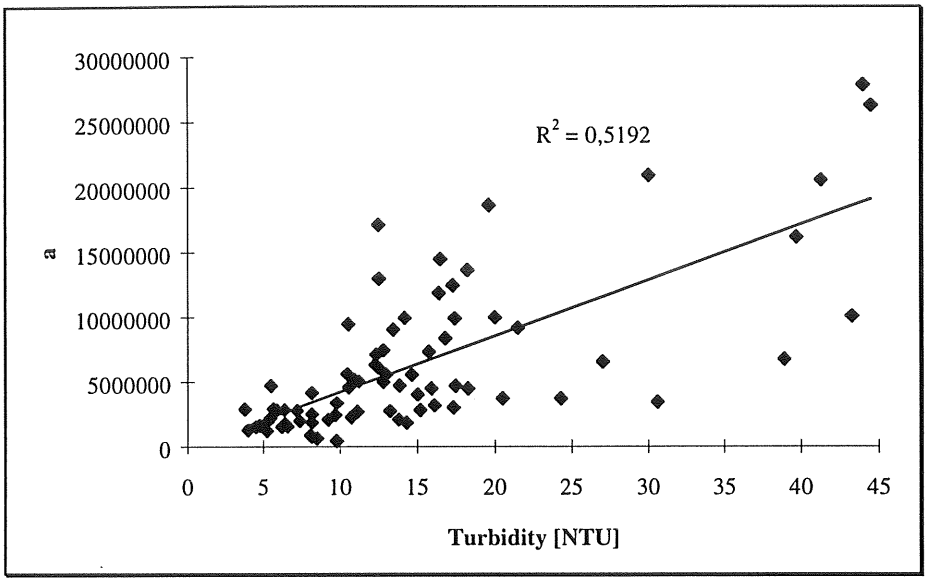


Figure 5.2-53 Correlation between the parameter *a* and turbidity (Part IV).

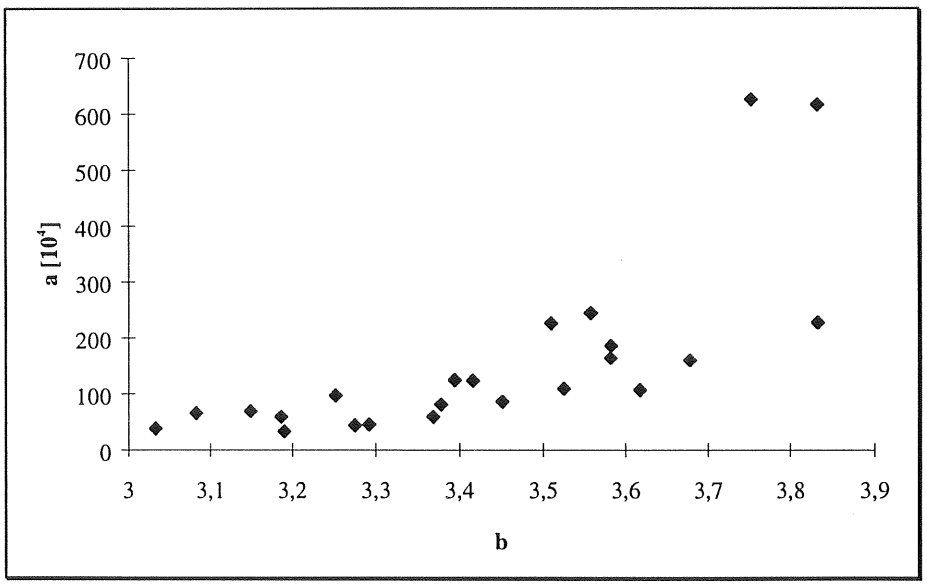


Figure 5.2-54 Correlation between the parameters a and b (Part III).

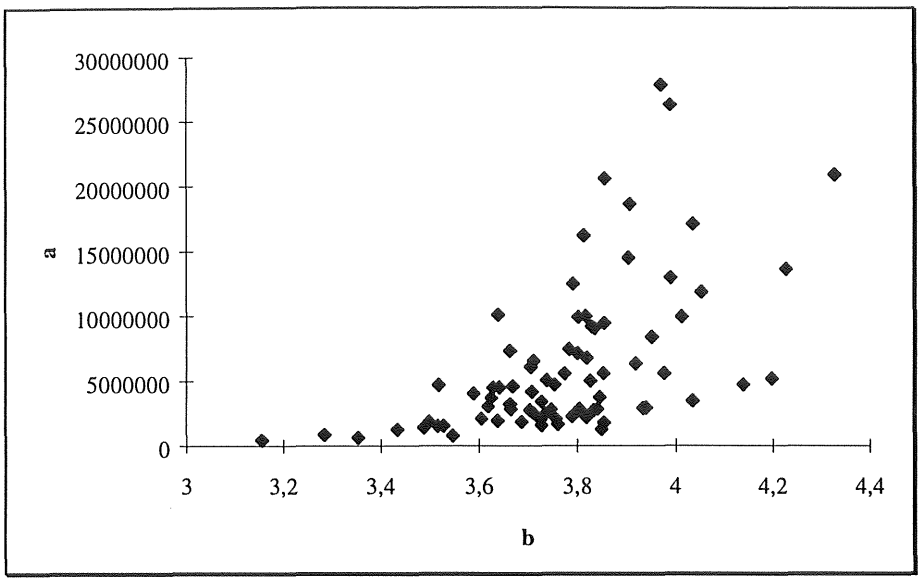


Figure 5.2-55 Correlation between the parameters a and b (Part IV).

An attempt was made to correlate the parameters *a* and *b* in the distribution function to the process parameters temperature, F/M, DO, pH and MLSS. However, no significant correlation could be found.

In summary it can be said that it is difficult to measure the size of activated sludge flocs. They are often irregularly shaped and it is difficult to define their size. They are also relatively fragile and can easily break during handling.

The presence of filamentous bacteria seemed to produce large flocs which may conceal the possible effects of DO concentration.

No clear relationship between DO concentration and floc diameter could be found. There was, however, a slight trend towards larger average floc diameter at higher DO concentrations could be noticed and the difference was largest in part IV of the experiment. There was also a trend towards smaller flocs at shorter SRTs.

Larger flocs are more important individually, and flocs with a diameter of > 50 μm contribute to most of the volume. Flocs within the size interval 11.6-1128 μm could best be fitted to log-normal distribution functions (based on floc volume) while flocs within the whole size interval could best be fitted to power distribution functions (based on floc number). More than 80% of the number of flocs in the supernatant were ≤2 μm. There were also more particles in the supernatant at lower DO concentrations than at higher. The shape of the volume distributions were similar which could indicate that there were relatively more smaller particles in the supernatant at lower than at higher DO concentrations (i.e. particles which cannot be measured with the particle analyser). Small particles are probably not measured very accurately with the particle analyser.

5.2.6 Microscopic investigation

The microscopic investigation revealed that the floc size in the pilot plant was considerably larger than in the full scale plant. The activated sludge with which the pilot plants was seeded, contained very small and fragile flocs (SRT≈2-3 days). About two sludge ages after the start-up of the experiments, the floc morphology changed quite dramatically. The flocs became much larger and mostly they contained large amounts of protozoa. It was also striking how fast the sludge properties could change. The experiments were run at fairly short sludge ages, 1.25-5 days, and as expected the sludge properties changed faster at shorter than at longer sludge ages. However, it was surprising that the sludge properties could change almost over night at a sludge age of 5 days. Especially Zoogloea bacteria could proliferate very fast. Also filamentous bacteria could grow very fast, but they could also disappear within a few days.

A summary of the main observations made during the experiments follows. Each experiment is treated separately.

Part I: During the start-up period, the flocs became more round and compact. Directly after the adjustment of the feed tank (from the beginning a larger tank was used with a HRT of a few hours), the floc morphology changed dramatically; they contained large numbers of Zoogloea bacteria which formed long arms protruding from the floc surface and the concentration of free bacteria increased as well.

About one week after the decrease in DO concentration to 1 mg/l, filamentous bacteria started to grow in larger numbers (they were present through the whole experiment). The dominating filament was judged to be Sphaerotilus natans. The second most common filaments were probably Thiothrix I-II (or possibly type 021N). After the initial decrease in DO concentration, the flocs became less compact, but after about one week they turned more compact again. The same thing happened when the DO was further decreased to 0.5 mg/l. A further decrease in DO concentration caused excessive numbers of filaments and the types were the same as before. The decrease in DO concentration did not affect the floc size (see section 5.2.5). More free bacteria could be seen just after a decrease in DO concentration. There were large numbers of protozoa (attached and free-swimming ciliates), rotifera and nematodes present throughout the experiments, except after two process failures (16-18 and 27th of Nov.) which led to DO depletion. The results from the microscopic investigation are illustrated in Figure.5.2-56 The morphology of the flocs was judged by means of a standard classification scheme which is described in Appendix II. In principle it is desirable to get as low total sum of indices as possible (apart from the number of protozoa), i.e. round and compact flocs containing no or small numbers of filaments and/or Zoogloea. These types of flocs settle fast and compact well. However, more irregularly shaped flocs tend to produce a more clear effluent. It is also desirable that the sludge contains large numbers of protozoa which also keep the effluent clear. It has also been suggested that small numbers of filamentous bacteria produce stronger flocs (Sezgin *et al,* 1978). In Figure 5.2-74 and 75, photographs illustrate the difference in floc morphology between a DO concentration of 2 and 0.5 mg/l.

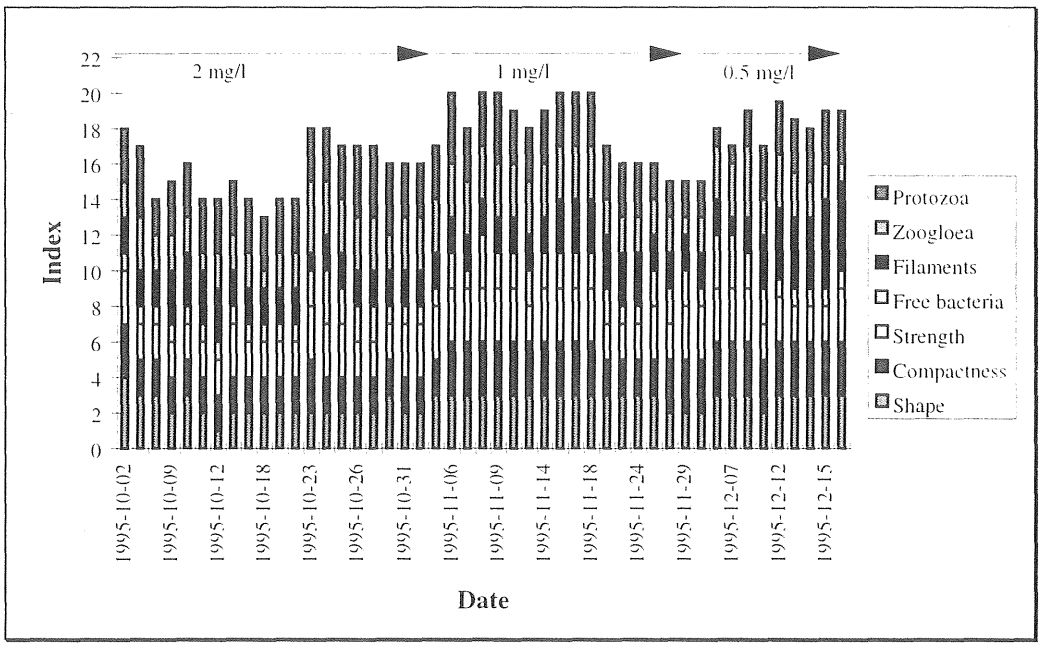


Figure 5.2-56 Classification of the sludge morphology according to a scale from 1-5 (number of free bacteria, filaments, Zoogloea, protozoa: 1=no present, 5=excessive numbers; shape, compactness, strength: 1=round and compact and strong, 5=very irregularly shaped, porous and weak (Part I).

Part II: The flocs grew in size from about 100 to 300 µm during the first two weeks of the start-up period. The number of filamentous bacteria increased gradually and this made the flocs very irregularly shaped and porous. After the selector was installed, the flocs became progressively more compact and round and the number of filamentous bacteria reduced drastically. If the selectors caused this or not is uncertain, but the filamentous bacteria did not come back in such excessive numbers (there were filaments present throughout the whole experiment).

In *period I* (reactor A subjected to alternating oxic/anoxic conditions of 1 hour and reactor B run at a constant DO concentration of 4 mg/l), there was no large difference in floc morphology between reactor A and B; the flocs became more irregularly shaped in both reactors and the number of filamentous and Zoogloea bacteria increased gradually.

During *period II* (reactor A subjected to alternating oxic/anoxic conditions of 2 hour and reactor B run at a constant DO concentration), the flocs in reactor B were compacter, rounder and contained less Zoogloea bacteria.

In *period III* (reactor A subjected to alternating oxic/anoxic conditions of 4 hour and reactor B run at a constant DO concentration of 4 mg/l), the flocs in reactor B were more compact. Otherwise there were no large differences except that the sludge in reactor A was a darker brown than that in reactor B throughout the major part of the experiment. There were many types of filamentous bacteria present and it was difficult to identify them all. The most dominating filament was Sphaerotilus natans and there were also many Thiothrix I-II (or possibly type 021N) present (type I, grew like spaghetti in large bundles). There were plenty of protozoa in both reactors throughout the experiment and there was no difference in the types present (many different types of attached and free swimming ciliates). In period III, there were spirochetes present in reactor A (in large numbers), which could indicate lack of DO.

There were also nematodes and rotifera present in both reactors. A summary of the microscopic investigation can be found in Figure 5.2-57 and 58.

Part III: At sludge ages of 1.25-5 days, the flocs were more compact at a DO concentration of 2 mg/l (reactor B) than at 0.5 mg/l (reactor A). The flocs were irregularly shaped in both reactors and there were filaments present throughout the experiment. The most dominating type was, as in the previous experiments, Sphaerotilus natans. There were also Thiothrix I-II (or possibly type 021N) present. At a sludge age of 5 days, there were more filamentous bacteria in reactor A (0.5 mg/l) than in reactor B (2 mg/l), and the filamentous bacteria started to grow later in reactor B. Just after the shift in sludge age to 2.5 days, the number of filamentous bacteria was reduced. After about 10 days they started to proliferate again. There was the same amount of filamentous bacteria in both reactors, but the flocs were more compact in reactor B. After a change in sludge age, the flocs became less compact in both reactors. However, about two sludge ages later they turned more compact again. The same phenomenon occurred when the sludge age was decreased to 1.25 days (unfortunately the plant could not be run for more than four days at a sludge age of 1.25 days due to a failure). The sludge contained various types of protozoa, nematodes and rotifera. A summary of the microscopic investigation can be found in Figure 5.2-59 and 60. In Figure 5.2-76 and 77 photos illustrate the floc morphology in reactor A and B at a SRT of 2.5 days.

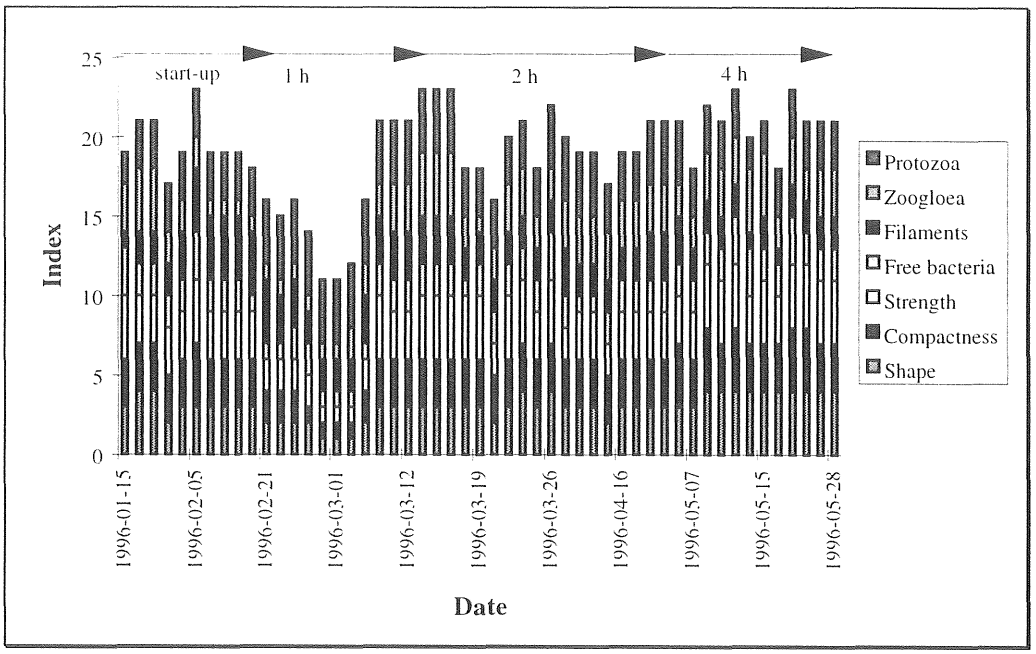


Figure 5.2-57 Classification of the sludge morphology according to a scale from 1-5 (Part II; reactor A).

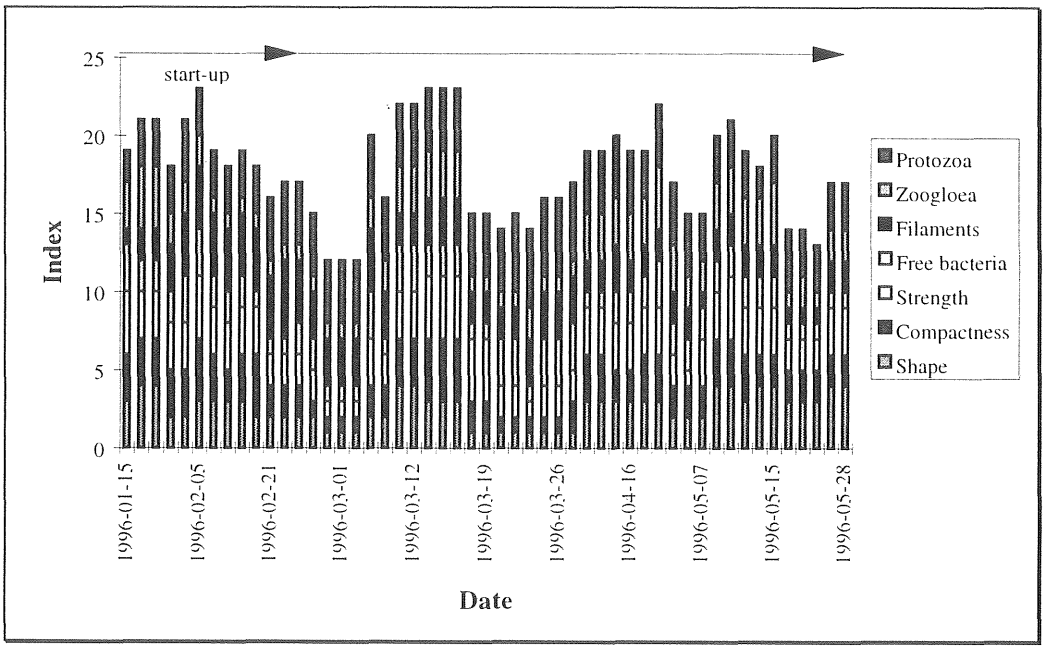


Figure 5.2-58 Classification of the sludge morphology according to a scale from 1-5 (Part II; reactor B).

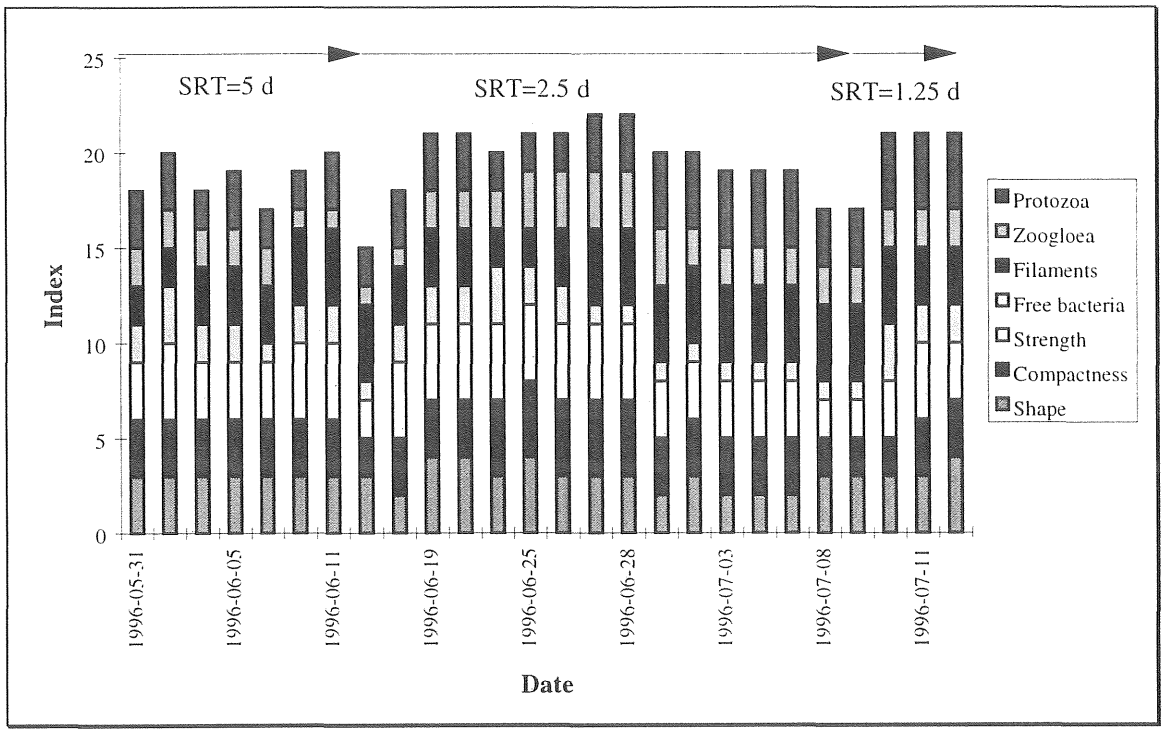


Figure 5.2-59 Classification of the sludge morphology according to a scale from 1-5 (Part III; reactor A).

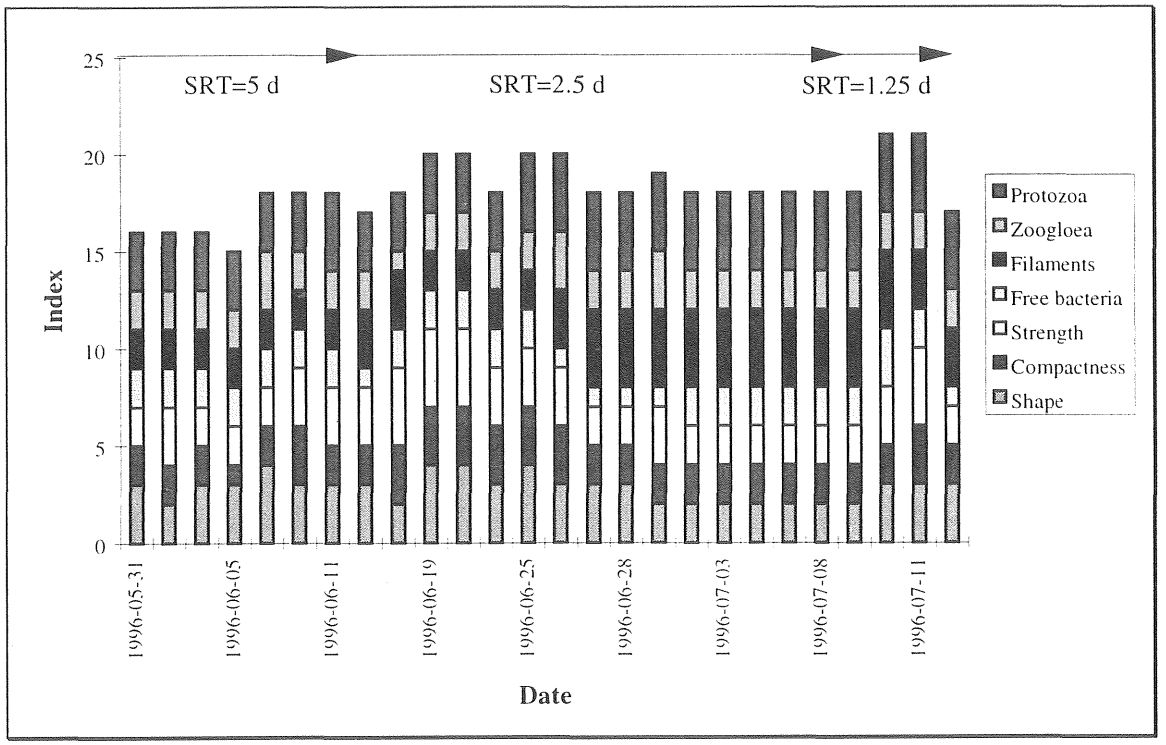


Figure 5.2-60 Classification of the sludge morphology according to a scale from 1-5 (Part III; reactor B).

Part IV: The sludge flocs were larger and more compact in reactor B (DO = 5 mg/l) than in reactor A (2 mg/l). For the first twenty days of operation, there were moderate numbers of filament present and the dominating type was Sphaerotilus natans. Thereafter, the numbers of filamentous bacteria increased in reactor B. This was surprising since reactor B was run at a

very high DO concentration. The flocs became, however, larger and more compact than in reactor A. Besides Sphaerotilus natans, there were other filaments present like 021N and Thiothrix I-II (probably). By the end of September the floc structure changed in both reactors: they became more porous, smaller and the number of dispersed bacteria increased. At the same time the filaments more or less disappeared. No explanation for this could be found. Thereafter the number of filaments started to increase in reactor A. The settling properties became very poor and the sludge hardly settled at all. There were the same filaments present as previously plus a new type of filament. They were Gram-negative, Neisser-negative and they contained p-β-h granules. Further they had rounded cell-septa. This indicates that it could have been type 1701. The 15th of October, the DO concentration was increased to 5 mg/l in reactor A. The filaments disappeared within a few days and the flocs became more compact, larger and less irregularly shaped. There was a large number of protozoa present throughout the experiment and there was no difference between reactor A and B. A summary of the microscopic investigation can be found in Figure 5.2-61 and 62.

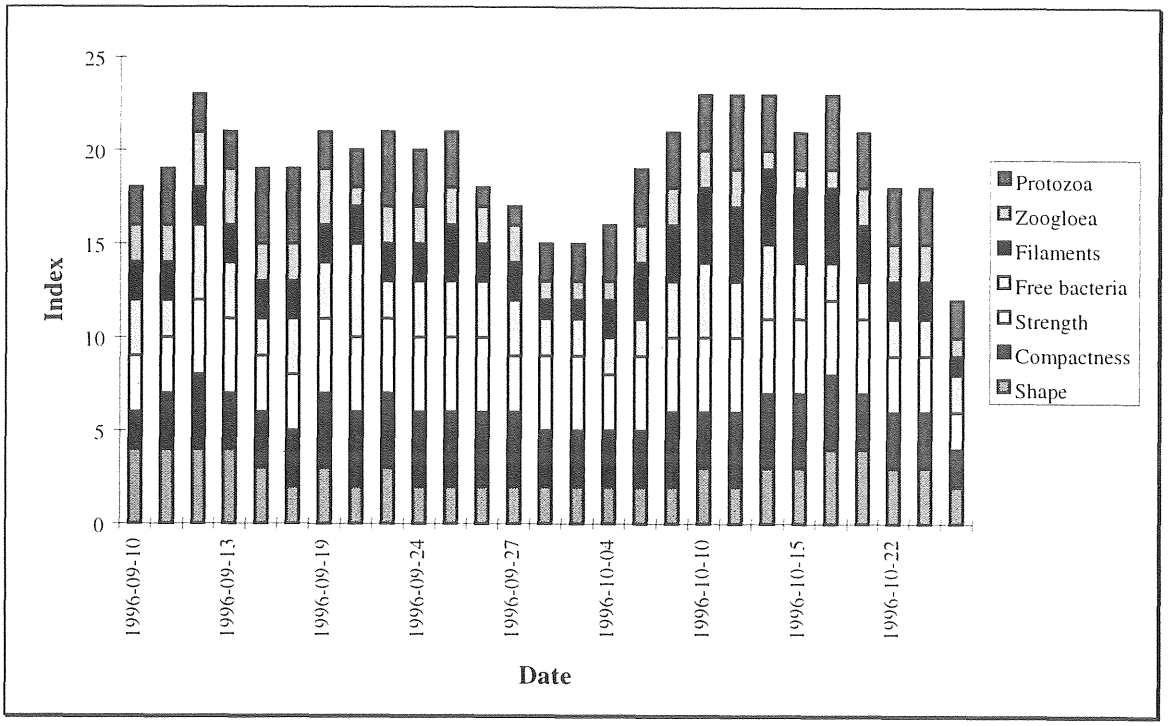


Figure 5.2-61 Classification of the sludge morphology according to a scale from 1-5 (Part IV; reactor A).

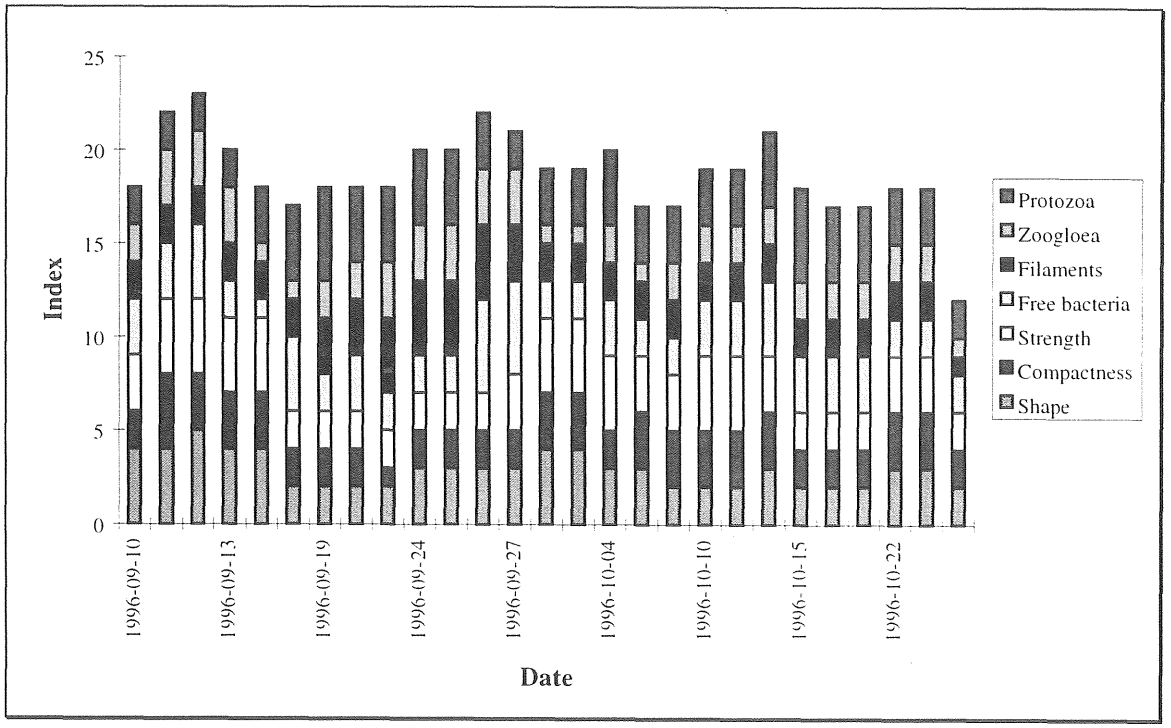


Figure 5.2-62 Classification of the sludge morphology according to a scale from 1-5 (Part IV; reactor B).

The proliferation of Thiothrix could be due to high concentrations of sulphides in the wastewater. During Part II-IV of the experiment, there were long periods with little rain which

could have led to anaerobic conditions in the sewer system. This was also verified, since the wastewater regularly smelled of sulphides and had often a black colour. It was also noticed that after a heavy rain, following a long dry period, the amount of filamentous bacteria increased (large amounts of sulphides were probably coming in to the wastewater plant).

The filament index (1=no filaments; 5=excessive numbers) could reasonably well be correlated to the SVI (Figure 5.2-63-69). Sometimes rather low SVI could be found at high filament indices and vice versa. The type as well as the number of filaments present is decisive for the SVI. Some filaments also affect the compactness of the flocs by growing inside them while others preferentially grow at the surface of the flocs. In this study, both types occurred.

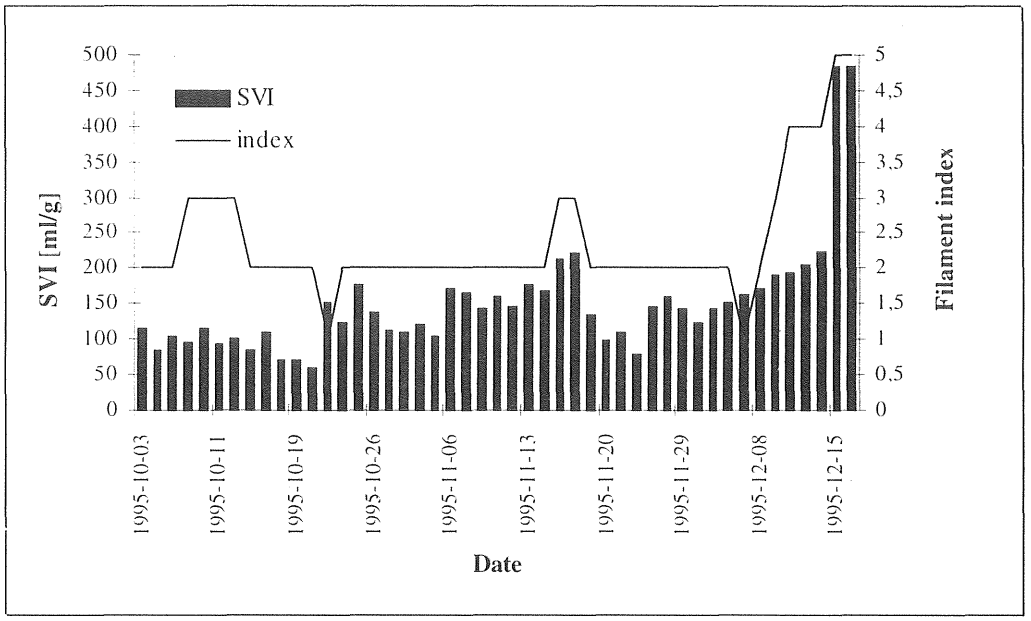


Figure 5.2-63 SVI and filament index (Part I).

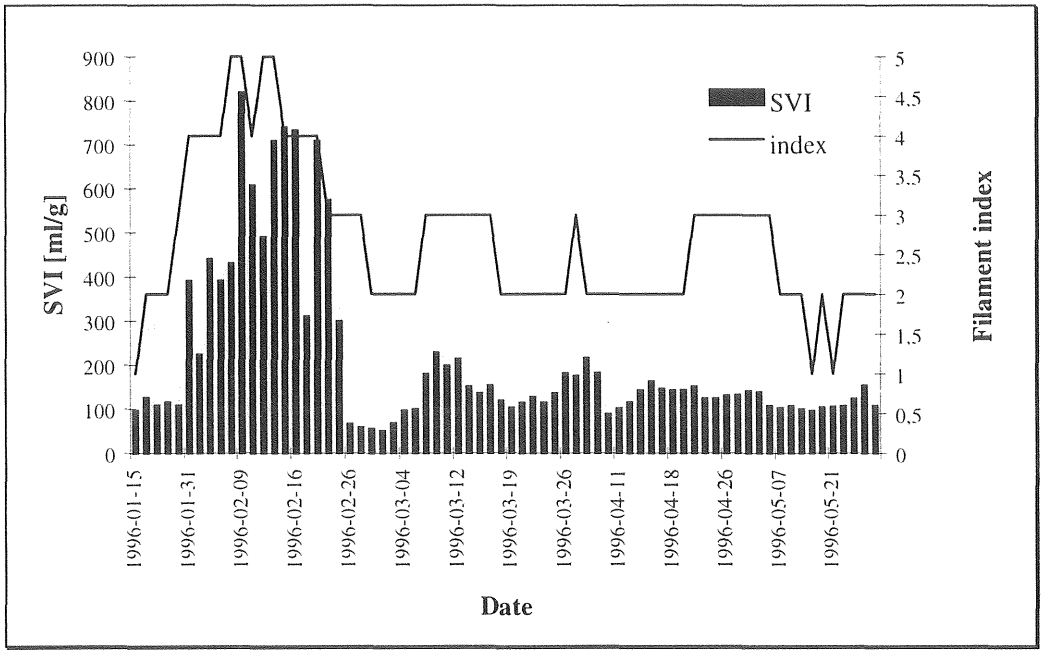


Figure 5.2-64 SVI and filament index (reactor A, Part II).

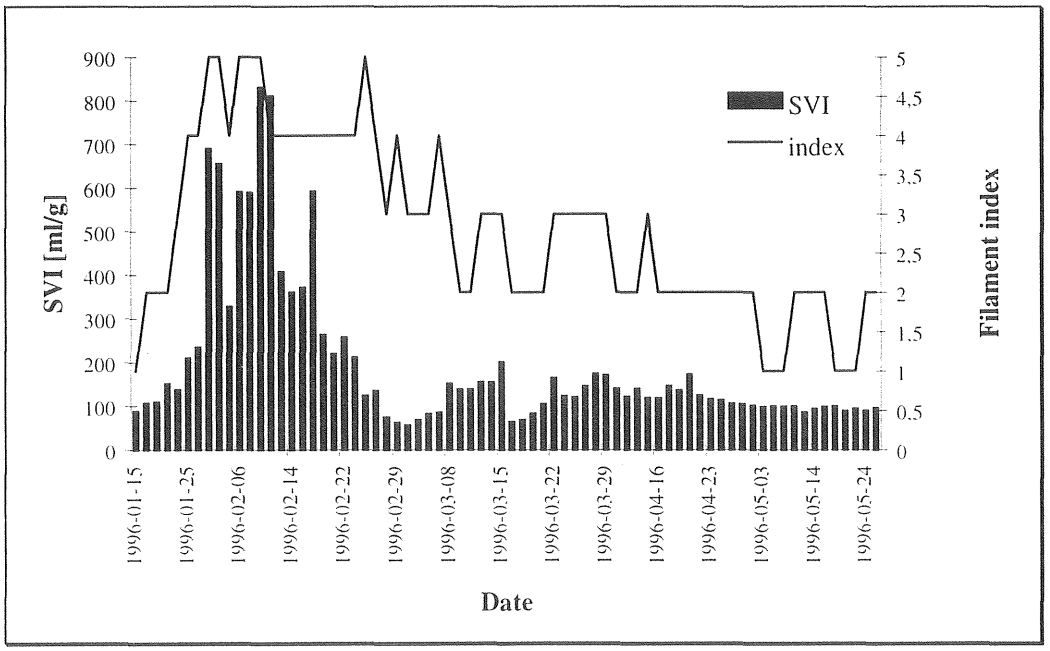


Figure 5.2-65 SVI and filament index (reactor B, Part II).

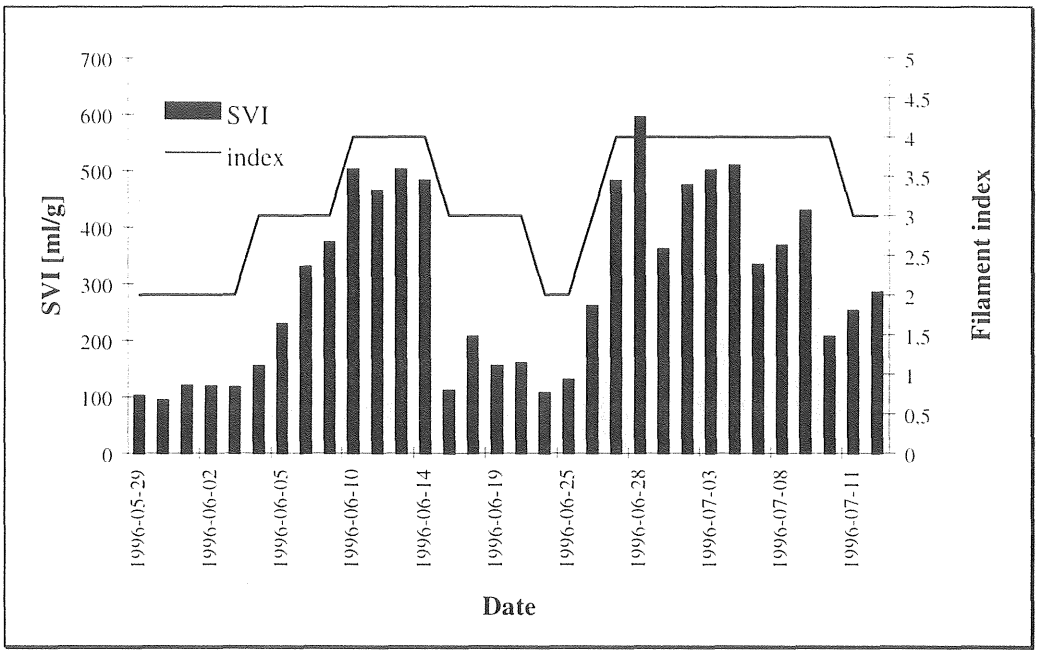


Figure 5.2-66 SVI and filament index (reactor A, Part III).

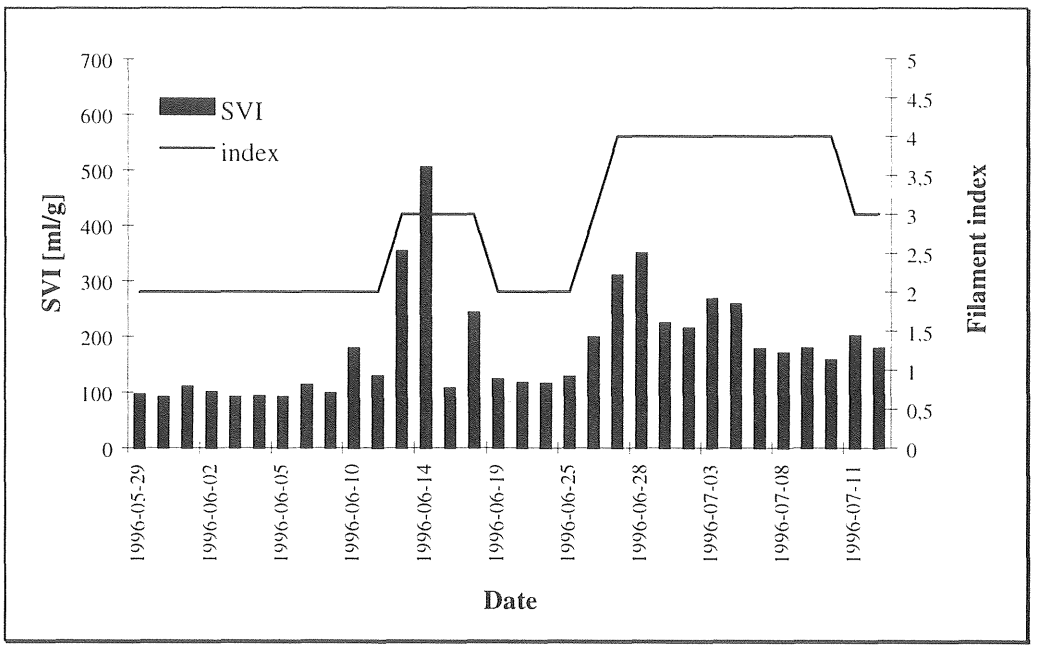


Figure 5.2-67 SVI and filament index (reactor B, Part III).

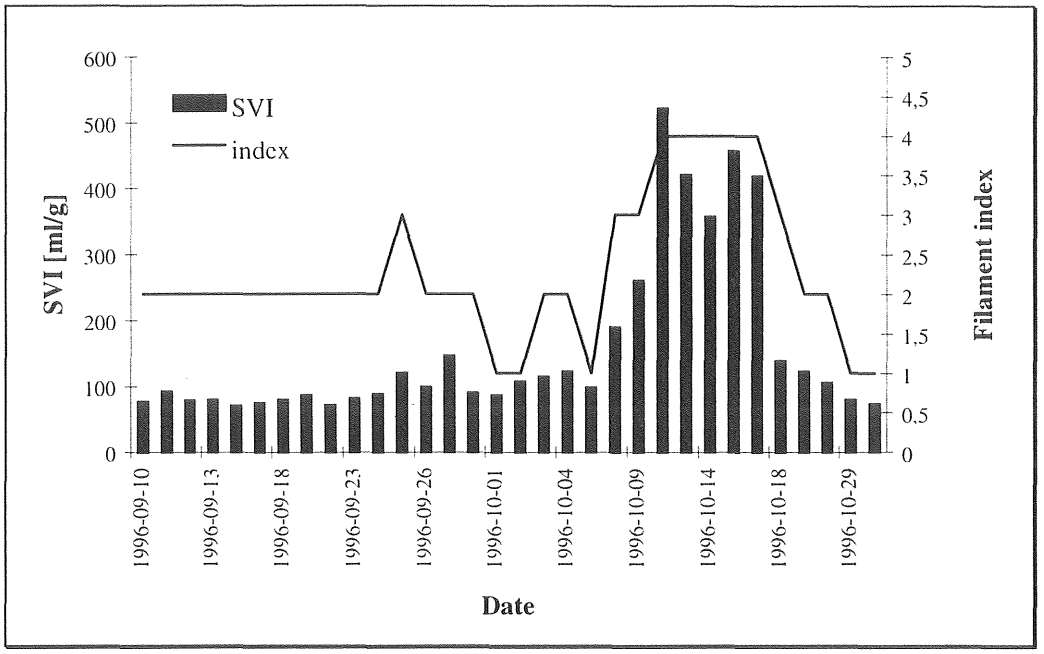


Figure 5.2-68 SVI and filament index (reactor A, Part IV).

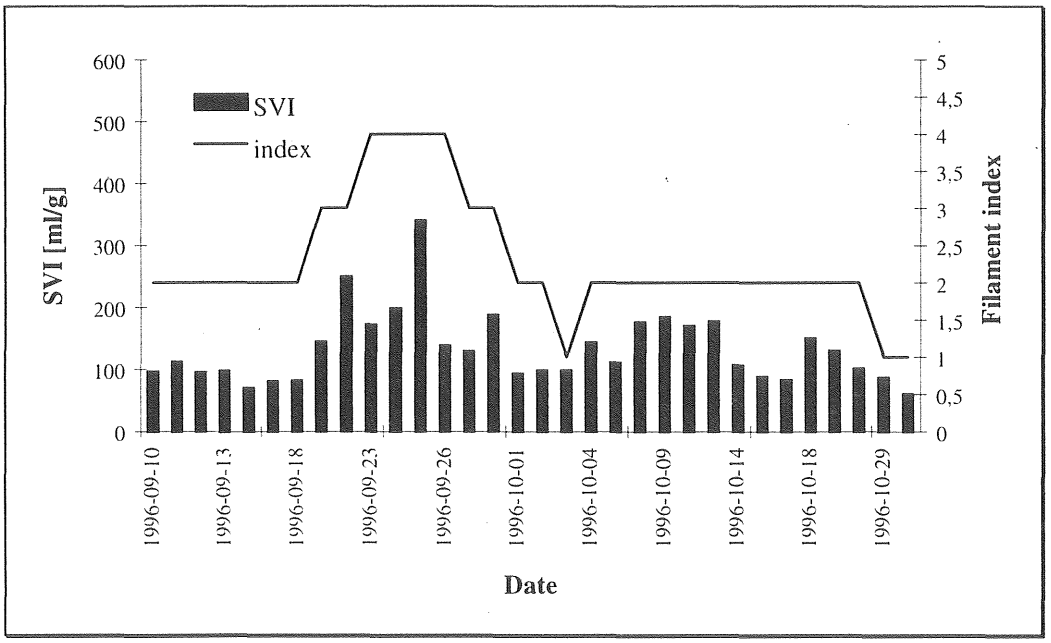


Figure 5.2-69 SVI and filament index (reactor B, Part IV).

Small pilot plants have a large wall surface-to-volume ratio, which makes it easy for microorganisms to proliferate in tubings etc. This could be the explanation to why the floc morphology could change so fast. To prevent wall growth as much as possible, the tubings were cleaned 2-3 times per week with hypo-chlorite. The reactor walls were cleaned with a brush every day except during the weekends. In spite of these actions, problems with filamentous bacteria occurred regularly.

The types of filamentous bacteria found in the pilot plant were compared with the ones found at the Rya WWTP (Robinsson, 1996). There is of course a large difference between the two plants (reactor type, organic loading, sludge age etc.) and you would not expect to find the

same types in two different systems. If something in the wastewater promotes the growth of certain types of filaments one might find these filaments in both systems. During *Part I* of the experiment, Sphaerotilus natans and Beggiatoa could be found at the Rya WWTP but not in excessive numbers; during *Part II*: type 1863 and Flexibacter; during *Part III*: Beggiatoa; during *Part IV*: no filaments. Only during period I, could Sphaerotilus natans be found in both systems. During the experimental period, the Rya WWTP was undergoing a plant expansion which led to extreme conditions such as very low SRT (about 2 days) and high organic loadings.

In Figure 5.2-70-73, the compactness index (1= very compact; 5 = very porous) is depicted. In *Part I*, the flocs became somewhat less compact when the DO concentration was decreased to 1 mg/l. By the end of this period, the flocs seemed to get used to the changed conditions and became more compact again. As the DO concentration was decreased from 1 mg/l to 0.5 mg/l, the flocs turned less compact again.

In *Part II*, the flocs in reactor A were generally less compact than in reactor B at an alternating period of 2 and 4 hours (the difference was largest at an alternating period of 4 hours). At an alternating period of 1 hour, the flocs in reactor A were sometimes compacter than in reactor B.

In *Part III*, the flocs were generally less compact in reactor A than in reactor B and the difference was more pronounced at shorter sludge ages.

In *Part IV*, the flocs were more compact at a DO concentration of 5 mg/l (reactor B) than at 2 mg/l (reactor A).

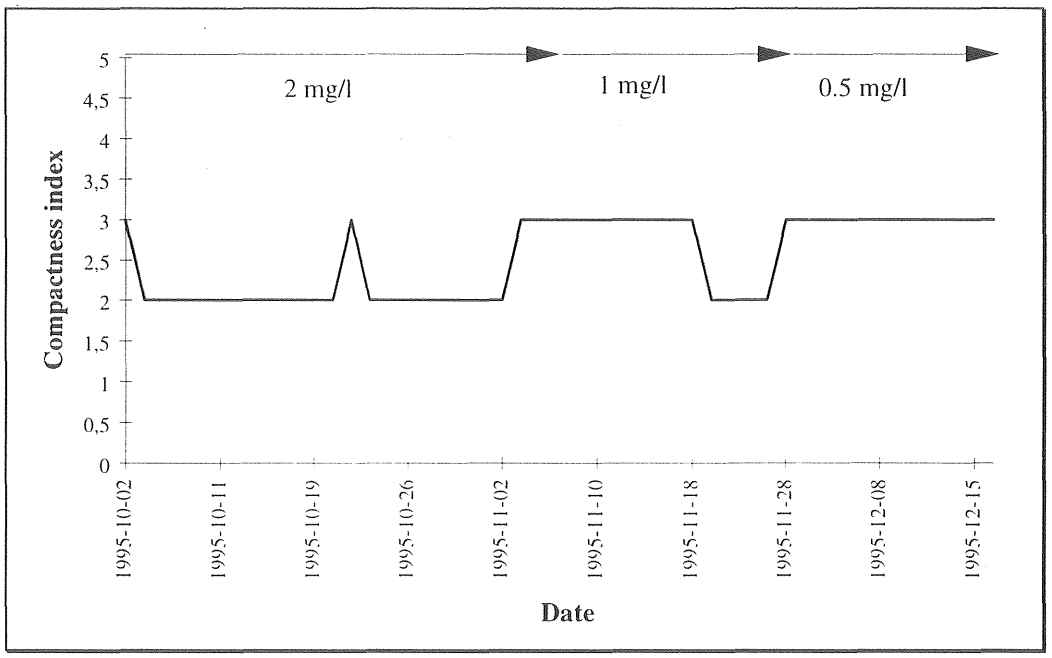


Figure 5.2-70 Compactness index, Part I.

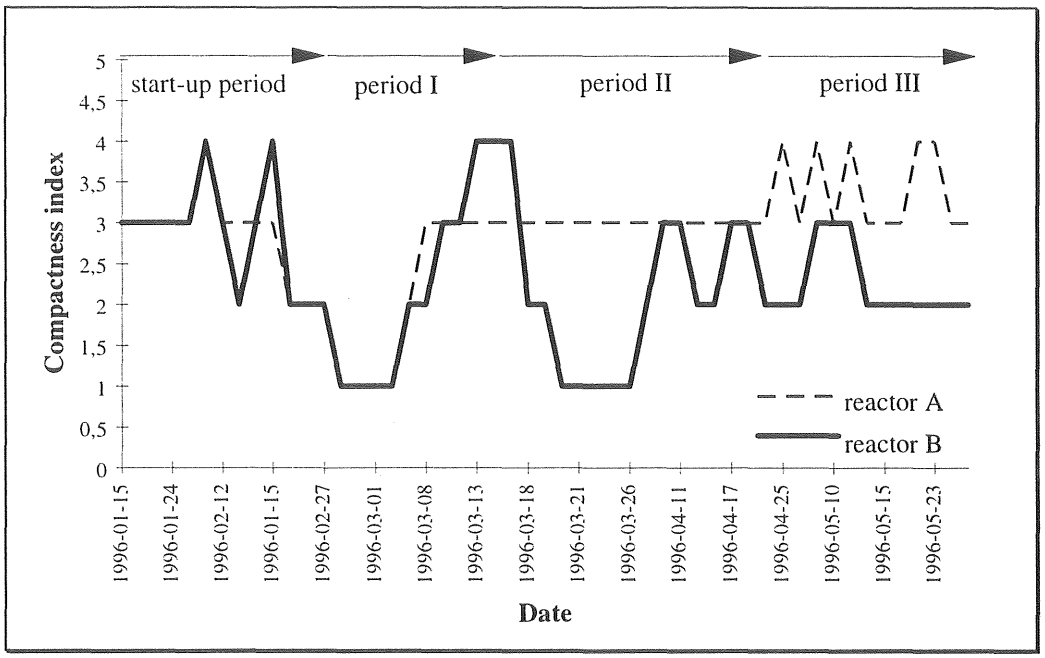


Figure 5.2-71 Compactness index, Part II.

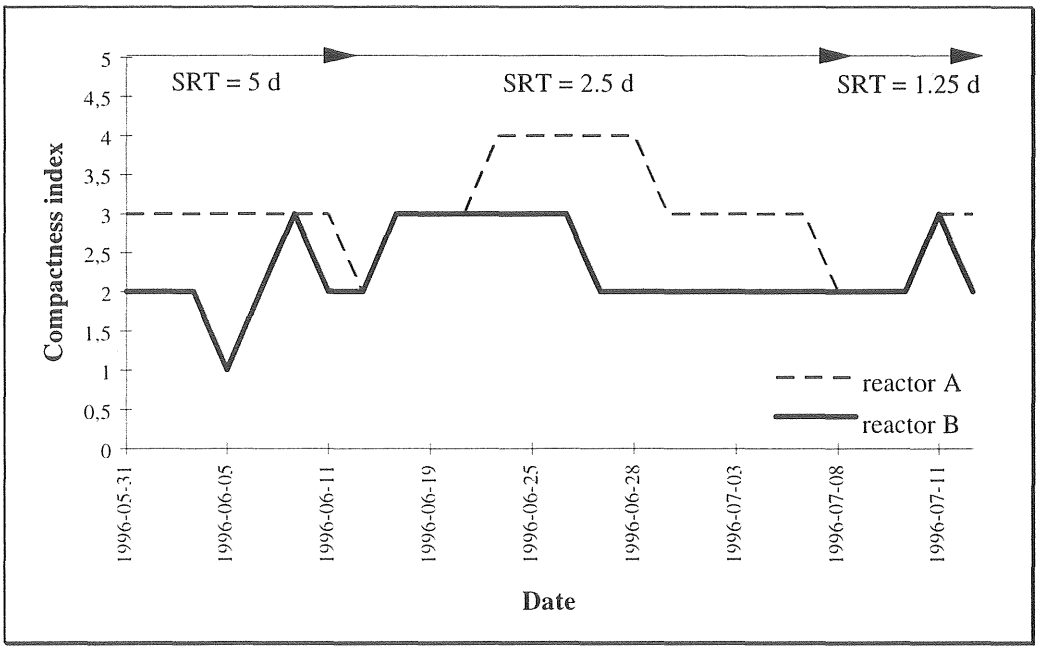


Figure 5.2-72 Compactness index, Part III.

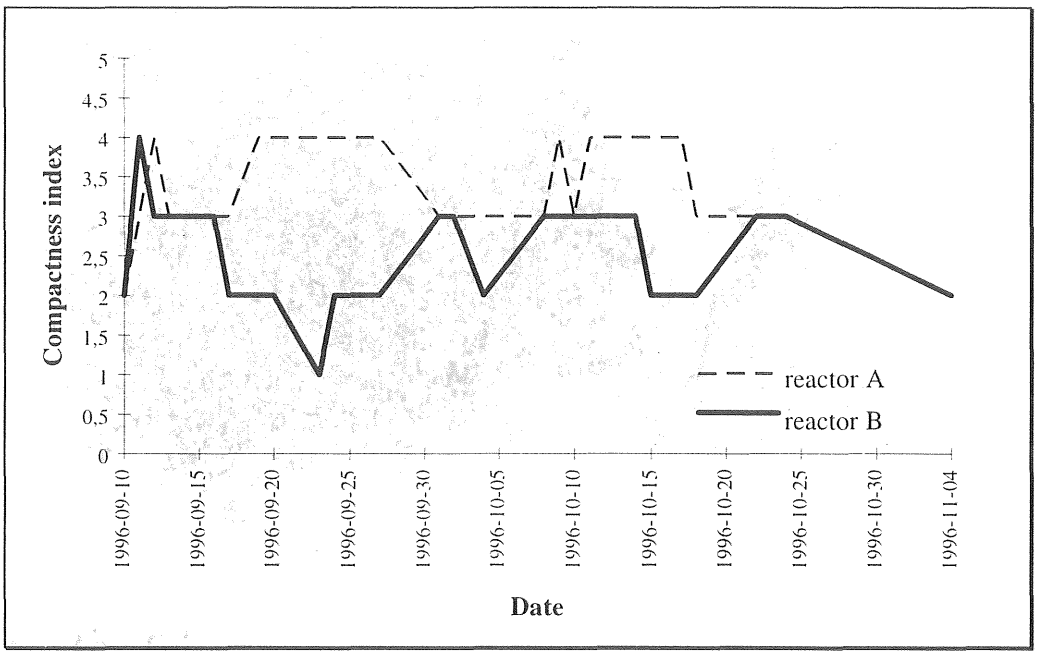


Figure 5.2-73 Compactness index, Part IV.

The results show that the numbers of filamentous bacteria were generally higher when the DO concentration was low compared to when it was high. The flocs became more compact when the DO concentration was high than when it was low.

When working with small pilot plants, the proliferation of filamentous bacteria is a large problem. It is also difficult to operate two parallel systems identically; the settling properties in one system can change even though it is operated in the same way as the other one. Therefore, it is very important to clean reactor walls and tubings regularly to minimize the risk of getting problems.

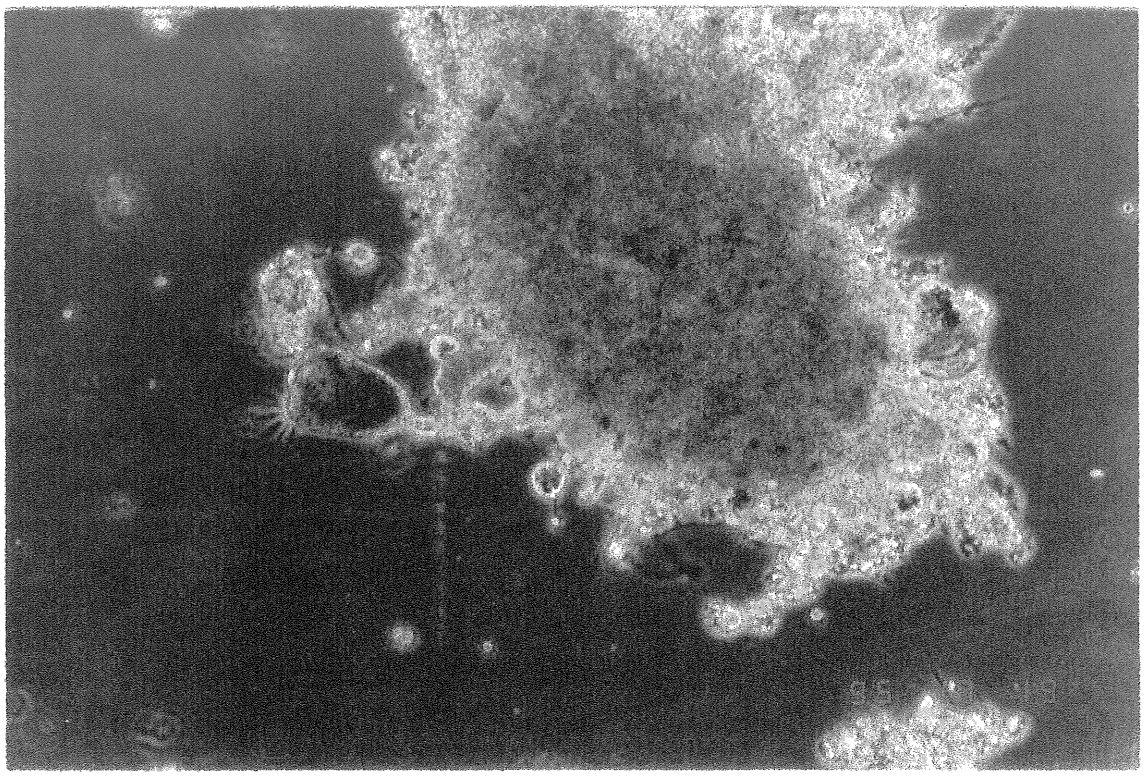


Figure 5.2-74 Round (category 2), compact (category 2) and large flocs containing no filaments (951019, Part I, magnification 100x).

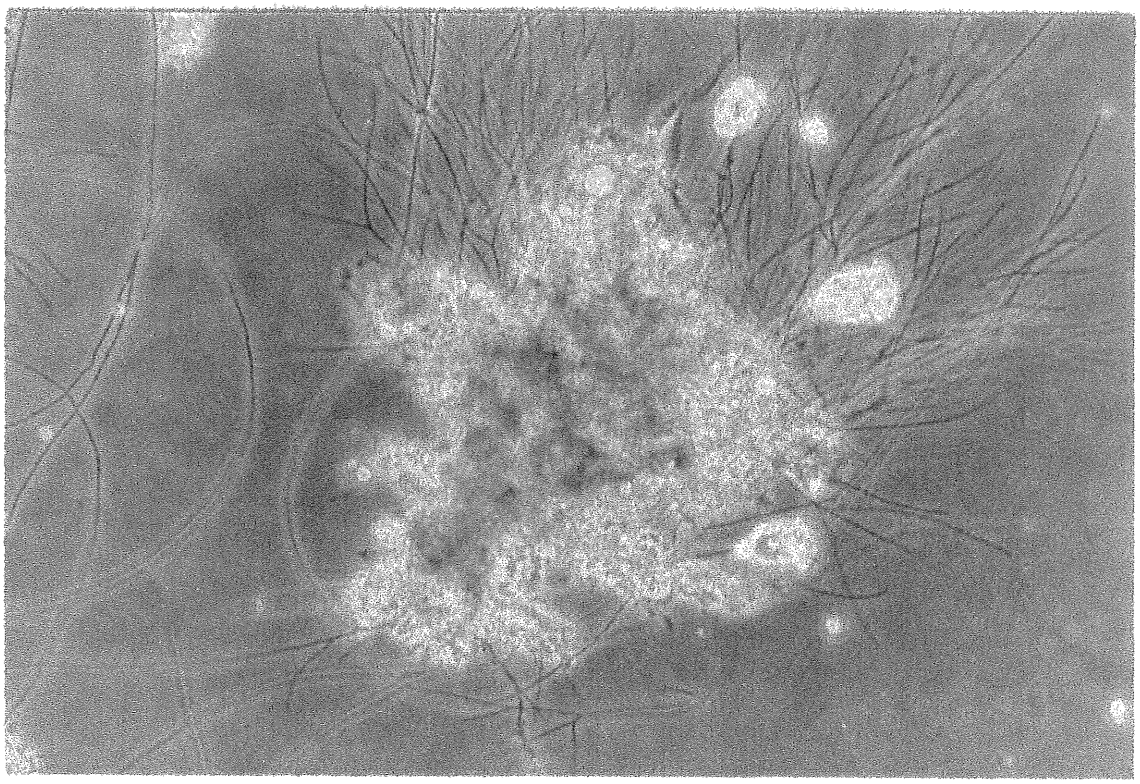


Figure 5.2-75 Irregularly shaped (category 3), and rather porous (category 3) flocs containing large numbers of filaments (category 5) (951215, Part I, magnification 100x).

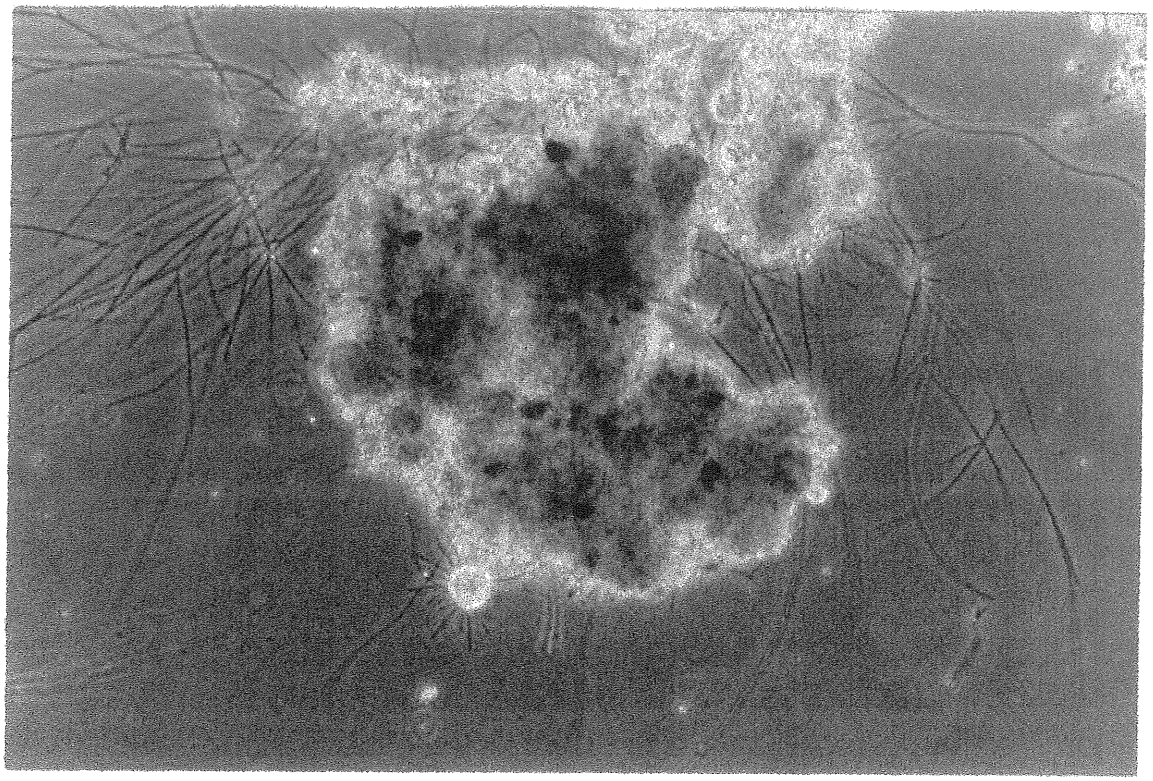


Figure 5.2-76 Round (category 2), large and compact (category 2) flocs containing large numbers of filaments (category 4) (960703, Part III: reactor B, SRT=2.5 d, magnification 100x).

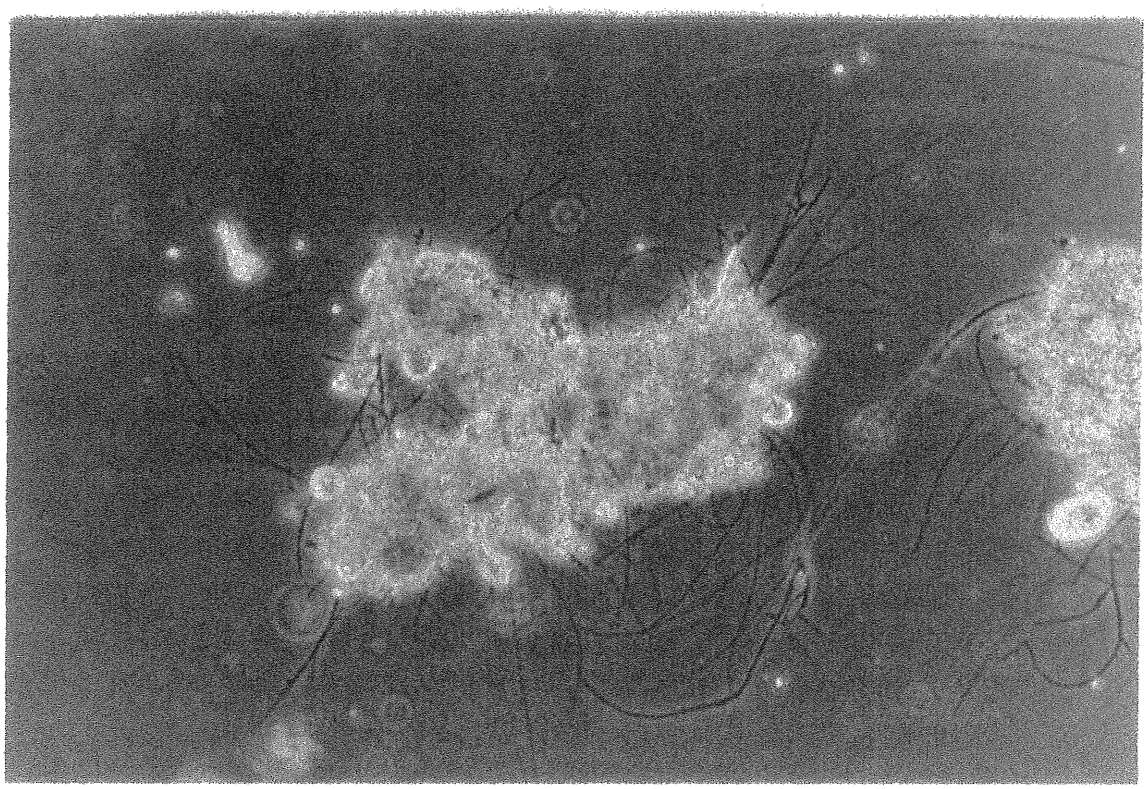


Figure 5.2-77 Round (category 2) and porous (category 3) flocs containing filaments (category 3) (960703, Part III: reactor A, SRT=2.5 d, magnification 100x).

5.3 Short term effects of dissolved oxygen concentration in a full scale plant

In the first experiment, the DO concentration was kept low at the beginning (< 1 mg/l), DO meter D, and high (4 mg/l) at the end, DO meter I, of the aeration tank. Figure 5.3-1 shows that no increase in turbidity was observed.

In the second experiment, the DO concentration was kept high (4-5 mg/l) at the beginning and low (1-1.5 mg/l) at the end of the aeration tank. A clear increase in turbidity could be found after 1.5 hours. The two different turbidity meters have different sensitivity to changes in turbidity. Therefore, the increase appears much larger with turbidity meter I (Sigrist). The Sigrist instrument is more sophisticated than the BTG instrument (II) and probably more reliable. When the DO concentration was increased to levels above 6 mg/l in the whole aeration tank, the turbidity started to decrease after 1.5 hours (Figure 5.3-2). If the flow to the secondary settlers was evenly distributed and the settlers hydraulic behaviour was ideal the peak in turbidity would be expected after about 3 hours. Lumley and Horkeby (1989) made tracer tests to investigate the hydraulic retention time in similar settlers (tests performed at the Rya WWTP before the expansion of the settlers to two stories). They found that the peak concentration of tracer in the effluent appeared after a period of time which corresponded to 40-60% of the ideal hydraulic retention time (assuming that the liquid flows in the whole volume of the settler).

In the third experiment, the DO concentration was kept low at the beginning (< 1 mg/l) and moderate (2-4 mg/l) at the end of the aeration tank. A small increase in turbidity was observed after about 1.5 hours. When the DO concentration was increased, the turbidity decreased gradually (Figure 5.3-3).

In the fourth experiment, the DO concentration was kept low (< 1 mg/l) both at the beginning and at the end of the aeration tank. A very large increase in turbidity could be noticed in the effluent. About 1.5 hours after that the DO concentration was increased again, the turbidity started to decrease and about 5-6 hours later the values were back to the initial ones (Figure 5.3-4).

The results show that a decrease in turbidity can be found when the DO concentration is low at the outlet end of the aeration tank. The effect of a change in DO concentration is probably dependent on the quality of the influent wastewater and the temperature. A few tests were performed during the summer when the water temperature was above 20 °C in the aeration tank. No increased turbidities were observed in the effluent even when the DO concentration was reduced quite drastically. The turbidimeters are not 100% reliable, and some changes in the turbidity might not be registered by the instruments. The main purpose with these experiments was to get a rough idea about how sensitive the process is for changes in the DO concentration. For a more detailed analyse, the DO concentration as well as turbidity of the effluent have to be measured manually throughout the experiment. The DO concentration profiles should also be monitored manually throughout the tests.

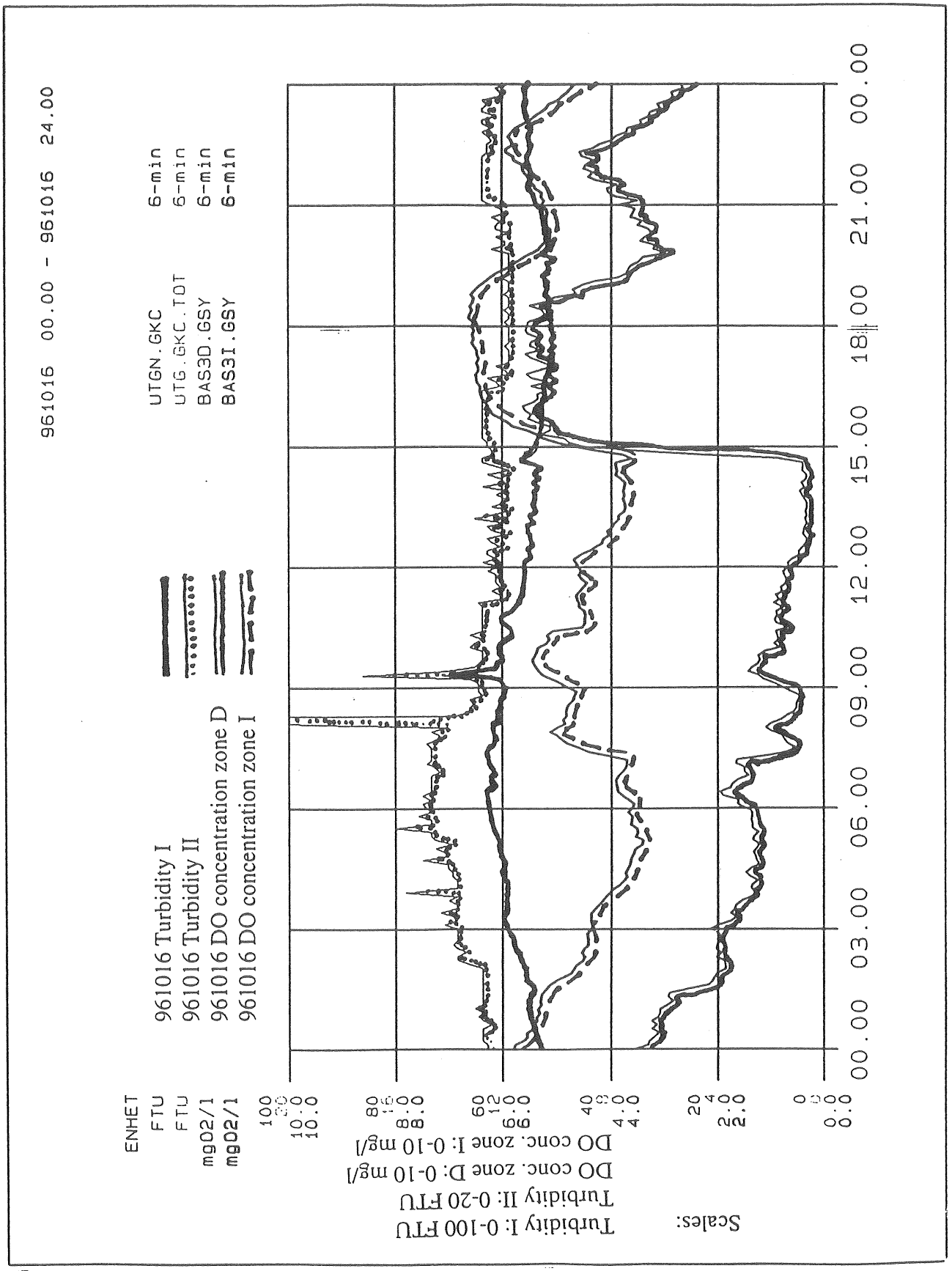


Figure 5.3-1 Experiment I (961016); inflow: 2 m^3/s; water temperature: 18 °C.

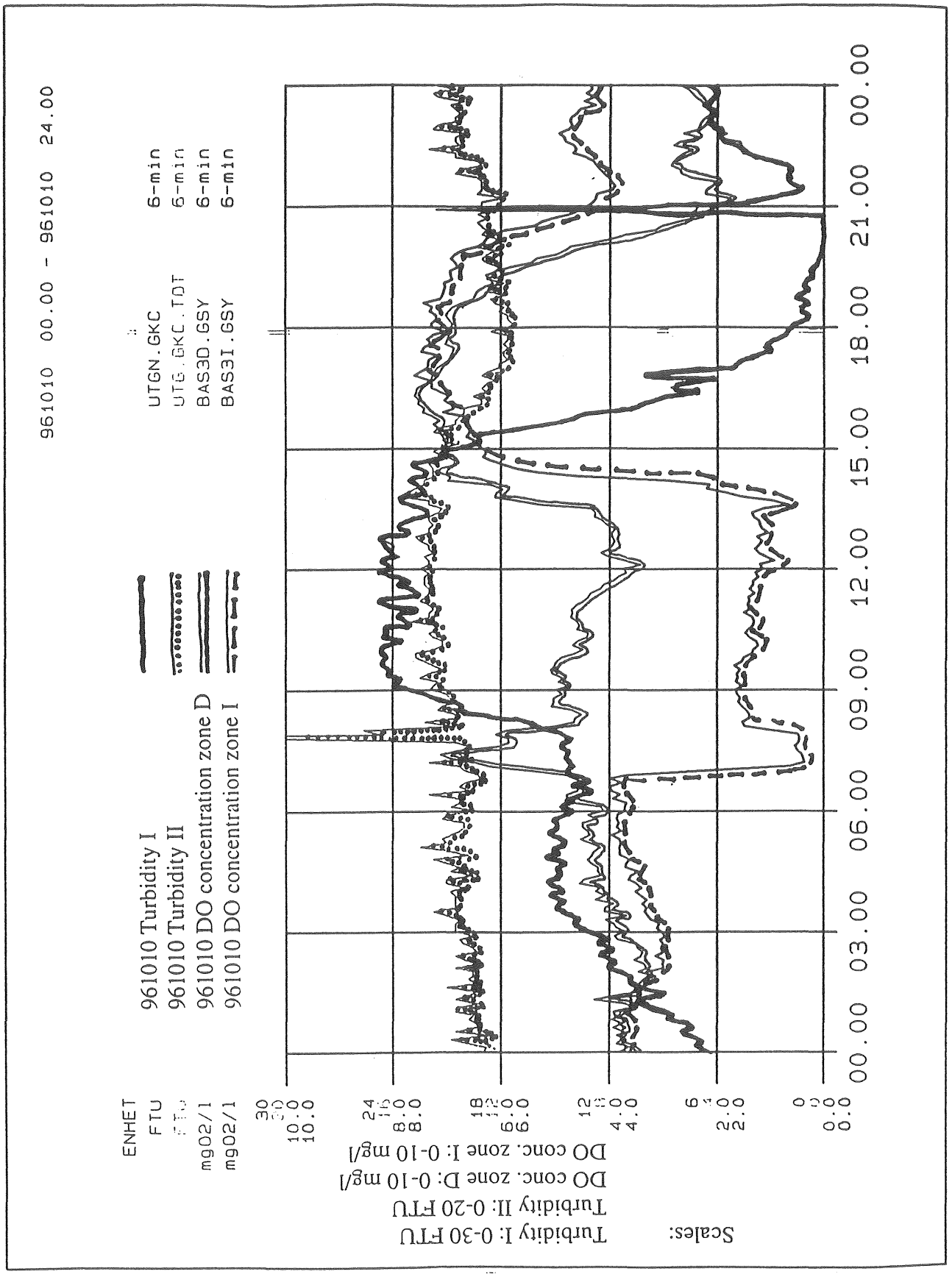


Figure 5.3-2 Experiment II (961010); inflow: 2.5 m^3/s; water temperature: 17-18 °C.

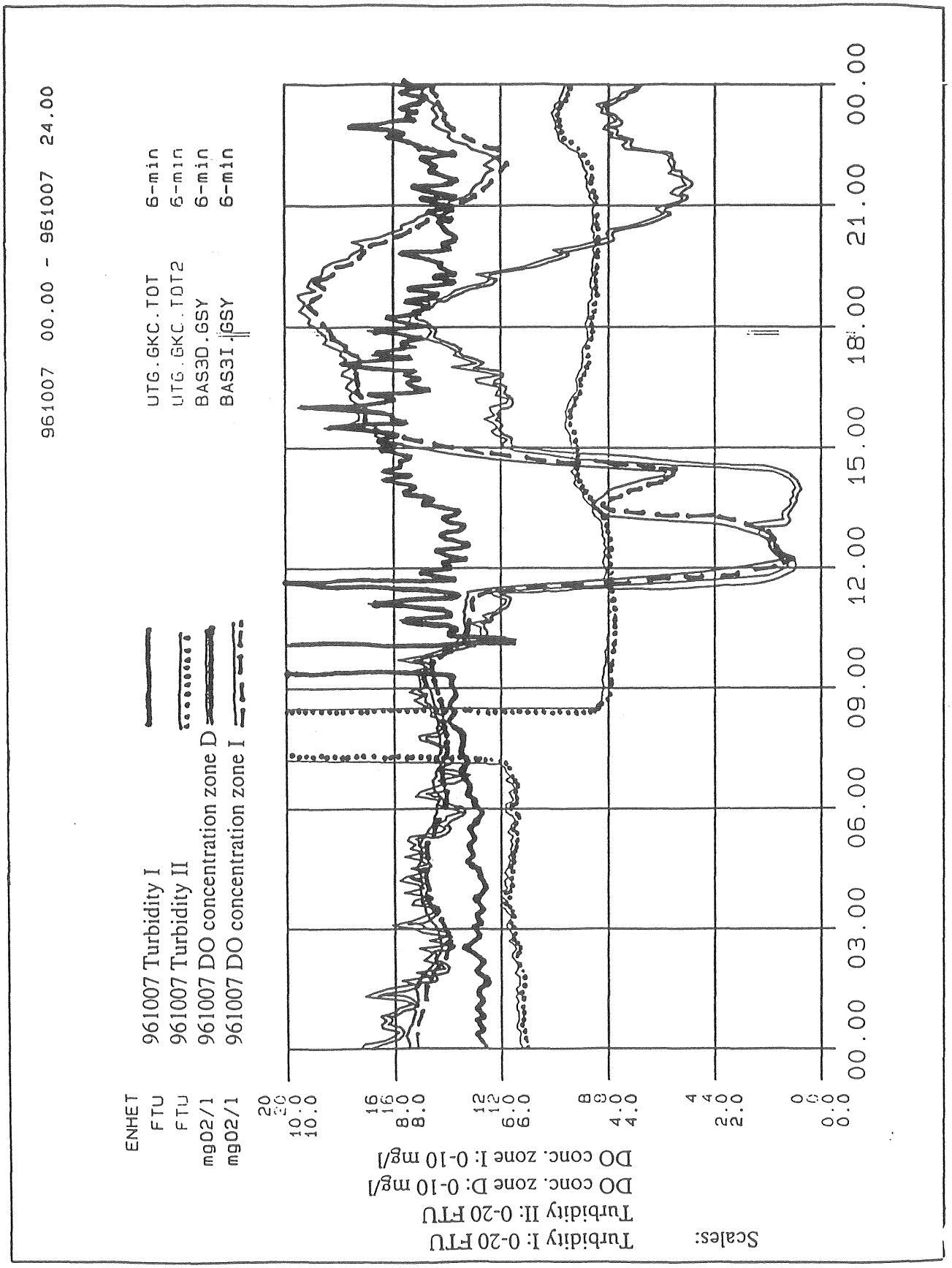


Figure 5.3-3 Experiment III (961007); inflow: 2.5 m^3/s; water temperature: 17-18 °C.

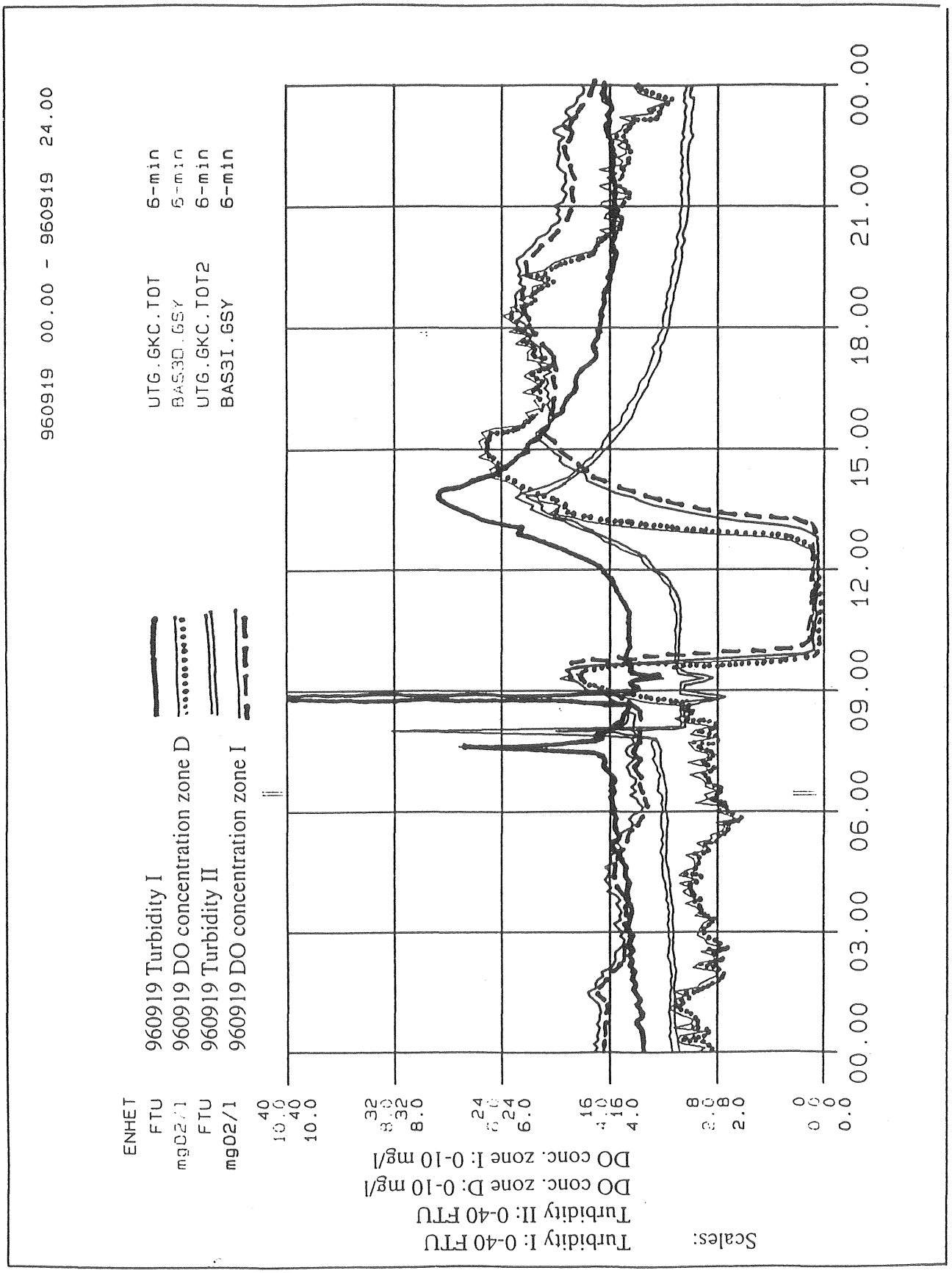
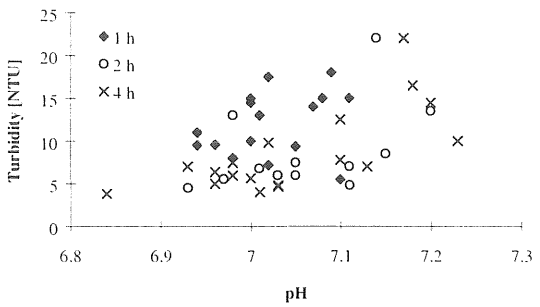


Figure 5.3-4 Experiment IV (960919); inflow: 2 m^3/s; water temperature 18.5 °C.

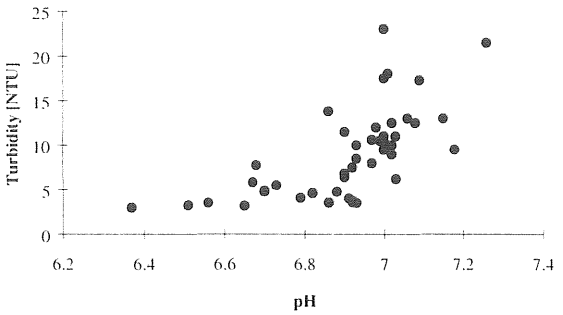
5.4 Effect of pH on the turbidity

5.4.1 Continuous experiments

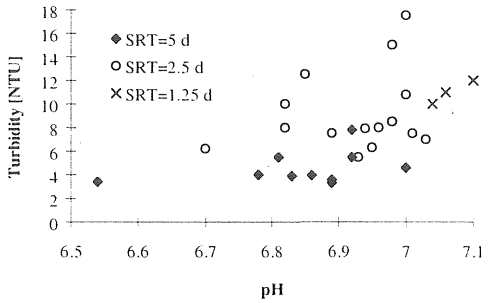
In the pilot plant experiments, it was noticed that there was a trend towards higher turbidities as the pH increased. The results from Part II-IV are shown in Figure 5.4-1.



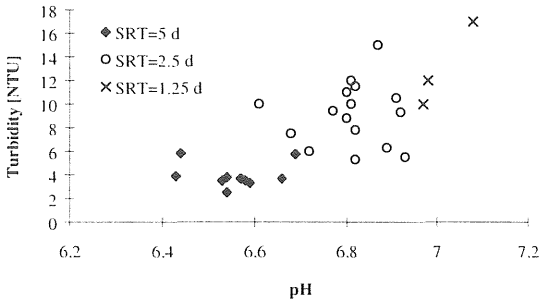
a) reactor A (1h, 2h and 4h correspond to the different alternating oxic/anoxic periods.



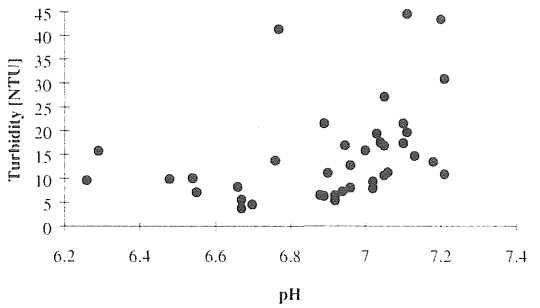
b) reactor B



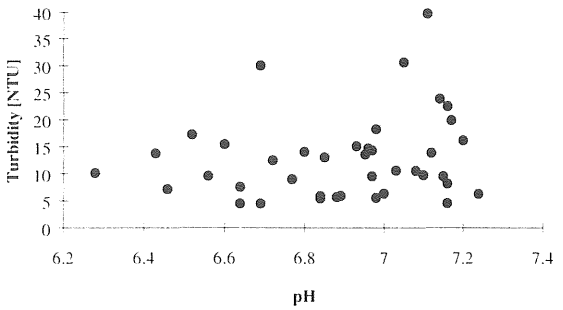
c) reactor A (SRT=1.25-5 days correspond to the different sludge ages).



d) reactor B (SRT=1.25-5 days correspond to the different sludge ages).



e) reactor A



f) reactor B

Figure 5.4-1 Turbidity versus pH: a-b) Part II; c-d) Part III; e-f) Part IV.

The pH in the aeration tank depends on the pH and alkalinity of the influent wastewater and on the alkalinity in the aeration tank. For domestic wastewater, the pH is to a large degree dependent on the lime-carbonic acid equilibrium. Therefore, changes in the alkalinity will affect the pH. Processes which affect the alkalinity in the aeration tank are: degradation of

organic material (production of CO_2), CO_2-stripping during aeration, nitrification, denitrification, production of biomass and chemical precipitation. The alkalinity of the wastewater depends mainly on the alkalinity of the drinking water, infiltration of stormwater and on ammonification.

In Part II-IV (alkalinity was not measured in Part I), there was a linear relationship between alkalinity and ammonium concentration (r^2 = 0.73, 0.84 and 0.88, respectively) and the alkalinity expressed as mg HCO_3^-/l was about 6.6-8.7 times the concentration of NH_4^+-N in mg/l.

The pilot plant was run at fairly short sludge ages (1.25-5 days), and no nitrification was expected. However, at favourable conditions such as high temperature and high DO concentrations, the system nitrified. The degree of nitrification is mainly dependent on substrate concentration, temperature, oxygen concentration, pH and the presence of toxic compounds. Further, nitrifying bacteria are more sensitive to low oxygen concentrations than heterotrophic bacteria (Henze *et al*, 1995). At a sludge age of 5 days a minimum temperature of 16-17 °C is necessary to achieve nitrification.

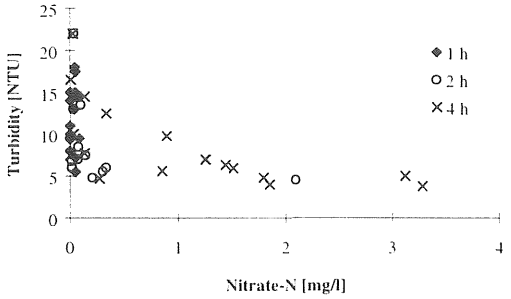
In *Part I*, hardly any nitrification took place. In *Part II*, nitrification started in the beginning of March and the reason could have been a slight increase in temperature (about 1-2 °C). The degree of nitrification increased gradually to 60-90% for reactor B and to 10-15% for reactor A (probably due to lack of oxygen during the anaerobic periods). This caused a drop in pH. The alkalinity of the influent wastewater is generally rather low at the Rya WWTP and during Part II-IV of the experiment it was on an average 240-260 mg HCO_3^-/l. Therefore, the pH will drop when nitrification occurs (i.e. the water has a rather low buffering capacity). In reactor B, there was a linear relationship between pH and alkalinity (r^2 = 0.82) and the decrease in alkalinity corresponded well with the formation of nitrate (a production of 1 mole NO_3^- corresponds to a consumption of about 2 mole HCO_3^-). In *Part III,* the degree of nitrification was 50-70% for reactor B and 15-20% for reactor A at a sludge age of 5 days. As the sludge age was decreased (first to 2.5 days and thereafter to 1.25 days), nitrification decreased to 2-11%. In *Part IV,* nitrification started in both reactors after about a month of operation. One month later nitrification was almost complete in both reactors.

No difference in COD removal could be found between reactor A and B in the different tests. According to Metcalf and Eddy (1995), oxygen is the limiting factor for nitrification when the DO concentration goes below 1 mg/l.

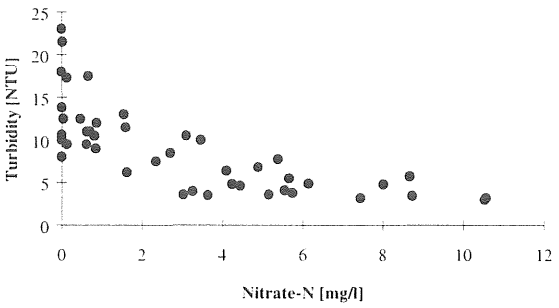
Examples of plots of turbidity against alkalinity and NO_3^- are shown in Figure 5.4-2 and 3. The same trend could be found in all experiments. The results show that there is a relationship between pH, alkalinity, NO_3^- concentration and the turbidity. One possible explanation could be that at slightly higher temperatures, the system starts to nitrify. At the same time, temperature probably has an impact on the turbidity. Further, low DO concentrations might decrease the degradation of organic material which produces a higher pH due to a decreased CO_2 production. This was however not observed. On the other hand, only grab samples were taken of the outlet composition since it did not seem to vary very much. The temperature was also measured once a day. The temperature probably changed slightly over the day and it was difficult to keep the tanks at a constant temperature. The temperature was just around the critical one necessary for nitrification which means that the system could have been extra

sensitive. Another possible explanation may be that nitrifying bacteria produce flocs which are well flocculated.

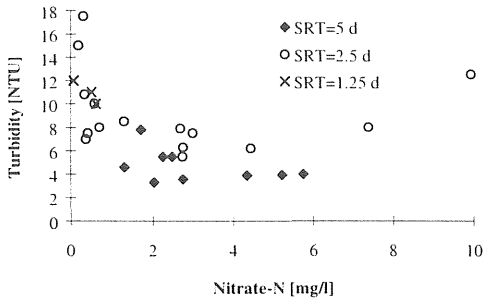
In order to be able to judge which parameter is responsible for the change in turbidity, more controlled systems have to be studied.



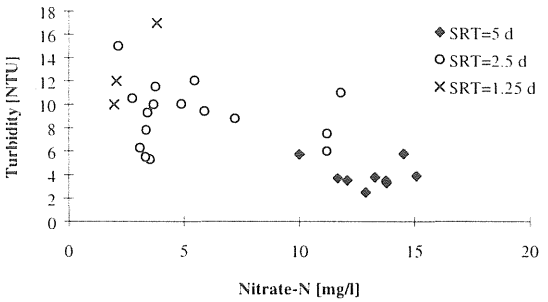
a) reactor A



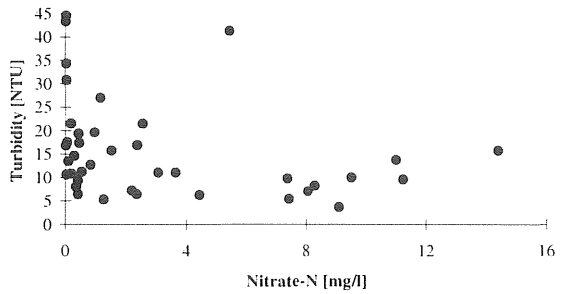
b) reactor B



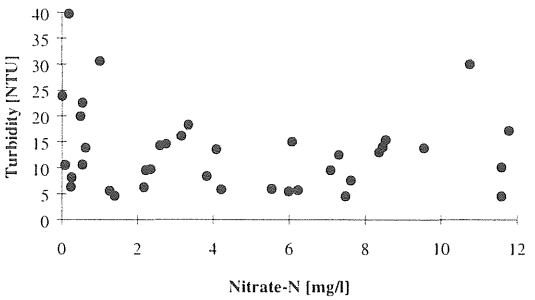
c) reactor A



d) reactor B

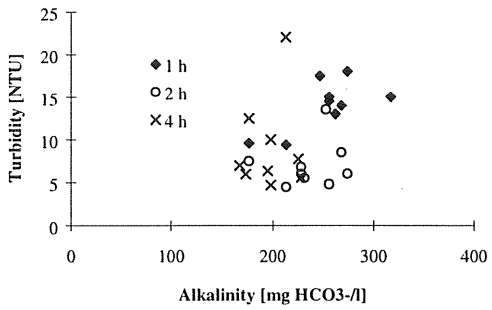


e) reactor A

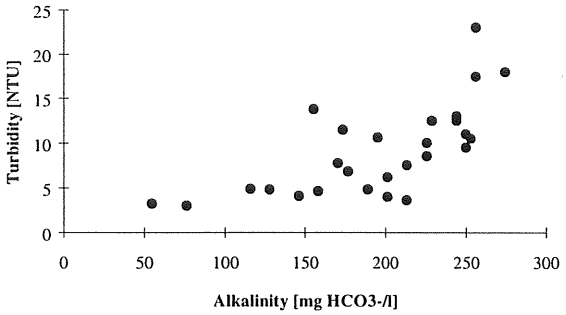


f) reactor B

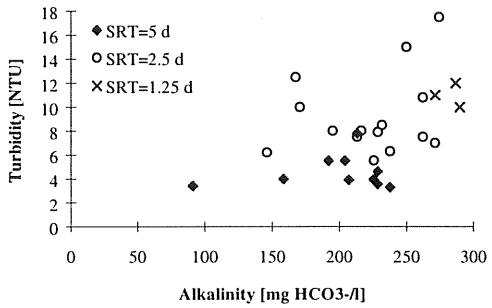
Figure 5.4-2 Turbidity versus nitrate concentration: a-b) Part II (the labels correspond to the different alternating oxic and anoxic periods for reactor A); c-d) Part III (the labels correspond to the different sludge ages); e-f) Part IV.



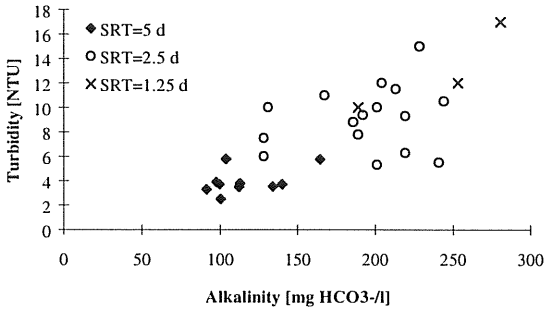
a) reactor A



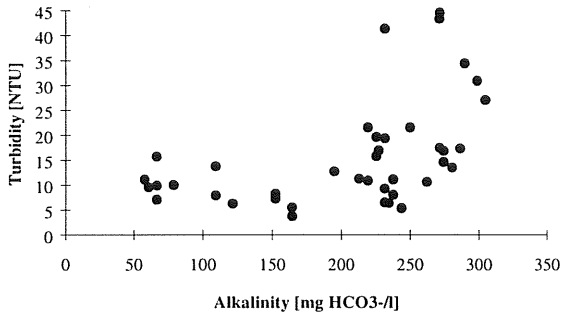
b) reactor B



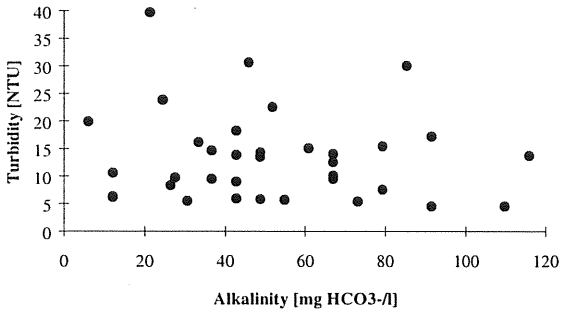
c) reactor A



d) reactor B



e) reactor A



f) reactor B

Figure 5.4-3 Turbidity versus alkalinity (mg HCO^-_3/l): a-b) Part II (the labels correspond to the different alternating oxic and anoxic periods for reactor A); c-d) Part III (SRT=sludge age, the labels corresponds to different sludge ages); e-f) Part IV.

5.4.2 Batch tests

To further verify the results from the previous section, a series of batch test were made to see how the pH affects the turbidity of the supernatant. NaOH and HCl was added to 1 litre beakers with activated sludge, to adjust the pH between 6 and 8. The results from one such test are shown in Figure 5.4.4. It seems as though there is a minimum in turbidity near a pH of 7 and that the turbidity increases as the pH decreases below a certain value (varied in the different tests from 6.4-6.8) as well as above a certain value.

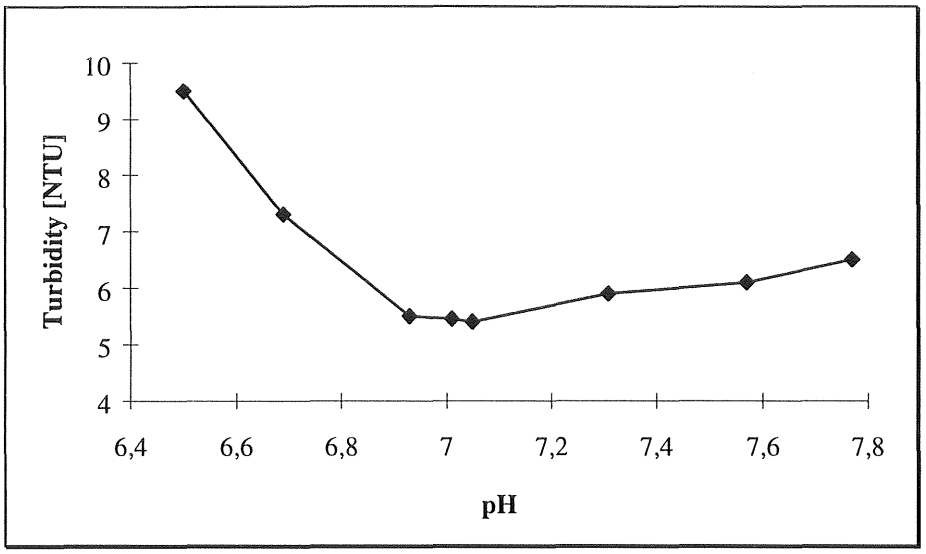


Figure 5.4-4 Turbidity of the supernatant at different pH (5 minutes stirring at 40 rpm; settling for 30 minutes).

The surface charge of activated sludge flocs is negative at neutral pH. Surface charge is obtained by ionization of functional groups of the exocellular polymers. The main constituents of the exocellular polymers are polysaccharides and proteins. The polysaccharides contain carboxyl groups (R-COOH) with pKa value of about 5 and the proteins contain amino groups ($R\text{-}NH_3$) and carboxyl groups with pKa values of about 9 and 2.5, respectively. The overall pKa value is than around 2-3 (Keiding, 1993). This means that at a pH of 7 the net surface charge is negative. A decrease in pH will reduce the net negative charge and an increase in pH will increase the net negative charge. The results show that turbidity increases with pH. The explanation for this could be that an increased negative surface charge increases the repulsive forces between the activated sludge flocs and the flocculation will be poorer. The turbidity increases at low pH could depend on cell lysis. Another explanation could be that Fe^{3+}ions in the activated sludge form complexes with hydroxide ions. Various complexes can be formed and the solubility for the different complexes is dependent on the pH. A change in pH could therefore affect the solubility of certain complexes and therewith change the turbidity in the supernatant.

The addition of NaOH and HCl changes the ionic strength of the solution and this might affect the turbidity as well (see section 3.3.1).

6 CONCLUSIONS

The following conclusions can be drawn from the results:

- The DO concentration has a large impact on the settling and thickening properties of activated sludge, mainly due to excessive growth of filamentous bacteria. Low DO concentrations produced less compact flocs than high DO concentrations; a difference was observed between 0.5 mg O_2/l and 2.0 mg O_2/l as well as between 2.0 mg O_2/l and 5.0 mg O_2/l.
- There was no clear correlation between DO concentration and average floc diameter. Only a trend towards larger flocs at higher DO concentrations could be found.
- Alternating oxic and anoxic conditions did not affect the settling and thickening properties of the activated sludge to any large extent.
- The turbidity of the supernatant (in the continuous experiments) was generally higher at low DO concentrations compared to at high DO concentrations. The reason for this could be floc dispersion, desorption of colloidal matter or increased dispersed growth of bacteria.
- It is difficult to study settling properties of activated sludge in a completely mixed pilot plant reactor due to problems with filamentous bacteria.
- The size distribution by volume of larger flocs in the activated sludge suspension fitted well to log-normal distribution functions.
- The size distribution by number of small flocs in the supernatant fitted well to power functions.
- The adsorption capacity of colloidal material in the wastewater onto the activated sludge flocs was greater at aerobic than at anaerobic conditions.
- The difference in adsorption capacity between high ($\geq$5 mg O_2/l) and low (<1 mg O_2/l) DO concentrations was small.
- Pre-aeration or periods of anaerobic conditions (0.5-2.5 hours) prior to mixing of activated sludge and pre-settled wastewater affected the adsorption capacity.
- The full scale experiments showed that low DO concentrations at the end of the aeration tank, increased effluent turbidity, while low DO concentrations in the first half of the aeration tank did not affect the turbidity to a large degree.

7 FUTURE STUDIES

The future work should concentrate on:

A more detailed study of what is happening with the bacteria in the activated sludge flocs when they are subjected to different dissolved oxygen concentrations. It would be interesting to study the change in composition and amount of the extracellular polymers which bind the bacteria together to flocs. It is also of interest to investigate what causes the increase in turbidity when activated sludge is subjected to anaerobic conditions (floc dispersion and/or desorption of particulate material). In these kinds of experiments, synthetic wastewater probably has to be used.

Further study of the mechanisms in the adsorption process: are the mechanisms physical and/or biological?

Study transient conditions to see how fast the floc properties and adsorption properties can change when the activated sludge flocs are subjected to changing DO concentrations.

Study the effect of DO concentration at longer sludge ages.

Study how the settling properties are changing in nutrient removal plants. This study showed that alternating oxic and anoxic conditions did not affect the settling properties significantly. However, practical experience shows that the introduction of nitrogen and biological phosphorus removal (processes with alternating oxic and anoxic conditions) often leads to changes in the settling properties. The conditions in the pilot plant used in this study. differed, however, from the ones in nutrient removal systems.

In the last few years, new "environmentally friendly" chemicals have been introduced on the market. This has changed the composition of the wastewater. Chemicals such as detergents might affect the surface of the activated sludge flocs which could affect processes such as oxygen transport through the flocs, adsorption properties of the activated sludge flocs and the bioflocculation processes.

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Appendix I

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THE EFFECT OF DISSOLVED OXYGEN CONCENTRATION ON THE SETTLING PROPERTIES OF ACTIVATED SLUDGE

Effekten av syrehalt på sedimenteringsegenkaperna hos aktivt slam

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Abstract

Long-term effects of dissolved (DO) concentration on the settling properties of activated sludge were studied in continuous completely-mixed pilot-plant reactors. Lower DO concentrations (0.5–2.0 mg/l) gave poorer settling properties and higher turbidities of the effluent than higher DO concentrations (2.0–5.0 mg/l). The main reasons to the deteriorated settling properties were excessive growth of filamentous bacteria and porous flocs. Alternating oxic and anoxic conditions (1–4 hours) did not effect the settling properties to a large extent. No clear relationship could be found between DO concentration and average floc diameter. The volume distribution of flocs in the size range 11.6–1128 µm fitted well to log-normal distribution functions. Flocs in the supernatant, after 20 minutes settling, had a diameter up to 50–60 µm. More than 80 % of the number of flocs were ≤ 2 µm, in most of the measurements. The size distribution of the flocs in the supernatant could best be fitted to power functions. The number of flocs in the supernatant after 20 minutes settling could relatively well be related to the turbidity.

Key Words – wastewater, activated sludge, settling, dissolved oxygen concentration, floc size distribution, settling properties, filamentous microorganisms.

Sammanfattning

Olika syrehalters inverkan på sedimenteringsegenskaperna hos aktivt slam, studerades i totalomblandade reaktorer i pilot-skala. Lägre syrehalter (0.5–2.0 mg/l) gav sämre sedimenteringsegenskaper och högre turbiditet i utflödet än högre syrehalter (2.0–5.0 mg/l). Huvudorsaken till de försämrade sedimenteringsegenskaperna var tillväxt av filamentbakterier och bildandet av porösa flockar. Alternerande oxiska och anoxiska förhållanden (1–4 timmar) påverkade inte sedimenteringsegenskaperna nämnvärt. Inget klart samband mellan syrehalt och medelflockstorlek fanns. Volymsfördelningen av flockar i storleksintervallet 11.6–1128 µm kunde väl passas till log-normalfördelningsfunktioner. Flockar i supernatanten efter 20 minuters sedimentering hade en diameter på upp till 50–60 µm. I de flesta mätningarna var mer än 80 % av antalet flockar ≤ 2 µm. Storlekfördelningen av flockarna i supernatanten kunde bäst passas till potensfunktioner. Antalet partiklar i supernatanten efter 20 minuters sedimentering kunde relativt väl relateras till turbiditeten.

Introduction

In wastewater treatment, the quality of the effluent is to a large degree dependent on the separation of activated sludge from the mixed liquor. Organic matter discharged from activated sludge plants is mainly particulate. Apart from exerting an oxygen demand, particles in the effluent may contain particulate bound phosphorus. The effectiveness of the separation process is mainly dependent on the ability of the activated sludge to form flocs. Often, however, flocs with poor settling properties occur and the most common reasons for settling problems are: pin-point flocs (Pipes, 1979); filamentous microorganisms (Eikelboom and van Buijsen, 1981); Zoogloea bacteria (Novák et al, 1993, 1994); rising sludge (Henze et al, 1993) and scumming (Kappeler and Gujer, 1994; Blackall, 1994).

Most research within this field has focused on the growth of filamentous bacteria (Eikelboom and van Buijsen, 1981; Wanner, 1994) while less attention has been given to the size, size distribution and structure of non-filamentous activated sludge flocs (Li and Ganczarczyk, 1993; Ganczarczyk, 1994; Urbain et al, 1993). The variations in particle size, density and porosity have a large impact on the settling properties. Various process parameters are known to affect the size and structure of activated sludge flocs: sludge age (Bisogni and Lawrence, 1971; Lovett et al, 1983; Cashion and Keinath, 1983; Knocke et al, 1986; Sheintuch et al, 1986); organic loading (Pipes, 1979; Li and Ganczarzyk, 1993; Barbusiński and Koscielniak, 1995); turbulence (Parker et al, 1971; Koníček and Burdych, 1988; Galil et al, 1991; Wahlberg, 1992; Das et al, 1993); dissolved oxygen concentration, DO, (Starkey and Karr, 1984;

Knudson et al, 1982) and starvation (Ericsson and Eriksson, 1988; Echeverría et al, 1993).

Surprisingly few studies have been carried out which relate the concentration of dissolved oxygen (DO) to the size and structure of activated sludge flocs. It has been shown (Starkey and Karr, 1984) that low DO concentrations inhibit the exocellular polymer production and lead to a poorer flocculated activated sludge, a more turbid effluent and a decreased adsorptive capacity of colloidal material onto the flocs. The operators of the Rya WWTP have reported in personal communication that they are convinced, from their operation experience, that there exists a relationship between DO concentration and effluent turbidity. In activated sludge plants built for nutrient removal, settling problems often occur (e.g. Andreasen and Sigvardsen, 1996; Hoffman. 1987), mainly due to excessive growth of filamentous bacteria. In these processes the activated sludge is subjected to alternating oxic and anoxic conditions which may affect the structure and size of the activated sludge flocs. Little is, however, known about this.

The size and size distribution of activated sludge flocs may change as a result of changes in the process conditions. Therefore, following the variation in floc size may be a valuable tool in understanding what process conditions affect the size of activated sludge flocs. Various methods have been used to measure the size of activated sludge flocs: direct microscopic observation with an eyepiece micrometer (Barbusiński and Koscielniak, 1995; Sadalgekar et al, 1988); photographs of individual flocs (Li and Ganczarczyk, 1987; Magara et al, 1976); image analysis system (Zahid and Ganczarczyk, 1990; Li and Ganczarczyk, 1991); Coulter counter (Li and Ganczarczyk, 1991; Andreadakis, 1993) and laser beam diffraction (Jorand et al, 1995). It is difficult to measure the size of activated sludge flocs since they are heterogeneous and the size range is very broad. Difficulties are also encountered in sampling to avoid reflocculation and other physical alterations of the flocs.

The objective of this study, was to investigate how different DO concentrations as well as alternating oxic and anoxic conditions influence the settling and thickening properties of activated sludge. This was studied in pilot-scale continuous reactors fed with domestic wastewater. The experiment was divided into four parts:

- part I: effect of low DO concentration;
- part II: effect of alternating oxic and anoxic conditions;
- part III: effect of DO at different solids retention times;
- part IV: effect of high DO concentrations.

As a measure of settling and thickening properties, the following parameters have been used: sludge volume index (SVI), initial settling velocity, turbidity in the supernatant after settling, particle size measurements and microscopic investigation. The SVI and initial settling velocity give information about the thickening and settling properties, while the size distribution and turbidity give information about the clarification properties. The floc sizes were measured with two types of laser beam diffraction apparatuses: flocs in the supernatant after settling (1–200 µm) were measured with a Met One instrument and the larger flocs (11.6–1128 µm) were measured with a Malvern instrument.

Materials and Methods

Experimental set-up

The activated sludge pilot-plant (Figure 1) consisted of a completely-mixed aerated tank (18 l) coupled with a secondary settler (volume 20 l; surface area 0.049 m^2). Return sludge was pumped continuously from the settler to the aeration tank with a ratio of 0.8–1.0 times the influent flow. Excess sludge was withdrawn directly from the aeration tank. The DO concentration was automatically regulated by means of a valve connected to a DO-meter and a tube with pure oxygen gas. The DO-regulator was set at an on/off mode with a hysteresis of ± 0.4 mg/l. Mixing was performed by means of a propeller stirrer (diameter = 10 cm; 148 rpm). The pilot-plant was fed with domestic pre-settled wastewater from Rya wastewater treatment plant (WWTP) in Göteborg, Sweden. Rya WWTP is a conventional activated sludge plant receiving wastewater from approximately 550,000 inhabitants and 220,000 equivalents of industry. To simplify the pumping of wastewater to the aeration tank, the pre-settled wastewater was continuously pumped to a feed tank (with a hydraulic retention time (HRT) of ca 1.2 hours), placed in a fridge at a temperature of 4°C. In part II–IV of the experiment, a pilot-plant pre-settler (volume 0.1 m^3; HRT 30 min; overflow rate 2 m/h) was installed in front of the feed tank into which raw wastewater was pumped. This was to avoid precipitation chemicals, which were dosed to the influent at Rya WWTP at that time, into the pilot-plant. A selector (volume 0.6 litre; HRT 6 minutes) was installed in front of each reactor in part II–IV of the experiment. To avoid warming of the reactors, they were placed in a water-bath through which tap-water was pumped.

In part I of the experiment, one pilot-plant was operated, while in part II–IV, two pilot-plant set-ups were operated in parallel: conditions were changed in reactor A while reactor B served as a reference.

Start-up

Initially, the pilot-plants were inoculated with activated sludge from Rya WWTP. The pilot-plants were run for at least three sludge ages to establish steady-state conditions.

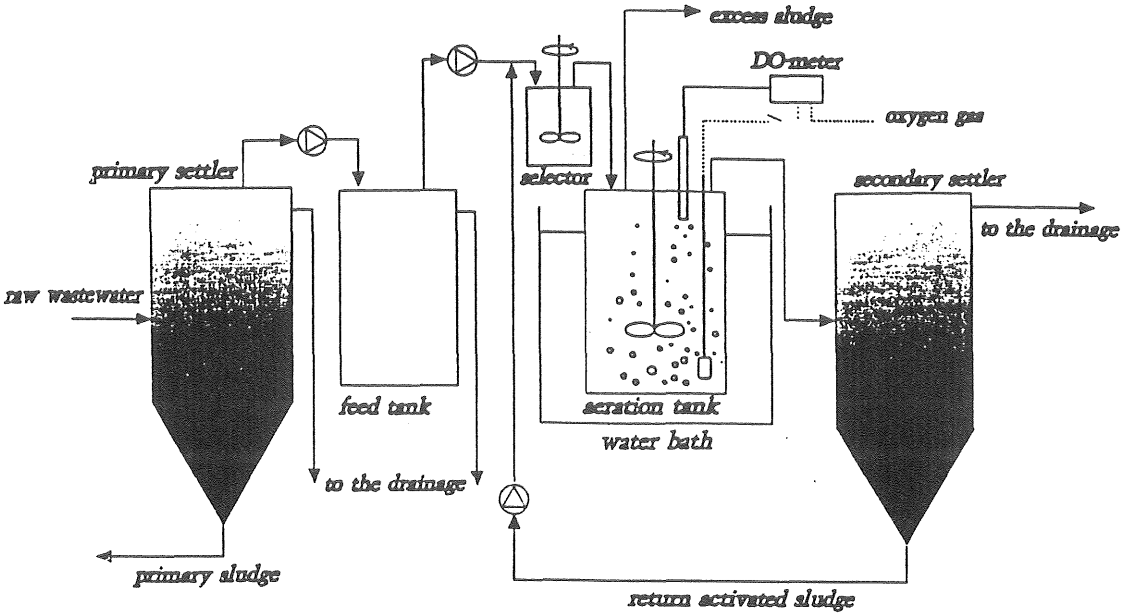

Figure 1. *Schematic drawing of the pilot-plant.*

Analyses

The performance of the reactors was followed by regularly (about 5 times per week) measuring chemical oxygen demand (COD) in unfiltered influent and in filtered effluent (Munktell filter paper no. 413005) according to Swedish Standard SIS 02 81 42; ammonium and nitrate in filtered influent and effluent with a Technicon II auto-analyzer (Zander & Ingeström: industrial method no. 857–871); alkalinity in unfiltered influent and in filtered effluent by titrating with 0.1 M HCl to pH 4.0; total phosphorus in unfiltered influent by adding Oxisolv and Phosver 3 reagens and measuring with a DR2000 Hach spectrophotometer; mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) according to Swedish Standard SS 02 81 12; turbidity after 20 and 60 minutes settling with a Hach turbidimeter. The analyses of the influent composition were made on 24-hour samples and the analyses of the effluent were made on grab samples. Sludge volume index (SVI) and initial settling velocity was measured in a 1 litre graduated cylinder (∅ = 60 mm) according to Standard Metods (nr 213). The sludge volume (SV), which is the volume (in ml) occupied by sludge after 30 minutes settling in a 1 l graduated cylinder, was diluted if it exceeded 300 ml before measuring the initial settling velocity. Temperature, DO and pH were measured continuously (Satron Instruments: POT200 and PH200). The instruments were calibrated twice a week.

Microscopic studies

The flocs were observed daily in a phase contrast microscope (Olympus BX40) equipped with an eyepiece micrometer for average floc size estimation. Only flocs with a longest dimension of about 50 µm were considered. The morphology of the flocs was judged by means of a classification scheme in which characteristics such as floc form, compactness (1=very compact, very round; 5=very porous, very irregularly shaped), average size, fraction of dispersed growth, fraction of Zoogloea and filamentous bacteria number of spiral bacteria, number/types of protozoa, number of rotifera and nematodes were classified on a scale from 1–5 (1=no present; 5=excessive numbers). For documentation, photos were regularly taken of the activated sludge flocs. The activated sludge was stained according to standard staining techniques (Gram+/Gram–, Neisser, PHB) a few times to be able to judge which types of filaments were present (Eikelboom and van Buijsen, 1981).

Floc size measurements

The floc sizes were measured with two types of laser beam diffraction apparatuses: flocs in the supernatant after settling (2–100 µm) were measured with a Met One (WGS-260) instrument (samples were always taken 10 cm below the water surface of the 1 litre cylinder) and the large flocs in the activated sludge suspension (11.6–1128 µm) were measured with a Malvern instrument. Due to the large size range of activated sludge

Table 1. *Process operation parameters.*

Experiment	Duration [days]	DO [mg/l]	SRT [day]	HRT (aeration tank) [hour]	HRT (settler)• [hour]	F/M [gCOD/gM LSS·d]
Part I	78	0.5–2	5	7	4	0.57±0.2
Part II: reactor A	90	0–4 (alternating periods: 1 h, 2 h, 4h)	5	7.5	4	0.99±0.4
Part II: reactor B	90	4	5	7.5	4	0.97±0.3
Part III: reactor A	48	0.5	1.25–5	5	3	0.88±0.1–1.92±0.05
Part III: reactor B	48	2	1.25–5	5	3	1.0±0.2–1.7±0.2
Part IV: reactor A	33	2	5	5	3	0.99±0.3
Part IV: reactor B	33	5	5	5	3	0.87±0.3

flocs, all floc sizes cannot be measured simultaneously. The Malvern instrument can measure different size intervals by changing lens; from 1128 µm down to 1.2 µm. However, the concentration of suspended solids in the supernatant was lower than the minimum concentration needed for measurement. This made it necessary to use an other instrument which can measure low concentrations of suspended solids. The Met One instrument uses a light blocking system in which the decrease in light corresponds to the particle size. A detector counts and categorizes the particles by size and the data is given as *frequency by number*. The Met One instrument can measure a maximum of 16,000 particles per ml, which made it necessary to dilute the samples with destilled water before measurement (20–100 times). The Malvern instrument use light scattering. Depending on the size of the particle, the diffraction pattern has different appearances. The diffraction pattern is computerized to give the *frequency by volume*. Both instruments assume that the particles are spherical. The samples for the Met One instrument were analysed directly after harvesting and the samples for the Malvern instrument were analysed within 60 minutes. The average floc size was also estimated by means of microscopic measurements.

Results and Discussion

The pilot-plant studies were run for a period of about one year. In Table 1–2, the duration of the different experiments and the process conditions are summarized. Although the objective was not to achieve nitrification, some ammonium was converted to nitrate, especially in the late spring and in the summer.

Change in SVI and floc morphology

Part I

The DO concentration was decreased from 2 mg/l to 0.5 mg/l over a period of about three moths. At a DO concentration of 2 mg/l, the activated sludge flocs gradually changed from being small, irregularly shaped and fragile to being more compact, regularly shaped and large. There were filaments present, but not in excessive numbers (category 2 = small number). The change in SVI is illustrated in Figure 2. As the DO concentration was decreased from 2 to 1 mg/l, filamentous and Zoogloeal microorganisms started to grow in larger numbers (from category 2 to 3) and the SVI increased to about 220 ml/g. The dominating type was by a microscopic inves-

Table 2. *Summary of the process conditions for the different experiments.*

Exp.	pH [–]	Temp. [°C]	COD (infl) [mg/l]	COD (effl) [mg /l]	COD-red [%]	MLSS [g/l]	VSS/ MLSS [–]	HCO_3^- (infl) [mg/l]	P-tot (infl) [mg/l]	NH_4^+ (infl) [m g/l]
Part I	6.9±0.2	15±3	208±53	60±10	70±8	1.6±0.4	0.66±0.07	–	–	18±5
Part II										
reactor A	7.01±0 .08	15.5±1.5	286±76	68±18	75±7	1.0±0.4	0.75±0.04	241±40	3.4±0.7	23±5
reactor B	6.96±0 .17	15.5±1.5	286±76	64±18	77±6	1.0±0.3	0.75±0.04	241±40	3.4±0.7	23±5
Part III										
reactor A	6.9±0.1	18±1.3	292±65	57±14	80±5	1.3±0.3	0.72±0.018	260±30	4.4±0.9	21±3
reactor B	6.7±0. 2	18±1.3	292±65	56±14	80±5	1.3±0.3	0.73±0.019	260±30	4.4±0.9	21±3
Part IV										
reactor A	6.9±0. 2	18±1	296±85	69±21	75±9	1.5±0.3	0.74±0.04	260±53	5.1±1.2	23±6
reactor B	6.9±0.2	18±1	296±85	65±18	76±9	1.7±0.3	0.73±0.06	260±53	5.1±1.2	23±6

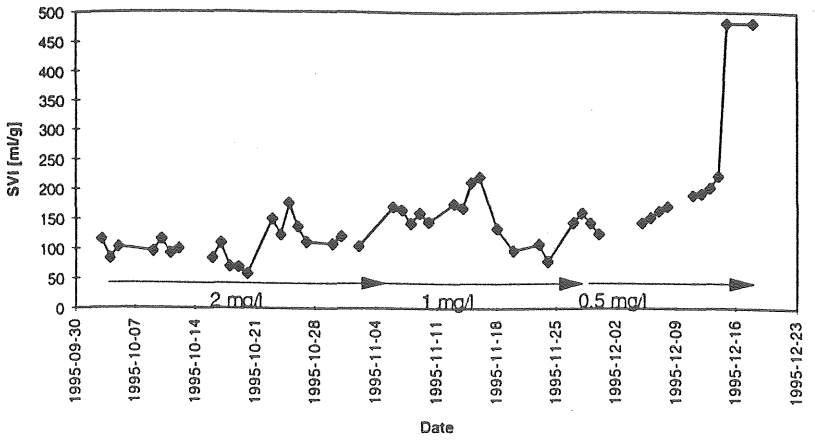

Figure 2. *Change in SVI for part I of the experiment.*

tigation judged to be Sphaerotilus natans and the second most common filaments were probably Thiothrix I–II (or possibly type 021N). There was a sudden drop in SVI after 10 days (16–18th of Nov.) of operation at 1 mg O_2/l, which was due to a process failure. This led to anaerobic conditions for about 20 hours which damaged the flocs and many of the filamentous and Zoogloea bacteria disappeared. When the DO concentration was further decreased to 0.5 mg/l, the number of Zoogloea and filamentous bacteria increased dramatically (category 5). The SVI increased to almost 500 ml/g within 15 days and the flocs became porous.

Part II

Three different lengths of alternating oxic/anoxic periods were studied: 1 h, 2 h and 4 h (the alternation took place in reactor A and reactor B was operated at a constant DO concentration). During the start-up period, the DO concentration (2 mg/l) was the same in the both reactors. After about 10 days of operation, large numbers of filamentous microorganisms (category 5) started to grow in the same proportions in both reactors and the SVI increased from about 100 to 800 ml/g. Staining and microscopic investigation indicated that the dominating filament was Sphaeotilus natans and the second most common filaments were probably Thiothrix I–II (or possibly type 021N). To combat this, a selector in which return sludge and influent wastewater was mixed (without aeration), was installed in front of each reactor (hydraulic retention time ≈ 6 minutes). This succesfully reduced the numbers of filaments within three sludge ages. Thereafter, the DO concentration was increased to 4 mg/l for the rest of the experiment to avoid filaments. When the SVI had decreased to 50–60 ml/g, the alternating period of 1 hour was initiated (there were small numbers of filaments present: category 2).

There was no large difference in SVI between reactor A (alternating oxic/anoxic) and reactor B (Figure 3). Alternating oxic/anoxic conditions gave slightly higher SVI. At alternating periods of 1 and 2 hours there were

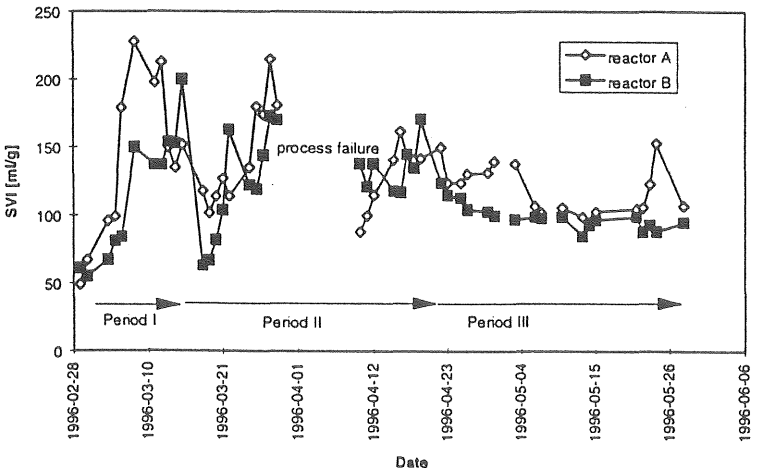

Figure 3. *Change in SVI for part II of the experiment* (period I=1 h; period II=2 h; period III=4 h; A: alternating oxic/anoxic conditions; B: constant oxic conditions).

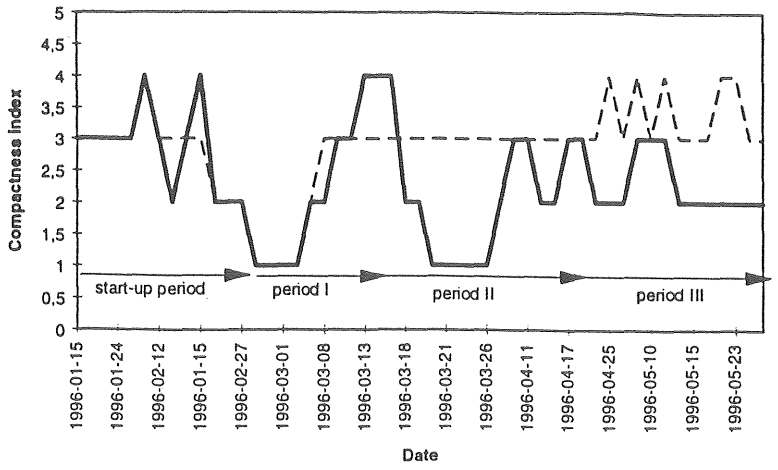

Figure 4 a. *Compactness index for part II of the experiment* (dotted line = A; continuous line = B; period I = 1 h; period II = 2 h; period III = 4 h; A: alternating oxic/anoxic conditions; B: constant oxic conditions).

slightly less numbers of filaments present than at constant aerobic conditions and at an alternating period of 4 hours it was the other way round. The microscopic investigation showed also that longer (2–4 hours) alternating periods produced less compact flocs than at a constant DO concentration (Figure 4 a). The change in SVI followed the same pattern for reactor A and B which indicates that it was something in the wastewater and/or in the reactor design that favoured the growth of filamentous bacteria. The slightly lower SVI for reactor B was mainly due to the higher compactness of the flocs compared to the ones in reactor A. There were plenty of protozoa present in both reactors throughout the experiment and there was no difference in the types present (different types of attached and free-swimming ciliates). In period III, there were large numbers of spirochetes present in reactor A, which could indicate lack of DO.

Part III

The effect of DO concentration was studied at three different solids retention times (SRT): 5, 2.5 and 1.25 days. In reactor A the DO concentration was kept at 0.5 mg/l and in reactor B at 2 mg/l. At a sludge age of 5 days, the SVI increased in both reactor A and B (Figure 5). It increased from about 100 ml/g to 500 ml/g within 5 days for reactor A and within 15 days for reactor B. There were filamentous bacteria present in both reactors, but the numbers were larger in reactor A (category 2–4 in A and 2–3 in B). The dominating types of filaments were the same as in part II of the experiment. Before the sludge age was decreased to 2.5 days, the activated sludge in reactor B was divided into two and the activated sludge in reactor A was thrown away. The change in sludge age caused a sudden drop in SVI and the number of filaments was drastically reduced. About three sludge ages later, the SVI started to increase in both reactor A

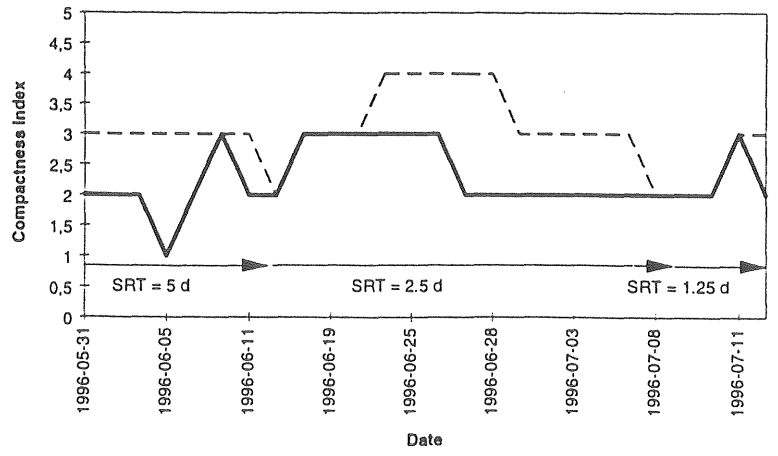

Figure 4 b. *Compactness index, part III* (dotted line=A; continuous line=B; A: 0.5 mg O_2/l; B: 2.0 mg O_2/l).

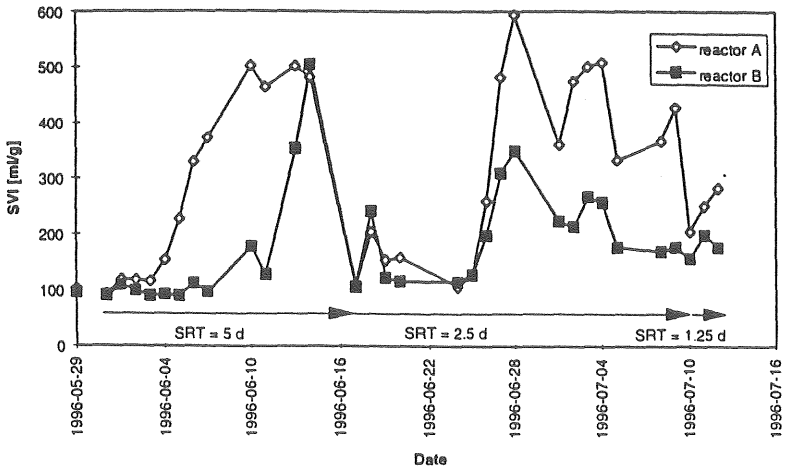

Figure 5. *Change in SVI for part III of the experiment* (A: 0.5 mg O_2/l; B: 2.0 mg O_2/l).

and B (from about 100 ml/g to 600 and 350 ml/g, respectively). There was the same amount of filaments in both reactors (category 3–4), but the flocs were more compact in reactor B. Similar results were obtained when the SRT was decreased from 2.5 to 1.25 days (Figure 4 b).

Part IV

Reactor A was run at a DO concentration of 2 mg/l, which in general is considered as being enough to avoid oxygen limitation. Reactor B was run at a DO concentration of 5 mg/l. After about ten days the SVI increased, unexpectedly, in reactor B which was run at a DO concentration of 5 mg/l (Figure 6). Filamentous bacteria started to grow in large numbers (category 4). The were many types of filamentous bacteria present but the dominating type was Sphaerotilus natans. There were also other types of filaments present like 021N and Thiothrix I–II. However, two sludge ages later, they suddenly disappeared. No explanation to this could be found. Some ten days later, the SVI increased dramatically in reactor A due to excessive numbers of filamentous bacteria (category 4). There were the same types of filaments present as previously plus a new type which was Gram-negative, Neisser-negative containing PHB granules (a food-storage polymer, poly-β-hydroxybutyric acid,, which some bacteria can produce). Further they had rounded cells which indicates that it could have been type 1701 which is known to proliferate at low DO concentrations. The activated sludge flocs were very porous and they did not settle at all. The SVI in reactor B remained at a lower level (80–180 ml/g). When the DO concentration in reactor A was increased to 5 mg/l, the SVI decreased to 150 ml/g within three days. Thereafter the both reactors were run at the same conditions for about three sludge ages to see if this would produce sludge with similar characteristics. The SVI remained low and there were hardly any filamentous bacteria present. The flocs were also very round and compact. During the major part of the experiment, the floc com-

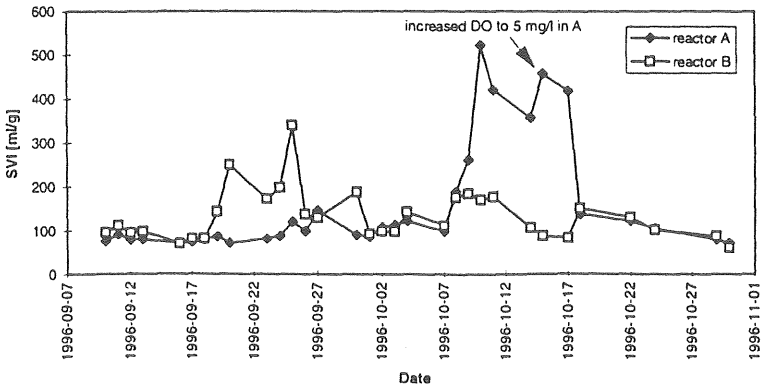

Figure 6. *Change in SVI for part IV of the experiment* (A: 2.0 mg O_2/l; B: 5.0 mg O_2/l).

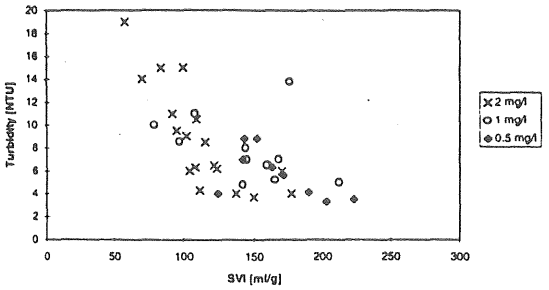

Figure 7. *Turbidity vs SVI, part I.*

pactness was higher in reactor B than in reactor A. There were large numbers of protozoa present throughout the experiment and there was no difference between reactor A and B.

The proliferation of filamentous bacteria is a big problem when operating completely-mixed pilot-plants. Sphaerotilus natans is rather rare in full-scale plants but very common in pilot-plants. They are favoured by low DO concentrations. Thiothrix I–II and type 021N are known to be favoured by sulphides and lack of nutrients like N and P. In these studies there was probably no lack of N and P while the wastewater occasionally smelled of sulphides. During period II–IV of the experiment, there was no or little rain and the wastewater was more concentrated, and anaerobically processes took probably place in the sewer system.

Turbidity

Part I

No correlation could be found between the DO concentration and the turbidity in the supernatant after settling. The turbidity seemed to be more affected by the structure of the activated sludge flocs; excessive growth of filamentous and Zoogloael microorganisms causes a sweep-effect; small flocs are enmeshed in the network of filaments and Zoogloeal microorganisms (Eriksson et al, 1992). In Figure 7, the turbidity is plotted against the SVI, and this shows a trend towards lower turbidities at higher SVI.

Part II

No large difference in turbidity of the supernatant after settling could be noticed between the two reactors. When the turbidity in the supernatant was followed over the 2 h and 4 h periods, a clear difference could be noticed: the turbidity decreased gradually during the oxic period and increased during the anoxic period. This happened both in the presence and absence of influent wastewater (Figures 8 a–b). The turbidity increase at anoxic conditions when the influent was turned off could be a result of floc dispersion and/or desorption of adsorbed matter in the wastewater. The pH was also followed over the cycles and it increased directly upon a decrease in DO concentration. This might change the surface charge of the activated sludge flocs and thus affecting the flocculation process.

Part III

The turbidity was during the major part of the experiment higher at a DO concentration of 0.5 mg/l than at 2 mg/l. However, the difference was not very large. Further, the turbidity increased as the SRT was decreased from 5 to 1.25 days. The decreased SRT gave increased organic loadings (expressed as g COD/g biomass · d). In Figure 9 the turbidity is plotted against the organic loading. Large numbers of filamentous microorganisms were present in reactor A and this might have produced lower turbidities due to sweep effects.

Part IV

The turbidity was higher in reactor A than in reactor B during the major part of the experiment (Figure 10).

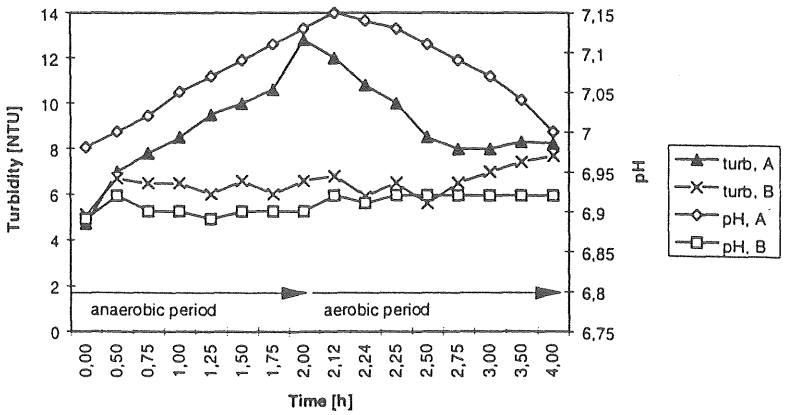

Figure 8 a. *Change in turbidity and pH during a 4-hour-cycle with influent, part II of the experiment* (A: alternating oxic/anoxic conditions; B: constant oxic conditions).

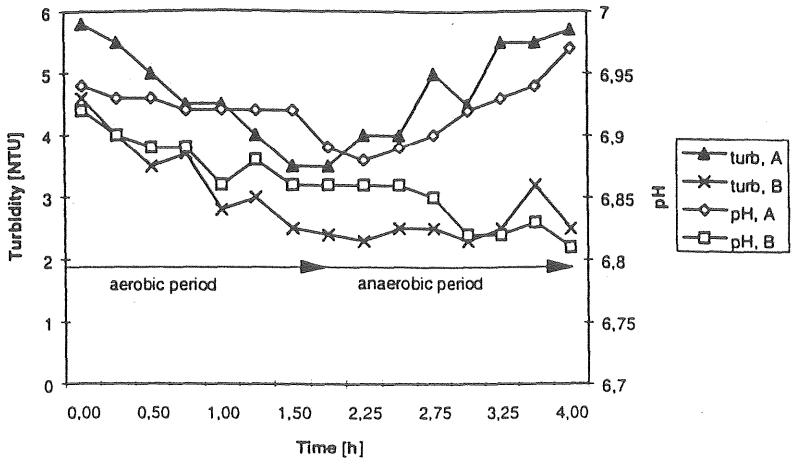

Figure 8b. *Change in turbidity and pH during a 4-hour-cycle without influent, part II of the experiment* (A: alternating oxic/anoxic conditions; B: constant oxic conditions).

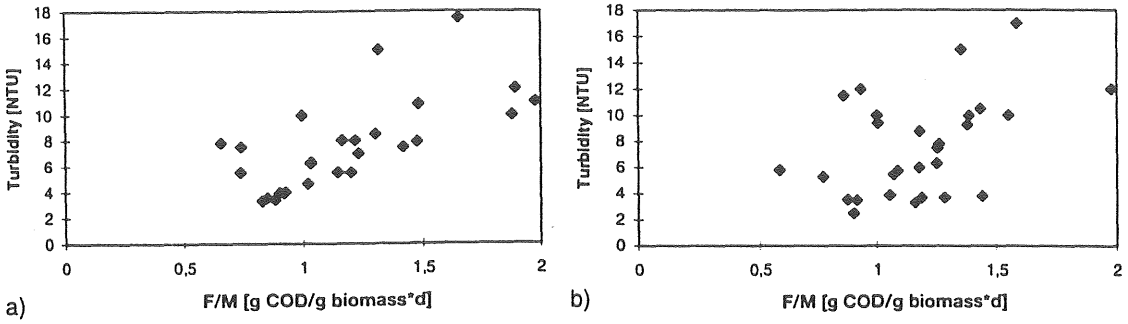

Figure 9. *Turbidity vs organic loading, part III;* a) reactor A, 0.5 mg O_2/l; b) reactor B, 2 mg O_2/l.

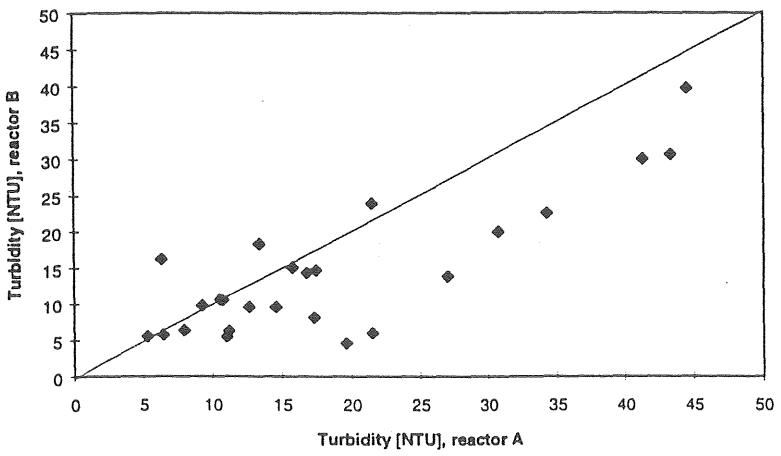

Figure 10. *Turbidity after 20 minutes settling, part IV* (A = 2 mg O_2/l; B = 5 mg O_2/l.

Floc size and size distribution

Activated sludge flocs (11.6–1128 µm)

The shape of the size distributions (based on floc volume) for flocs larger than about 11.6 µm had a similar form in all measurements except that they were shifted more or less to the right. A typical example of a size distribution of activated sludge flocs is depicted in Figure 11. The average diameter of the activated sludge flocs were large in all experiments (200–500 µm). Further,

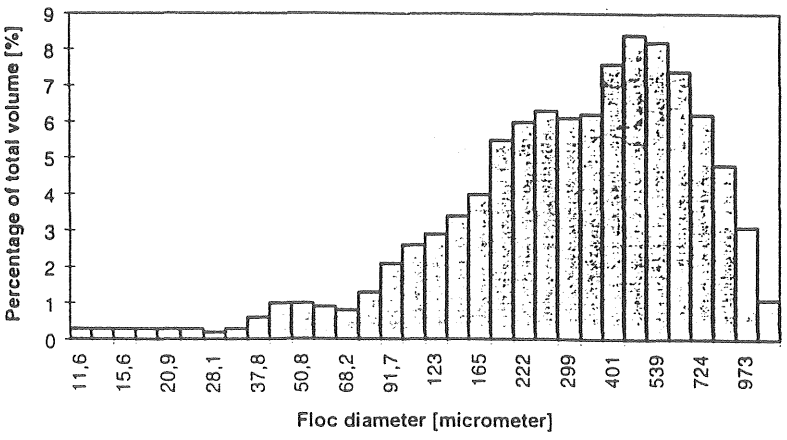

Figure 11. *Example of a size distribution by volume.*

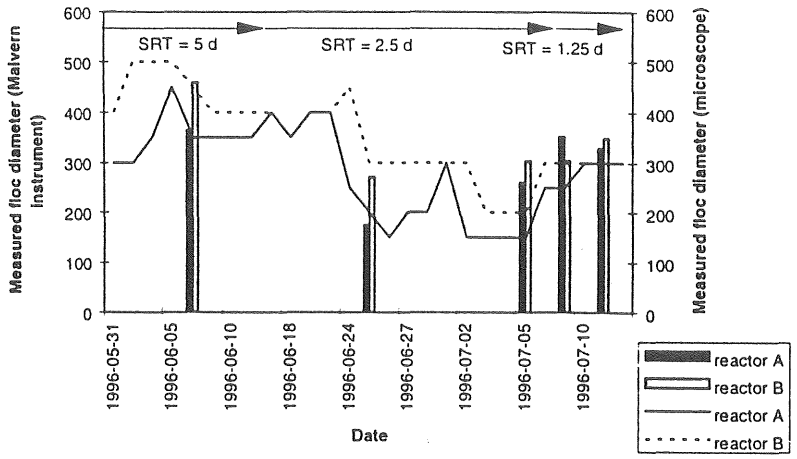

Figure 12. *Average floc diameter, part III* (line = microscopic measurement; bar = particle analyser measurement).

there were during the major part of the experiment filamentous bacteria present which seems to produce large flocs. Table 3 summarizes the percentage of floc volume in six different size intervals. In part I, the distribution was shifted towards smaller flocs after a process failure which coincided with the decrease in DO concentration to 0.5 mg/l. Activated sludge flocs with an average floc diameter > 100 µm contributed to most of the volume.

The microscopic measurement agreed quite well with the particle analyser measurements (Figure 12–13).

Part I

The activated sludge flocs were large (about 300 µm) and irregularly shaped during the experiment (except for a period after a process failure which led to floc dispersion due to DO depletion). No relationship between

Table 3. *Volume distribution of flocs into six different size intervals.*

Size interval [µm]	Percentage of total volume [%]				Average
	I	II	III	IV	I–IV
11.6–50.8	3–11	1–5	3–9	8–11	5
50.8–123	2–29	6–13	6–23	21–33	13
123–222	12–57	10–24	12–33	27–39	23
222–346	16–42	12–28	14–26	21–23	22
346–539	47–75	25–40	21–41	12–23	34
>539	25–55	20–59	5–47	7–19	34

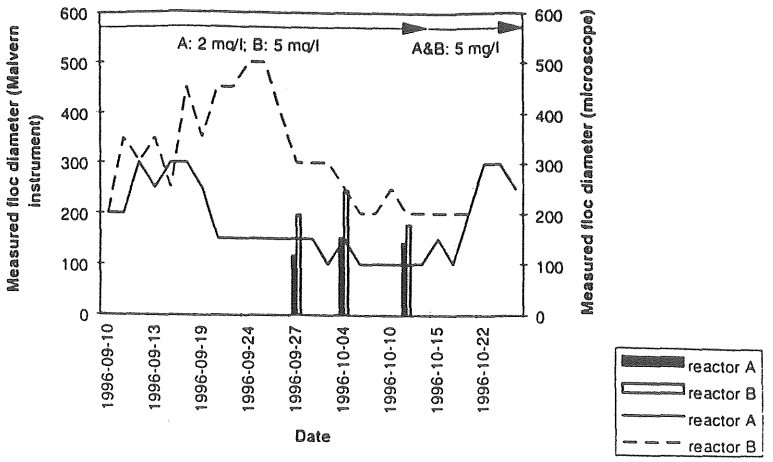

Figure 13. *Average floc diameter, part IV* (line = microscopic measurement; bar = particle analyser measurement).

floc size and DO concentration could be found. This may be explained by the fact that Zoogloeal and filamentous microorganisms produce large flocs. However, to eliminate the effect of the variability of the wastewater composition, two reactors have to be operated in parallel.

Part II

The average floc size was large in both reactors. Alternating oxic and anoxic periods did not seem to affect the floc size significantly; they were only 3–20 % larger in reactor B.

Part III

The change in floc size followed the same pattern for both reactors. A DO concentration of 2 mg/l produced slightly larger flocs than a DO concentration of 0.5 mg/l (6–50 % larger). As the SRT decreased from 5 to 2.5 days, the average floc diameter decreased. A further reduction of the SRT to 1.25 days did, however, not cause even smaller flocs. The results are illustrated in Figure 12.

Part IV

A DO concentration of 5 mg/l produced larger flocs than a DO concentration of 2 mg/l (Figure 13). The measurements made with the particle analyser the 4th and the 11th of October coincided well with the microscopic measurements, but on the 27th of October there was a large deviation. The explanation to this may be that the flocs were very porous by that time and they might fell apart during handling. When the DO concentration in reactor A was increased to 5 mg/l, the average floc diameter increased to the same level as in reactor B.

The floc size distributions could best be fitted to log-normal distribution functions:

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

where x is the average diameter of the floc, and μ and σ are the average and standard deviation of $\ln x$, respectively. The μ was in the range 4.81–6.12 and σ in the range 0.67–1.13 (r^2 = 0.64–0.94). This is in accordance with the literature (Li and Ganczarczyk, 1991; Barbusiñski and Koscielniak, 1995). No relationship between μ and σ and the DO concentration could be found.

Activated sludge flocs in the supernatant

There were activated sludge flocs present in the supernatant (after 20 minutes settling) up to a diameter of about 50–60 µm. In most of the measurements, more than 80% of the number of the flocs were smaller than 2 µm. The distribution by number can be converted to distribution by volume and area (assuming spherical flocs). A comparison of distribution by number, area and volume can be found in Table 4. The four size intervals corresponds to dispersed bacteria, floc fragments, pin-point flocs and small flocs. Flocs larger than about 16 µm contribute to most of the volume even though they are much fewer than the smaller ones.

The size distribution of small flocs (based on number) in the supernatant after settling could best be fitted to power functions:

$$f(x) = ax_i^{-b}$$

Table 4. *Summary of the distribution of particles in the supernatant into different size intervals* (20 minutes settling).

Size interval [µm]	Number	Distribution [%] by: Area	Volume
≤ 2	84	31	6
2–16	15	48	33
16–50	< 1	21	53
50–100	negligible	< 2	8

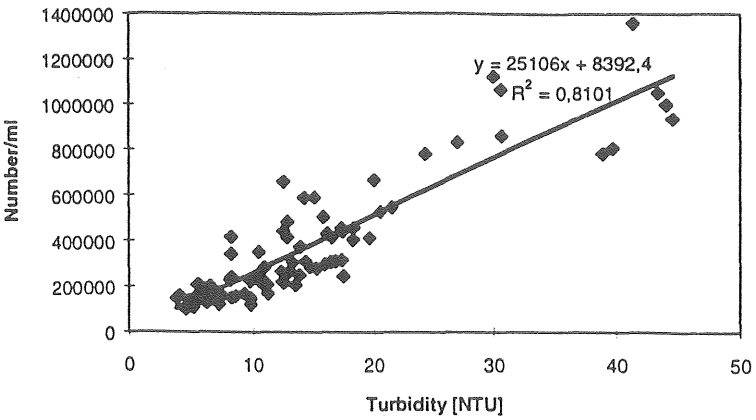

Figure 14. *Correlation between number of particles/ml and turbidity, part IV.*

where the constant *a* can be related to the number of flocs within a certain volume and *b* can be related to the number of flocs within each size class.

In part III and IV of the experiment, the particles in the supernatant were analysed several times per week. At the same time as the sample was analysed with the particle analyser, the turbidity was measured. The correlation between number of particles and turbidity was good in part IV ($r^2 = 0.81$) (Figure 14) while it was inferior in part III ($r^2 = 0.52$). The turbidities were low in part III and the measurements of turbidity were more uncertain compared to the ones at higher turbidities. No difference in parameter *a* and *b* between reactor A and B could be found, probably due to the relatively small difference in turbidity. The relationship between the parameter *a* and turbidity was poor.

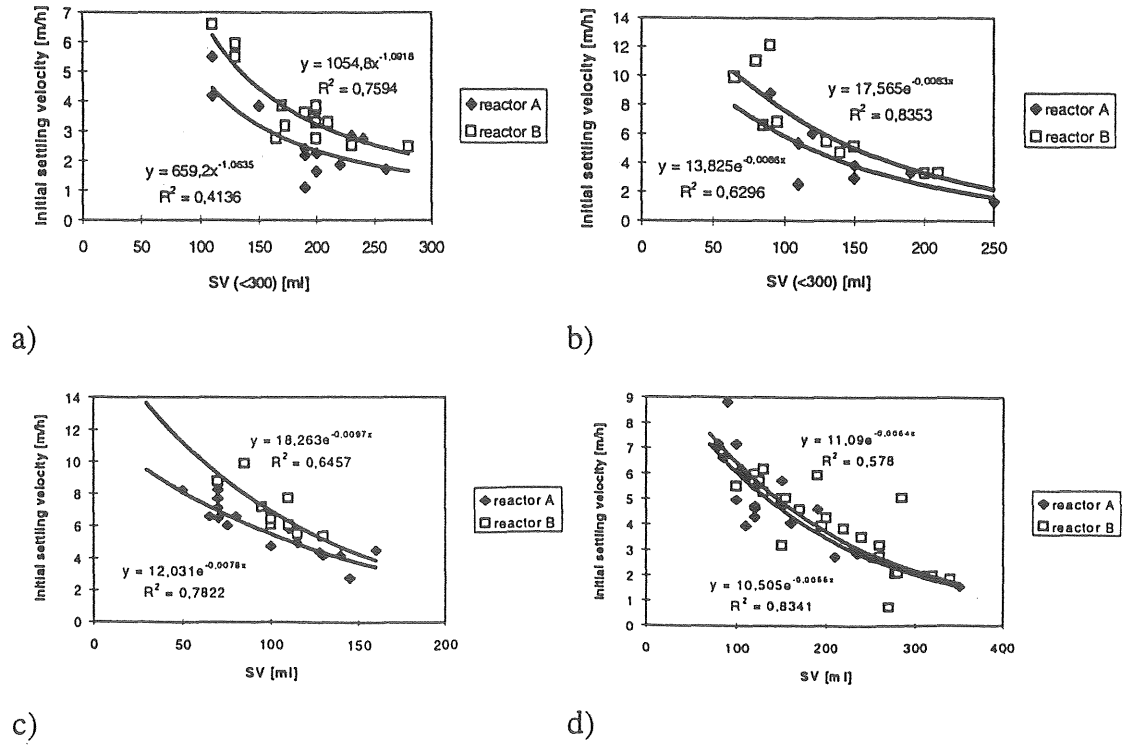

Figure 15. *Relationship between SV and initial settling velocity at:* a) part III (SRT = 2.5 d); b) part III (SRT = 5 d); c) part II (alternating period of 4 hours); d) part IV (A = 2.0 mg O_2/l; B = 5.0 mg O_2/l).

The samples had to be diluted with destilled water before measurement (20–100 times). This affect the ionic strength of the solution and this might affect the floc stability (Zita and Hermansson, 1994). This has not been investigated in this study. The Met One instrument is very sensitive to the number of particles in the solution and it was difficult to obtain a particle free solution by diluting the sample with filtrated wastewater.

Initial settling velocity

The initial settling velocity was best correlated to the sludge volume (≤ 300 ml) in all experiments. In most of the cases, an exponential function gave the best correlation. The difference in settling velocity between reactor A and B was largest in part III and IV of the experiment. This is in agreement with the fact that the flocs in reactor B were, on an average, larger and more compact than in reactor A in those experiments. In part II of the experiment, there was only a difference in initial settling velocity between reactor A and B at an alternating oxic/anoxic period of 4 hours (50 % lower settling velocities in reactor A). In part III of the experiment, the initial settling velocity was higher (30–40 %) for reactor B (operated at a DO concentration of 2 mg/l) than for reactor A (operated at a DO concentration of 0.5 mg/l) at all the tested sludge ages (5–1.25 days). In part IV of the experiment, there was hardly any difference in settling velocity (slightly higher in reactor B). However, the flocs were larger and compacter in reactor B than in reactor A. The flocs were on an average smaller in this experiment compared to the other experiments and there were also filamentous bacteria present which could have minimized the difference in settling velocity between the two reactors.

The initial settling velocity depends on factors such as cylinder diameter, concentration of mixed liquid suspended solids, sludge volume, floc size and morphology and water temperature. To reduce the effect of SV, the sludge was diluted if the SV exceeded 300 ml and a cylinder with a relatively large diameter (∅ = 60 mm) was used in all measurements. However, the type and number of filaments present has a large impact on the settling velocity.

Conclusion

- The DO concentration has a large impact on the settling and thickening properties of activated sludge, mainly due to excessive growth of filamentous bacteria.
- Lower DO concentrations produced less compact flocs than higher DO concentrations. A difference was observed between 0.5 mg/l and 2.0 mg/l as well as between 2.0 mg/l and 5.0 mg/l.
- There was no clear correlation between DO concentration and average floc diameter. Just a trend towards larger flocs at higher DO concentrations could be found.
- Alternating oxic/anoxic conditions did not affect the settling and thickening properties to a large extent.
- The turbidity of the supernatant was mostly higher at low DO concentrations compared to at high DO concentrations.
- It is very difficult to study settling properties of activated sludge in a pilot-plant completely-mixed reactor due to the problems with filamentous bacteria.

Acknowledgement

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Appendix II .

Microscopic investigation of the activated sludge

The classification scheme used in this study is an adaptation of the one developed by Eikelboom and van Buijsen (1981). Different floc characteristics were judged on a scale from 1-5 (Table 1). A magnification of 100-200x was generally used. Filament identification was performed at a magnification of 1000x.

Table 1 Classification scheme,

Sample: *Date:*	*Classification:*	*Comments:*	
Average floc size (µm)	0-------100-------200-------300-------400-------500		
Floc shape	1 2 3 4 5		
Compactness	1 2 3 4 5		
Floc strength	1 2 3 4 5		
Free bacteria	1 2 3 4 5		
Filament	1 2 3 4 5	primary type:	secondary type:
Zoogloea	1 2 3 4 5		
Protozoa	1 2 3 4 5	types:	
Rotifera	1 2 3 4 5		
Nematodes	1 2 3 4 5		
Spirochetes	1 2 3 4 5		

Average floc size: The average floc diameter was estimated at a magnification of 100x with an eyepiece micrometer. Several flocs were investigated to get a representative sample. Normally flocs are considered large when the diameter is > 500 µm, medium when the diameter is 150-500 µm and small when the diameter is < 150 µm. Regularly, samples were taken for particle analyse with a Malvern instrument (see section 5.2.5) as a comparison.

Floc shape: The activated sludge flocs were divided into round and irregularly shaped: 1 = very round and 5 = very irregularly shaped. Round flocs settle generally better than irregularly ones and they have a higher compaction capability. Irregular flocs often contain filamentous bacteria or Zoogloeal bacteria. They often settle with a lower settling velocity than round ones and they often give high SVI. An advantage is that irregularly shaped flocs often leave a clear supernatant after settling: small flocs and dispersed bacteria are enmeshed in the network of filaments and irregularities during settling.

Compactness: Very compact flocs were classified as 1 and very porous, open flocs were classified as 5. High compactness can depend on two things: high concentrations of bacteria within the flocs (desired) or high concentrations or 'slime-forming' bacteria (Zoogloea) which is undesired and can, in severe cases, lead to settling problems. Very porous flocs (=5) settle very badly. Often, very porous flocs contain large numbers of filamentous bacteria which produce a network of flocs.

Floc strength: This is a very difficult parameter to judge. A very strong floc (=1) has a distinct floc edge while a weak floc has a very diffuse edge, often surrounded by free bacteria. There are also often many small flocs (pin-point flocs) between the larger flocs. Sometimes, the floc strength is very difficult to judge: very compact flocs can have porous finger-like arms protruding from the edge (often Zoogloea bacteria).

Free bacteria: Very low levels of free bacteria were classified as 1 and very high concentrations classified as 5. The amount of free bacteria can be related to the turbidity of the supernatant after settling. A turbidity up to 5-6 NTU corresponds approximately to 1 and a turbidity above 35-40 NTU corresponds approximately to 5. This is of course quite a rough approximation, but if the sludge is investigated daily, a good judgement can be obtained.

Filament: At 1 on the scale, no filaments are present. Settling problems start around 3 and at 5 the sludge hardly settles at all. Sometimes, however, very compact flocs can settle quite well even at 3-4 on the scale. The types of filaments present is also decisive for the influence on the settling properties. The amount of filaments within the flocs can be more difficult to judge and staining is a method to make it easier. To be able to judge which filaments were present, conventional staining techniques were used (see section 4.1.2). Many different types of filaments were often present and it was impossible to identify them all. Instead attempts were made to identify only the 2-3 dominant types.

Zoogloea: This type of bacteria produces a gelatinous slime (contains large amounts of water). If there are excessive numbers present, it can be a sign of imbalance in the process and they can cause severe settling problems. At 1 on the scale, there are no Zoogloea bacteria present, while at 5 the whole flocs are covered. They could proliferate very fast, especially in connection with changes in the process or at process-failures.

Protozoa: A healthy activated sludge contain large amounts of protozoa. At scale 1 there are no protozoa present and at 5 there is a large amount present. There are four main types: attached ciliates, free-swimming ciliates, flagellates and amoeba. They eat free bacteria and contribute to a clear effluent. The presence of attached and free-swimming ciliates is an indicator of a well operating process. When the attached ciliates dominate, there is a shortage of free swimming bacteria. Throughout the experiments comments were made on which of the four categories protozoa were present

Rotifera: Moderate numbers of rotifera is an indicator of a well operating process. They often exist in low loaded activated sludge plants. The judgement (1-5) was similar to that of the protozoa.

Nematodes: They often occur at high sludge ages and they can be a problem since they eat sludge flocs. They are not important for the treatment process, but they can act as an indicator of a well operating process. Normally there are moderate levels (2-3) in activated sludge plants.

Spirochetes: This is mainly an indicator of low DO concentrations. At very high concentrations (5), the bulk liquid between the flocs is full of these small spiral-formed bacteria which move very intensely. They are best seen at a magnification of 400-1000x.

Filament staining

Gram staining is important in the identification procedure of filamentous bacteria. Bacteria can be divided into gram positive and gram negative, depending on the type of cell wall. Gram-negative bacteria are light red after staining, while gram-positive bacteria are dark blue after staining. Some filamentous bacteria are gram-negative and some are gram-positive. Old cells which are gram-positive can be decolourized because of their weak cell walls and they can therefore be taken as gram-negative. This risk is higher at high sludge ages.

Some filaments are neisser-negative (light yellow after staining) and some are neisser-positive (dark blue after staining). A neisser-negative filament can have granules of poly-phosphate which are dark blue after staining (these cannot clearly be observed without staining).

Many bacteria can form intracellular pools of poly-β-hydroxybuturate or analogues fatty acids. They can be seen in a microscope by staining them in sudan-black (P-β-H accumulating bacteria have small black granules within them). A problem is that P-β-H accumulation only occurs at certain growth conditions which can lead to misjudgements.

A small droplet of activated sludge was placed on an object glass to be air-dried for about one hour. The following conventional staining procedures were used (Eikelboom and van Buijsen, 1981):

Gram-staining: 1) Stain the slides for 1 minute in a crystal-violet solution (2% crystal-violet and 0.8% ammoniumoxalate in a 20:80 ethanol:distilled water solution); 2) Rinse with destilled water for 2 seconds to remove excess staining solution; 3) fix with an iodine solution (0.33% I2 and 0.67% KI in distilled water, stored in dark bottles in a refrigerator); 4) Rinse with distilled water for 2 seconds; 5) Decolourize in ethanol until the rinse solution is light blue; 6) Rinse with distilled water for 2 seconds; 7) Counterstain in saphranine solution (0.25 g saphranine, in 10 ml 95% ethanol and dilute to 100 ml with distilled water) for 1 minute; 8) Rinse with distilled water for 10 seconds.

Neisser-staining: 1) Stain in a mixture of solution 1A (0.3 g methylene-blue in 15 ml 95% ethanol and 15 ml crystalline acetic acid is diluted to 300 ml with distilled water) and 1B (1 g crystal violet in 30 ml 95% ethanol is diluted to 300 ml with distilled water) for 30 seconds; 2) Rinse for 2 seconds in distilled water; 3) Counterstain for 1 minute in a Bismarck brown solution (1 g Bismarck brown in 300 ml distilled water); 4) Rinse in distilled water.

P-β-hyroxybuturate-staining: 1) Stain for 10 minutes in a sudan-black solution (0.9 g sudan-black in 300 ml 60% ethanol); 2) Rinse for 2 seconds in distilled water; 3) Stain for 10 seconds in a saphranine solution (1.5 g saphranine in 300 ml destilled water); 4) Rinse in distilled water.

Appendix III.

Relationship between turbidity and suspended solids concentration

Turbidity is often, in wastewater treatment, used as a measure of the concentration of suspended solids in a solution. Turbidity is a consequence of light scattering and its intensity depends, among other things, on the size, the shape and the optical density of the particles. Therefore, the relationship between turbidity and suspended solids concentration can vary. In this study, turbidity was used instead of suspended solids concentration because of its simplicity and sensitivity. Various tests were made in which turbidity as well as suspended solids concentration were measured simultaneously to be able to estimate a correlation factor. Good linear correlations were found with regression coefficients (r^2) of 0.90-0.99. The suspended solids concentration was in the range 1.2-2.1 times the turbidity (NTU). This is in agreement with literature values (Wahlberg, 1992). If all points from the various measurements were put together, the suspended solids concentration was 1.3 times the turbidity with a r^2 of 0.93 (Figure 1). When data was divided into two different turbidity intervals: 2-26 NTU and 11-162 NTU, the suspended solids concentration was 1.45 (r^2=0.81) and 1.34 (r^2=0.98) times the turbidity (NTU), respectively.

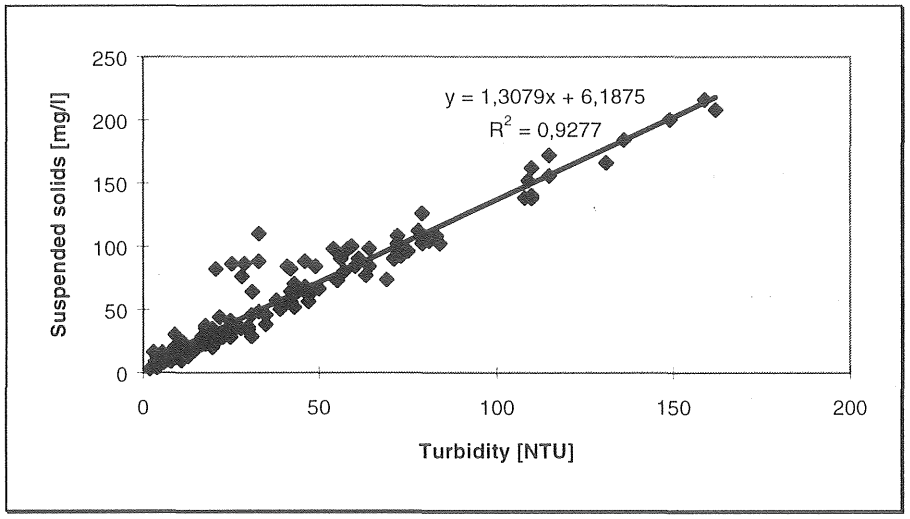

Figure 1 Turbidity as a function of suspended solids concentration.

The difference in relationship between turbidity and suspended solids concentration has probably to do with the fact that colloidal particles (<1 μm) contribute to the turbidity but they may slip through the filter paper and will not contribute to the suspended solids concentration. Therefore, measurements taken from just one activated sludge sample will give a very good correlation while measurements from different samples will give a poorer correlation.

To check if homogenization of the activated sludge would give a better correlation between turbidity and suspended solids concentration, samples were treated with an ultrasound probe (sonication). Turbidity is dependent on the size and size distribution of particles. By sonicating the activated sludge, the larger flocs will be disrupted and the size distribution will be more uniform. An ultrasound probe (Virtis Company Inc., equipped with a ½" standard titanium disrupter horn) with an frequency of 20 kHz was used. Activated sludge, taken from the outlet of the aeration tank of the Rya WWTP, was poured in a 3.5 litre cylinder. After 20 minutes settling a part of the supernatant was decanted. This sample was treated with the

ultrasound probe (50 ml) at different intensities (20, 40 and 60% of the maximum effect) and at different periods (1, 5 and 10 minutes) according to a 3^2-factor design. The purpose was to create different degrees of floc break-up. The samples were left to settle for 15 seconds prior to turbidity measurement. Each test was made in triplicate to be able to calculate the standard deviation. These standard deviations were compared with the standard deviation of untreated samples. The results are summarized in Table 2 and 3.

Table 2 Summary of ultrasound treatment.

Test	Effect [% of maximum]	Period [minutes]	Average [NTU]	Standard deviation (s)
1	20	1	62.7	2.01
2	20	5	75.3	1.43
3	20	10	82.3	2.52
4	40	1	68.3	1.46
5	40	5	88.0	3.78
6	40	10	109.2	4.48
7	60	1	71.8	0.81
8	60	5	98.9	3.16
9	60	10	113.9	6.59
10	no treatment	no treatment	57.2	0.94

The standard deviation was smallest for a treatment at 60% effect for 1 minute. However, the standard deviation for the untreated samples was only slightly larger. The test was repeated, but the treatment period was 1, 2 and 5 minutes. This test gave similar results, i.e. high intensities at short treatment periods gave the lowest standard deviation.

Table 3 Summary of ultrasound treatment.

Test	Effect [% of maximum]	Period [minutes]	Average [NTU]	Standard deviation (s)
1	20	1	37.2	0.95
2	20	2	39.1	0.89
3	20	5	43.8	1.98
4	40	1	40.4	0.17
5	40	2	45.0	1.46
6	40	5	57.4	1.68
7	60	1	41.7	0.86
8	60	2	46.3	0.65
9	60	5	61.9	3.92
10	no treatment	no treatment	33.5	0.39

The correlation between turbidity and suspended solids concentration after ultrasound treatment was investigated. Activated sludge settled in a 3.5 litre cylinder and samples of the supernatant was taken after 10, 20, 30, 40 and 60 minutes to get a range of turbidities. The turbidity of samples treated at 40% and 60% of the maximum effect for 1 minute (which gave the smallest standard deviation in the previous test), and untreated samples were correlated to the suspended solids concentration (100 ml sample was filtrated). The correlation was good for treated as well as for untreated samples with r^2 values between 0.97-0.98 (Figure 2). The same experiment was made at a higher turbidity interval: 67-160 NTU. The regression coefficients were 0.98 for sonicated samples and 0.97 for untreated samples.

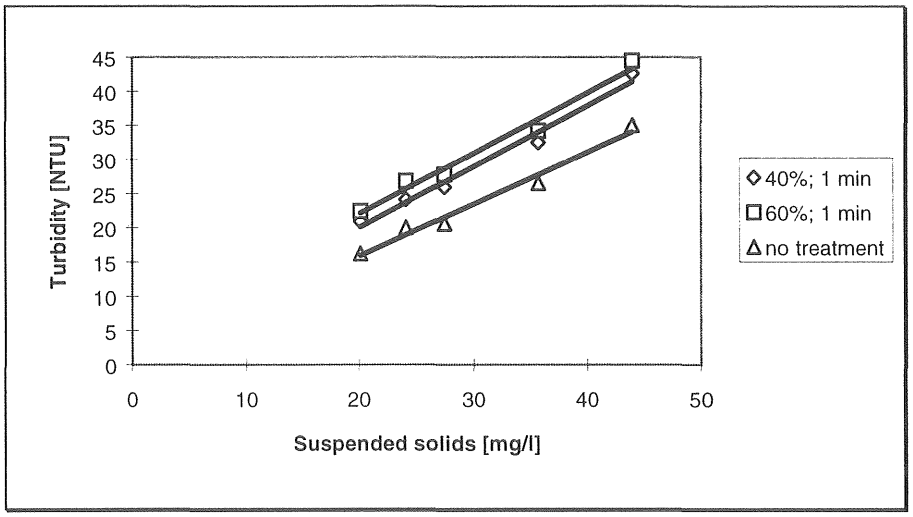

Figure 2 Relationship between turbidity and suspended solids concentration at different degrees of ultrasound treatment.

It can be concluded that ultrasound treatment gives marginally smaller standard deviations when short treatments (1-2 minutes) and high effects (40-60%) are applied compared to untreated samples. This probably cause a disruption of flocs present in the supernatant and the size distribution will be more uniform. A further sonication might cause disruption of some of the free cells and the size distribution will be less uniform (shifted towards smaller particles) and the deviation between different measurement will be larger. The problem is to find the optimum ultrasound treatment and to avoid reflocculation of the homogenized sample. In this test this was not taken into consideration. Chemicals like metaphosphate can be added to stabilize the suspension. Another problem is that the sample heats up during treatment (especially at higher effects and at longer times) which can affect the sample. That the standard deviation for low effects were larger than for untreated samples can be due to the fact that this could have flocculated the activated sludge particles to a certain degree.

Since the standard deviation was low for untreated samples and that the correlation between turbidity and suspended solids concentration was linear with a high regression coefficient, it seemed justified to use turbidity as a measure of suspended solids concentration and that ultrasound treatment was not necessary.