Thermophilic aerobic post treatment of anaerobically pretreated paper process water

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Aan mijn vader en moeder

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Thermophilic waste- or process water treatment increases in importance as industries shift from end-of-pipe treatment towards integrated process water treatment. The need for process water treatment becomes evident as the levels of pollutants in industrial water circuits need to be controlled whereas the intake of fresh water generally diminishes. In the paper and board industry, high process water temperatures prevail and thus water treatment needs to take place under thermophilic conditions. In many cases, an anaerobic pretreatment method can be used but aerobic post treatment is required for polishing of the anaerobic effluent. This thesis describes research in which the aerobic post treatment of anaerobic effluent of a board mill was investigated under thermophilic conditions.

As a boundary condition for aerobic conversions, sufficient oxygen needs to be transferred from the gas phase to the liquid in which the bioconversion takes place. It was shown that although the oxygen saturation concentration decreases with a rise in temperature, this effect is fully compensated by the increased oxygen diffusion rate with the same temperature increase. The overall oxygen transfer rate thus remains constant in the temperature range of 20-55 °C.

Post treatment of anaerobic effluent in activated sludge reactors revealed several fundamental differences between mesophilic and thermophilic treatment. Firstly, batch and continuous experiments showed a lesser removal of complex soluble COD under thermophilic conditions when compared to mesophilic reference experiments. This could not be attributed to a higher production of soluble microbial products (SMP) at elevated temperatures. It is therefore expected that thermophilic biomass is unable to oxidize the same variety of complex soluble components as the mesophilic biomass is capable of.

Secondly, thermophilic effluents are often cloudy while effluents of mesophilic activated sludge reactors are generally clear. This is caused by smaller cohesion forces within thermophilic activated sludge flocs resulting in a higher sensivity towards shear forces and smaller floc sizes. Furthermore, fewer colloidal particles from the influent are adsorbed on the thermophilic sludge flocs and are washed out with the effluent. However, a clear thermophilic effluent can be obtained provided the influent contains little colloidal material.

The underlying causes for the weaker cohesion forces within the flocs are still unclear. The absence of protozoa at 55 °C was shown to be of minor importance regarding the effluent turbidity and could not account for this effect. Binding of hydrophobic pollutants on a hydrophobic surface was hardly affected by temperature and could not explain the observed effects either. Calculations using the DLVO theory showed that bacterial exo-polymers are of crucial importance in the flocculation process. These polymer interactions are highly temperature dependant and are therefore expected to be the underlying cause for the differences in flocculation behavior.

Besides differences in removal efficiencies and flocculation behavior, also the kinetics of mesophilic and thermophilic activated sludge treatment differ. The maximum growth (and thus conversion rate) of biomass cultivated at 55 °C was a factor 1.5 higher than for a similar type of biomass cultivated at 30 °C. Decay rates are doubled with the same temperature increase whereas the theoretical biomass yields were similar. As a result, higher substrate conversion rates can be obtained under thermophilic conditions provided that a high concentration of thermophilic biomass is cultivated in the reactor by application of a high organic loading rate.

These kinetic advantages are however of little use when polishing the effluent of an anaerobic bioreactor. Under thermophilic conditions biomass growth will be limited since the organic loading rate is restricted by the need to retain and convert particulates from the anaerobic effluent and by the absence of readily biodegradable COD. Furthermore, biomass decay rates have doubled under thermophilic conditions. The combination of these factors diminishes the amount of active biomass in the thermophilic reactor and can not be compensated fully by the intrinsic higher conversion rates. Overall conversion rates in a thermophilic bioreactor can thus be lower as compared to a mesophilic reference system, depending on the applied loading rates.

Nevertheless, for application in the board industry these disadvantages can be dealt with as the water quality demands are relatively low. Additional treatment methods are however required in case higher water quality demands prevail.

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1

GENERAL INTRODUCTION

1. INTRODUCTION

Paper and board production has been a very energy intensive and water consuming practice since a long time. The European paper industry produces 70 million tons of paper annually and at the same time uses an average of 20 cubic meters of freshwater per tonne of paper produced, in total 1.4 billion cubic meters of freshwater annually (Kappen *et al.,* 1999). In The Netherlands, the figures are somewhat lower due to a more efficient water usage. The specific water consumption per tonne of paper produced very much depends on the type of paper produced, the quality of the mechanical equipment used in the mill, availability of water and energy and customs/traditions in papermaking. Additional bleaching steps for production of a high quality product such as graphic papers will increase the water demands as well. Producing a lower quality product, such as board or newsprint generally requires less water. In some cases, paper mills are not allowed to discharge any (purified) wastewater or only in limited amounts whereas in Canada and Scandinavia water consumption is generally higher due to the abundant availability of water and the lower energy prices. In transition countries such as India, specific water consumption can be as high as 110-170 m^3 tonne of $paper^{-1}$ caused by a low pulp quality, based on straw, bagasses or jute and by the use of outdated paper machines (Gupta, 1994; Habets, personal communication). Table 1 depicts the specific water consumption in the paper and board industry in The Netherlands for each product group and the estimated values in the near future. In The Netherlands, in nearly all cases, groundwater is used as the primary water source. In the year 2000, approximately 35 million cubic meters of groundwater was used by the Dutch paper and board industry.

Table 1. Specific water usage in The Netherlands (Joore, 1999)

	specific water usage in time (m^3 $tonne^{-1}$)				
product group	1970	1985	1993	2000	2010
massive board		8.9	7.5	3	0 ?
corrugated board		9.2	5.6	3.5	0 ?
graphic paper		29.8	23.1	10	<5 ?
tissue		25.1	21.3	15	< 5 ?
total	80	20.0	14.8	7.5	3 ?

In the paper production process, energy consumption is linked to the specific water consumption. Heat dissipation takes place from mechanical equipment that is used for grinding, mixing and pumping of pulp and water. As a result, process water temperatures increase to approximately 30 °C. However, for optimum runability of the paper machines a higher process water temperature of 50-60 °C is desirable. To attain this temperature, steam is generally injected in the process. Steam is generated at the mill, partially obtained from combined heat and power systems used to generate electricity and from burning of natural gas. Consequently, minimizing the specific water consumption

also results in lower energy demands as a smaller water volume needs to be heated. Decreasing the specific water consumption by 10 m^3 tonne of $paper^{-1}$, assuming a groundwater temperature of 10 °C and a process water temperature of 55 °C results in energy savings of approximately 10^3 MJ $tonne^{-1}$ of paper.

In the past, water savings mainly took place by good housekeeping. Reducing the specific water consumption even more, or even achieving a zero effluent situation would require additional measures. A more stringent recycling can result in a process water deterioration which may lead to a product value loss and a less efficient production process and thus additional measures need to be taken.

According to Habets and Knelissen (1997) different groups of compounds accumulate in the process water as water cycles are closed. These are: microorganisms, volatile fatty acids (VFA), calcium, salts, stickies and anionic trash. Starch and calcium carbonate are used as fillers and coatings on newly produced paper and are reintroduced in the process in case recycled wastepaper is used as a raw material for paper or board production. Microorganisms grow in the process water due to the abundant amounts of substrate (starch) and the high process water temperature and convert starch into volatile fatty acids (VFA). Furthermore, these microorganisms try to immobilize themselves by developing biofilms and producing bacterial slime. This enables bacteria to maintain optimal environmental conditions for growth. When slime patches detach and move along with the process water they can cause cause spots or holes in the product and in the worst case cause a paper break. The produced volatile fatty acids in turn cause calcium carbonate to dissolve in the process water. Calcium causes scaling problems while the VFA's mainly cause bad smells in the product and the surroundings of the mill. Accumulation of salts may lead to corrosion of machinery while stickies, which as compounds of a sticky/tacky nature reduce the product quality. Anionic trash is a generic term for those compounds that cause an increase in the use of cationic polymers on the paper machine and are in most cases negatively charged organics. These cationic polymers are dosed on the paper machine to flocculate the negatively charged cellulose fibers, creating a fiber mat. Dosage of cationic polymers is thus a crucial step in the paper production process and increasing polymer demands lead to additional production costs.

Preventing the accumulation of these various types of compounds can take place by a combination of biological and physico-chemical treatment methods, depending also on the required process water quality. The combination of an anaerobic pretreatment followed by aerobic post treatment has shown to be the most cost effective option for application in a mill producing corrugated cardboard from recycled wastepaper (Habets and Knelissen, 1997). Virtually all easily biodegradable COD (lactate and VFA) is removed in the anaerobic pretreatment while hydrogen sulphide is partially stripped out with the biogas. The aerobic post treatment mainly removes rest BOD, sulphide, calcium hardness, and a part of the anionic trash and stickies by a combination of biosorption and biodegradation. Inert

salts and non biodegradable humic acids remain in the process water. For most board mills, this treatment scenario will suffice since corrosion of the equipment by salts is marginal in case high quality stainless steel is applied while humic acids only color the end-product which can even be beneficial. In case color removal is required, for instance for production of fine papers, additional physico-chemical treatment methods such as coagulation-flocculation, ozonation or ion-exchangers can be applied.

The above mentioned treatment scenario was shown to function satisfactory in a German paper mill with a completely closed water system (Habets and Knelissen, 1997). Kappa Zülpich Papier in Germany is producing corrugated cardboard from recycled wastepaper for more than 20 years now without discharging any effluent. Board production takes place at 55 °C providing optimum run-ability of the paper machines and reducing bacterial slime production while water purification takes place at 35 °C. A part of the process water is thus continuously cooled down, purified and returned to the pulping process. The next step towards a more efficient energy usage is to treat the process water at 55 °C. Additional energy gains of approximately 250 MJ tonne of paper^{-1} can be achieved in this scenario.

In 1997, an EET (Economy, Ecology, Technology) project was launched aiming at the development of appropriate technologies to purify hot process waters from the paper and board industry in order to recover water and energy. The total process chain would consist of a thermophilic anaerobic pretreatment for efficient C and S removal (Van Lier *et al.,* 2001) combined with a thermophilic aerobic post treatment system subsequently followed by thermophilic physico-chemical treatment methods. This thesis describes the lab-scale research concerning thermophilic aerobic post treatment of anaerobic effluent of a paper mill. The research project aims at obtaining fundamental knowledge concerning thermophilic aerobic wastewater treatment processes. This could eventually help in full scale implementation of a thermophilic aerobic post treatment system. The purpose of the aerobic polishing step is to remove rest BOD, malodorous compounds such as sulphides, anionic trash, stickies and calcium.

The subsequent paragraphs of this chapter describe a literature review on microbiological aspects of thermophilic aerobic conversions and on applied aspects of thermophilic aerobic wastewater treatment. Chapter 1 concludes with the thesis objectives and the thesis outline.

2. MICROBIOLOGY OF THERMOPHILIC AEROBIC CONVERSIONS

2.1 What are thermophiles?

Sonnleitner and Fiechter (1983a) define a thermophile as a microorganism that requires high temperatures for growth; in other words: they are not active under mesophilic conditions. Thermophiles can be categorized into thermotolerant, facultative thermophilic, moderate thermophilic, thermophilic or extremely thermophilic, depending on their optimal temperature for growth. This definition of thermophily by means of cardinal temperatures is however subject to changes as new microorganisms are continuously discovered (Wiegel, 1990). Furthermore, some organisms were found to have an extremely wide temperature range for growth extending over the mesophilic and thermophilic temperature range (Wiegel, 1990) and are now defined as cryptic thermophiles. From a engineering point of view, LaPara and Alleman (1999) define a thermophilic process as any process operating at a temperature exceeding 45 °C. This definition is also used in this thesis.

Thermophilic organisms can be found among bacteria, archaea, fungi and algea (Sonnleitner and Fiechter, 1983b). The group of moderate thermophiles that predominates in thermophilic aerobic wastewater treatment systems operating at 55 °C belong to the bacteria domain while most hyperthermophilic bacteria are archaea (with some exceptions such as *Aquifex* and *Thermotoga*). Apart from their high optimal temperature for growth, moderately thermophilic microorganisms resemble their mesophilic counterpart to a large extent (Brock, 1986). In an early theory of thermophiles (Allen, 1953, cited by Brock, 1986), it was suggested that thermophilic microorganisms are able to grow at high temperatures because, by active metabolism, they rapidly replace the cell material that is destroyed by the high temperature conditions. It is now recognized that growth under thermophilic conditions is primarily based on the thermo stability of the cell components. The critical cell components that require thermostabilisation are proteins, the biological membranes, RNA and DNA and co-factors such as ATP and NADH.

Thermophilic biological membranes tend to have longer hydrocarbon chains and methyl branched chains as compared to bacterial membranes of mesophiles while unsaturated hydrocarbon chains are rare. A longer carbon chain makes the membrane more rigid and increases its thermostability. It has been shown that thermophilic bacteria can alter their chain composition and length as a function of temperature, thereby maintaining a constant membrane viscosity (Brock, 1986). The apolar chain length of thermophilic archaea is almost invariably fixed at 20 or 40 carbon atoms. However, membranes of archaea contain tetraether bonds that provide a covalently bound bilayer membrane that cannot melt apart at high temperatures and furthermore, the membrane is resistant to an acidic environment.

Thermophilic proteins are stabilized by extra hydrogen bonds, ionic interactions and hydrophobic interactions in certain crucial parts of the protein macromolecules. Brock (1986) notes that thermophilic proteins are in most respects similar to mesophilic proteins such as size, sub-unit structure, helicity and β-structure. It was shown that the thermostability of a protein can be changed significantly already by a single amino acid change implying that subtle rather than gross structural changes are expected for thermophilic proteins as compared to mesophilic analogues.

DNA and RNA are by itself relatively thermostable and additional stability can be attained by binding to specific proteins, as has been found in hyper thermophilic archaea (Wiegel and Adams, 1998). In some thermophiles, a higher G-C content was found compared to their mesophilic analogues but this is not an invariant characteristic of these microorganisms (Brock, 1986).

The upper temperature limit for growth , exceeding 120 °C is most likely governed by the stability of cofactors such as NADH and ATP (Wiegel and Adams, 1998). Some of these cofactors have quite short half lifes at 105 °C while biological macromolecules all seem to have the potential to remain stable up to 125 °C. For instance, only 5 % of active NAD remained after it was exposed for 1 hour to 95°C in 1 mM potassium iodine. In any way, archaea are able to grow at 105 °C by using these coenzymes thus in some way they are able to circumvent the intrinsic instability of the cofactors that is observed in the laboratory.

2.2 Habitats and growth requirements of thermophiles

Natural habitats of thermophilic microorganisms are in any case exotic locations such as thermal springs and geysers, thermal basins and deep sea geo thermal vents (Brock, 1986). However, most moderate thermophiles can be found everywhere on earth (Sonnleitner and Fiechter, 1983a,b) and experience with sterile thermophilic bioprocesses has shown that they become just as easily contaminated as mesophilic processes. This issue is of importance in wastewater treatment as it suggests that seed sludge can be obtained from mesophilic sources and that continuous re-inoculation takes place with moderately thermophilic bacteria via the aeration. So far, thermophilic aerobic processes have mainly been started up by using mesophilic seed sludge and by imposing a certain temperature adaptation period (Table 2).

Many thermophilic bacteria have been reported to require growth factors that are usually supplied in the form of yeast extract, tryptone, peptone or other frequently used complex substrates. The growth requirement for methionine is quite common for thermophilic *Bacillus* spp. (Sonnleitner, 1983a). Sürücü (1999) studied the growth requirements of a mixed culture of thermophilic bacteria degrading a highly concentrated substrate. He found the minimal nutritional requirements for growth to be methionine, magnesium, calcium and iron besides carbon, nitrogen and phosphorous. Histidine, thiamine and riboflavin stimulated growth but were not absolutely required. Furthermore, the mixed culture was less demanding in nutritional requirements as compared to the pure cultures that

comprised the mixed biomass. From a practical perspective, continuous addition of growth supplements to wastewater treatment systems brings additional cost and should, if possible, be avoided. In literature, in many cases, yeast extract or peptone is added when a synthetic wastewater is degraded under thermophilic conditions (LaPara *et al*., 2000a, 2001a; Lim *et al*., 2001; Kurisu *et al*., 2002). In more practical oriented research with actual wastewaters, no extra additions besides nitrogen and phosphorous were made, seemingly without affecting the process performance (Barr *et al*., 1996; Tardiff and Hall, 1997; Tripathi and Allen, 1999). Thus far, it is assumed that additional organic growth requirements are not necessary in thermophilic biodegradation, especially in rather low loaded wastewater treatment systems although actual proof is lacking.

2.3 *Microorganisms involved in thermophilic aerobic conversions*

In the past, many microbiological studies have been made on pure cultures of thermophilic microorganisms (e.g. Kuhn *et al*.,1980; Sonnleitner *et al*., 1982; Cometta *et al*., 1982; Becker *et al*., 1997). Bacterial diversity in ATAD (Autothermal Thermophilic Aerobic Digestion) and thermophilic composting processes were mainly studied in the eighties using classical microbiological methods (Sonnleitner and Fiechter, 1983c,d; Strom, 1985; Bomio *et al*., 1989; Hensel *et al.*, 1989; Beaudet *et al*., 1990; Burt *et al*., 1990; Fujio and Kume, 1991). Most of the isolates obtained from thermophilic sludge digestion were *Bacillus* species (Sonnleitner and Fiechter, 1983c,d; Strom, 1985; Beaudet *et al*., 1990)) or *Thermus* species (Fujio and Kume, 1991; Beffa *et al.*, 1996). Hensel *et al.* (1989) used a combination of classical isolation techniques and indirect immunofluorescence to check whether the isolated thermophilic strains were representative for their respective habitat. They found that approximately 20% of the physiologically active population in their systems of study could grow on standard cultivation media, implying that 80% could thus not be detected using the classical isolation techniques. These observations show that the previously mentioned isolates are apparently present in their respective habitats but are not necessarily representative for the entire microbiological community.

Since recently, the microbiological community structure of thermophilic aerobic processes can be studied with modern, culture independent methods. These techniques, such as the quinone profile method, denaturing gradient gel electrophoresis (DGGE) and fluorescent *in-situ* hybridisation (FISH) overcome to a large extent the problem that many microorganisms are hard to cultivate in the laboratory. The inevitable limitations of the modern molecular methods are discussed by for instance Head *et al*. (1998).

Lim *et al.* (2001) studied the microbiological community in a thermophilic aerobic solid phase reactor using the quinone profile method. They found increasing mole fractions of the MK7 quinone with increasing reactor temperatures. MK7 is known to be the dominant quinone of thermophilic *Bacillus* species. However, quinone profiles are not very specific and only reveal the community structure at

the pylum or division level. Kurisu *et al.* (2002) studied a similar thermophilic bioprocess by a combination of quinone profiles as well as DGGE analyses and FISH. They also observed the predominant presence of the MK7 quinone. Based on sequencing of DGGE bands and FISH analyses they found that the community was primarily composed of *Bacillus* spp. including close relatives of *B. Thermocloacea* (obligate thermophile), *B. Licheniformis* (facultative thermophile) and *B. Lentus* (mesophile). The presence of mesophiles in their bioreactor is understandable since the system was operated batch wise with temperatures ranging from 35-60 °C. LaPara *et al.* (2000c, 2002) used similar techniques when applied to thermophilic aerobic bioreactors treating a pharmaceutical wastewater. They found a predominance of β-proteobacteria. Furthermore, a smaller microbial diversity was observed in the thermophilic bioreactors as compared to the mesophilic bioreactors. This could have implications for the stability of a thermophilic wastewater treatment process as a smaller diversity makes the system more vulnerable to changes in influent composition and concentration. However, in their experiments, the effluent of the thermophilic reactors operating without sludge retention was used as influent for the mesophilic reactors. In the mesophilic reactors, DNA from thermophilic bacteria was detected as amplification of DNA does not require the biomass containing the DNA to be active. The microbial diversity in the mesophilic reactors was in these experiments thus overestimated.

When comparing parallel operated bioreactors, a decrease in species richness with increasing temperatures was observed as well (Konopka *et al.,* 1999; LaPara *et al.,* 2000a). However, in both studies an inoculum from a mesophilic activated sludge plant was used and both reactors were subsequently operated under sterile conditions. The bacterial community in the thermophilic bioreactor thus originates solely from the inoculum and it is not surprising that a mesophilic inoculum contains more mesophilic microorganisms than thermophiles. Concluding: it seems plausible that more extreme environmental conditions actually reduce the microbial diversity but so far this has not been proven for (moderate) thermophilic processes operating at 55-60 °C.

3. THERMOPHILIC AEROBIC WASTEWATER TREATMENT

3.1 Introduction

Thermophilic aerobic wastewater treatment is a relatively new research field with few full scale implementations so far. Exploratory research dates back to the early 1950s (e.g. Rudolfs and Amberg, 1953; Gehm, 1956; Dougherty and McNary, 1958) but these researches provide little insight in the actual process phenomena. In the 1980s, the first full scale autothermal thermophilic aerobic digestion (ATAD) plants came into operation. These were mainly installed in the USA, Germany and Switzerland since these countries value extensive pathogen destruction highly. A 10^5 fold reduction in

E.Coli counts is generally found over a 24 h period at 50-65 °C (Sonnleitner, 1983). ATAD processes are based on the release of heat during the aerobic conversion of high strength waste streams. Provided a high concentration of biodegradable COD in the influent and an efficient aeration system, thermophilic conditions can be maintained without any external heating (Vismara, 1985; Pagila *et al*., 2000; Chiang *et al*., 2001).

In recent years, thermophilic aerobic wastewater treatment has again gained increasing interest due to the more stringent water system closure in paper and board mills. In the pulp and paper industry, water re-use has always been intensive due to the high energy demands associated with the heating of cold groundwater up to the high process water temperatures. More stringent legislation and expected economic benefits drive paper and board mills towards a more intensive re-use of process water. This also requires efficient wastewater treatment systems operating under these high temperature conditions. Most of the current research found in literature therefore deals with waste streams from the pulp and paper industry.

In this chapter an overview is given of all relevant literature on thermophilic aerobic wastewater treatment systems. A division is made between suspended growth systems (Table 2) and other reactor configurations such as biofilm processes or membrane bioreactors (Table 3). Kinetics of thermophilic bioconversions are dealt with in Chapter 3.

3.2 Thermophilic activated sludge treatment

In the Nordic countries, activated sludge is the most frequently applied treatment system in the pulp and paper industry (Saunamäki , 1997). So far, results concerning the application of activated sludge beyond temperatures of 40 °C have been mostly negative and sometimes contradictory. There appear to be two major characteristics of thermophilic activated sludge hindering its application. These are: 1. an increased effluent turbidity (Rudolfs and Amberg, 1953; Carpenter *et al*., 1968; Flippin and Eckenfelder, 1994; Visvanathan and Nhien, 1995) and 2. bad sludge settling properties (Hunter *et al*., 1966; Tardiff and Hall, 1997; Tripathi and Allen, 1999). Only Gehm (1956) and Barr *et al*. (1996) were able to operate an activated sludge system with well settling sludge and no effluent turbidity. The applicability of activated sludge strongly depends on the sludge settleability which enables one to maintain the biological activity in the reactor and this factor is thus of crucial importance.

Barr *et al*. (1996) mention that most of the older studies on thermophilic activated sludge treatment have been inconclusive. They state that differences in source feed, acclimatization periods for the sludge and the rates at which temperature changes were imposed confounded the attempts to study the temperature effects on the reactor performance. Furthermore, they state that the decrease in the sludge settleability might not have been a result of operation under thermophilic conditions but is most likely a result of low sludge ages (Rudolfs and Amberg 1953, Carter and Barry, 1975,

T range °C	Influent	System	SRT-loading rate g COD· gMLVSS^{-1}·day^{-1}	temperature adaptation period	Observations	References
35-50	pre settled board mill white water	B	short, not mentioned SRT loading: 0.08 - 0.29	2 weeks	good solids separation but higher effluent turbidity at 50 °C	Rudolfs and Amberg (1953)
30-53	kraft mill wastewater	AS	SRT unknown loading: 0.5	not mentioned, probably 3 months.	excellent sludge settling characteristics, nothing mentioned about effluent turbidity	Gehm (1956)
21-46	citrus wastes	AS			poor settling mixed liquor at temperatures beyond 43 °C	Dougherty and McNary (1958)
26-52	simulated kraft effluent	AS	SRT unknown loading: 1.3	10 days	decreasing treatment efficiency at temperatures beyond 37°C	Carpenter et al. (1965)
4-55	synthetic sewage	B-AS	SRT and loading unknown	2-3 weeks	lower SVI at 55°C but more suspended solids in the effluent.	Hunter *et al.* (1966)
35-50	powdered milk	SBR	SRT 5 days- 2 days loading: 0.58	not mentioned	96% soluble BOD removal but biological solids did not settle properly compared to 35 °C.	Carter and Barry (1975)
20-37	industrial wastewater	pilot AS	SRT unknown loading: 1.4	not mentioned	effluent TSS increased with increasing temperatures	Duke *et al.* (1981)
53	cardboard waste water	CSTR	SRT: 1-10 days	not mentioned	colloidal material was present in the influent and efluent.	Jackson (1983)
53	cardboard waste water	pilot AS	SRT: 1-10 days loading: 1-3	not mentioned	effluent turbidity due to colloids could only be removed by addition of a polymer.	Jackson, (1983)
28-52	bleach kraft and chemical wastes	full -scale AS	SRT and HRT unknown loading: 0.7-1.8	not mentioned	increased effluent turbidity at elevated temperatures.	Flippin and Eckenfelder (1994)
35-50	pre settled BKME	AS	SRT: 5-15 days loading: ≅ 0.8 at 50 °C	total 4 months for a 15 °C temperature increase.	BOD removal, reactor MLVSS and effluent solids thermophilic and mesophilic were comparable.	Barr et al. (1996)
20-50	simulated mechanical newsprint white water	SBR	SRT: 20 days loading: ≅ 0.5 at 50 °C	acclimatization period not mentioned	poor sludge settling and low biomass growth resulted in low contaminant removals at 50 °C	Tardiff and Hall (1997)
35-60	BKME	SBR	SRT: 10-15 days loading: ≅ 0.6 at 60 °C	total 8 weeks for a 25 ° C temperature increase	decreased COD removal and higher suspended solids concentration in the thermophilic reactors.	Tripathi and Allen (1999)
24-44	pharmaceutical wastes	pilot AS	SRT: 3 days loading: 1.6	no specific acclimatization	bulking sludge obseved at temperatures beyond 39 °C turbid supernatant	Bastin *et al.* (1999)
25-56	recycle mill wastewater	lab SBR	SRT: 9 days loading: ?	30 °C temperature increase in 76 days	severe filamentous bulking at temperatures beyond 40 ° and a lesser COD removal beyond 45 °C.	Norris *et al.* (2000)

Table 3: Overview of thermophilic biofilm and MBR processes.

Temperature °C	Influent	Reactor system	Observations	reference
65 °C	NH_4Ac and evaporator condensate NSSC mill	Biofilm (diatomite carriers)	Acetate removal rates: 0.7 and 0.5 g $l^{-1}h^{-1}$ on both substrates	1
55 °C	BKME	Biofilm on PUR	Thermophilic anaerobic/aerobic treatment gave stable COD removal efficiencies	2
30, 38, 45, 55 °C	Modified domestic wastewater	Aerated filter	Suspended solids removal decreased with increasing temperatures	3
55 °C	Simulated whitewater	MBR and UF	MBR gave better results compared to UF alone	4
60 °C	Methanol and evaporator condensate	MBR	Methanol removal is more efficient using the synthetic wastewater	5
37-52 °C	whitewater	Suspended carrier biofilm	70-85 % sol COD removal, cloudy effluent	6
55°C	TMP whitewater	MBBR	60-65% soluble COD removal, yield similar to mesophilic treatment	7

Table 2 (page10): Overview of suspended growth systems at elevated temperatures.
Note: we could not obtain the references Dougherty and McNary (1958) ourselves and used citations from other literature concerning their research. Loading rates rates were calculated using a VSS/TSS ratio of the mixed liquor of 0.7, a BOD/COD ratio of 0.5 was assumed.

Table 3 (page 11): Overview of thermophilic biofilm and MBR processes.
1: Perrtula *et al*. (1991) 2: Rintala and Lepistø (1993) 3:Visvanathan and Nhien (1995) 4: Ragona and Hall (1998) 5: Bérubé and Hall (1999) 6: Malmqvist *et al*. (1999) 7: Jahren *et al*. (2002)

Abbreviations used in Table 2,3:
AS: activated sludge, B: batch, BKME: bleach kraft mill effluent, CSTR: completely stirred tank reactor, MBR: membrane bioreactor, MBBR: moving bed biofilm reactor, NSSC: semichemical neutral ammonium sulphite pulping process, SBR: sequencing batch reactor, TMP: thermo mechanical pulping process, UF: ultrafiltration

Bastin *et al.* 1999) and high loading rates (Carpenter *et al*., 1968; Jackson, 1982; Duke *et al*., 1981; Bastin *et al*., 1999) promoting logarithmic growth of the bacteria. In some researches no data concerning the sludge retention time were mentioned (Gehm, 1956; Carpenter *et al*., (1968); Hunter *et al*., (1966); Duke *et al*., 1981). It should also be noted that some of the literature frequently cited concern studies of mesophilic activated sludge systems that have to cope with short term temperature upsets (Duke et al., 1981; Flippin and Eckenfelder, 1994; Bastin *et al*. 1999). These studies do not provide insights in the true feasibility of activated sludge under thermophilic conditions.
However, more recent research conducted by Tardiff and Hall (1997) and Tripathi and Allen (1999) did confirm the results of the older literature sources. At 50-60 °C they mention a deterioration in sludge settling properties leading to sludge losses and lower COD removal efficiencies. They used

sequencing batch reactors (SBR) in the temperature range of 20-60 °C. The reactors were operated for sufficiently long periods at sludge retention times (SRT) of 10-20 days. Furthermore, long sludge acclimatization periods were applied (in total 8 weeks for a 25 °C temperature increase in case of Tripathi and Allen (1999).

Both Barr *et al*. (1996) and Tripathi and Allen (1999) used bleach kraft mill effluent as reactor influent. They used different reactor configurations (activated sludge and SBR) but the key characteristics describing their systems are comparable (suspended biomass with sludge recycle, 15 days SRT, comparable influent and long sludge acclimatization periods). Their contradictory findings still make it unclear whether activated sludge can be operated successfully under thermophilic conditions. Moreover, the crucial factors affecting the sludge settling properties and the effluent turbidity remain unsolved.

3.3 Thermophilic biofilm processes and membrane bioreactors

The reported difficulties with activated sludge treatment led to a focus on alternative reactor configurations such as biofilm reactors (e.g. moving bed biofilm reactors) and membrane bioreactors. Table 3 presents an overview of these researches. In these studies however, not much attention was paid to a possible effluent turbidity and most experiments were conducted solely under thermophilic conditions making a direct comparison with mesophilic treatment difficult. Only Visvanathan and Nhien (1995) and Malmqvist *et al*. (1999) mention a cloudy effluent with an increasing temperature. Studies of Perrtula *et al*. (1991), Rintala and Lepistö (1993) and Jahren *et al*. (2002) mainly focused on COD removal. They showed that biofilm processes can be applied successfully under thermophilic conditions. However, for application of a moving bed biofilm reactor, Jahren *et al*. (2002) report that an additional sludge separation step is required to remove all suspended solids from the effluent.

Application of thermophilic aerobic membrane bioreactors (MBR) has been shown to be successful in the pulp and paper industry (Tardiff and Hall, 1997; Ragona and Hall, 1998; Bérubé and Hall, 1999; Ramaeckers *et al*., 2001). Membrane bioreactors have the advantage of a complete biomass retention, regardless of the state of aggregation of the biomass, and a high effluent quality. As high biomass concentrations can be attained, excess sludge production can be minimized and high volumetric loading rates are possible (Muller *et al*., 1995). However, energy costs are very high since a transmembrane pressure needs to be maintained and aeration requirements are significantly higher as compared to conventional aerobic treatment systems (Muller *et al.,* 1995; Mulder *et al.*, 2001). Furthermore, membrane bioreactors are very susceptible to (partly) inert influent solids as they would accumulate in the reactor and reduce the biological activity (Mulder *et al*., 2001). However, for certain specific applications, a membrane bioreactor can be a valuable alternative. For instance Bérubé and Hall (1999) used an MBR for methanol removal from foul evaporator condensate of a kraft pulp mill at 60 °C. A cost calculation showed that capital and operating costs of a high

temperature MBR were significantly lower than for methanol removal by a conventional alternative such as steam stripping (Bérubé and Hall, 2000c).

3.4 Proposed thermophilic reactor choice for application in the paper and board industry

The literature review of the previous paragraph suggests that biofilm processes are the most attractive option for application under thermophilic conditions. However, the process water of a board mill using recycled wastepaper has a very high calcium content. This results in extensive calcium carbonate precipitates in the biofilm. Unpublished research with a rotating biological contactor, treating anaerobic effluent of a board mill, was stopped after already two weeks of operation due to a complete clogging of the rotating discs (Vogelaar, unpublished). Furthermore, in practice the discs would increase too much in weight resulting in construction failures. Practical experience with a moving bed biofilm reactor treating wastewater from a recycled wastepaper mill has shown the limitations of this system as well (Habets, personal communication). Calcium carbonate deposits on the floating carriers increase their weight to such an extent that they will not move along with the water phase anymore and remain on the reactor bottom. The high calcium concentrations also gave severe operating problems when applying a membrane bioreactor with submerged membranes (Vogelaar, unpublished data). Calcium deposits on the membrane resulted in a rapid build up of the transmembrane pressure and after several days of operation the membranes became irreversibly damaged.

The presence of calcium in the process water thus gives severe problems for application of biofilm and MBR processes and two possible approaches can be followed: 1. remove calcium from the water phase in a separate step by for instance a crystallization reactor and apply the best suitable reactor system for this type of wastewater under thermophilic conditions, or 2: use an aerobic bioreactor that can simultaneously remove calcium and rest BOD without being hampered by the calcium deposits.

In the activated sludge treatment plant in Zülpich, calcium carbonate precipitates in the activated sludge itself without affecting its biological activity (Habets and Knelissen, 1997). In fact, the precipitates increase the sludge density and improve the sludge settling characteristics. This treatment scenario is most likely the most cost effective option for application in the board industry and was therefore also chosen for the lab-scale experiments (Chapter 5). Furthermore, the contradictory results of different researches regarding activated sludge treatment (Barr *et al*., 1996; Tardiff and Hall, 1997; Tripathi and Allen, 1999) make this an interesting reactor system to study from a scientific point of view.

4. PROBLEM STATEMENT AND OBJECTIVES

The aim of this thesis is to obtain knowledge on fundamental aspects of thermophilic aerobic wastewater treatment aiming to assist in the design of a thermophilic aerobic post treatment system for application in a paper or board mill. The purpose of this treatment system is to remove rest BOD, malodorous compounds, stickies, anionic trash and possibly calcium from the anaerobic effluent.

5. THESIS OUTLINE

The high temperature conditions do not only affect the microbiology of the bioreactor but also the physico-chemical parameters. Oxygen saturation concentrations decrease as a function of temperature while the diffusion rates increase, both affecting the oxygen transfer rate. **Chapter 2** evaluates the temperature effect on the oxygen transfer rate in clean water and in process water.

In **Chapter 3**, intrinsic growth and decay rates are estimated for aerobic biomass cultivated at 30 and 55 °C on acetate.

The anaerobic effluent of a board mill is as influent for mesophilic and thermophilic activated sludge reactors in chapter 5,6 and 7. The anaerobic effluent itself is characterized by means of respirometry in **Chapter 4.** Furthermore, possible causes for differences in COD removal efficiencies between 30 and 55 °C are discussed.

Chapter 5 describes a feasibility study of a thermophilic activated sludge process. Continuous activated sludge reactor experiments are performed at 30 and 55 °C with COD removal as the main parameter of interest.

The thermophilic reactor was characterized by a lower COD removal and a higher effluent turbidity as compared to the mesophilic reactor. The nature of the effluent colloidal material, causing the turbidity was studied in **Chapter 6**. Furthermore, the effects of protozoa on the effluent turbidity in both reactors is investigated.

In **Chapter 7** a more fundamental approach is used to clarify the reason for the less efficient flocculation/biosorption under thermophilic conditions by investigating the temperature dependency of the hydrophobic interaction.

Chapter 5 and 6 showed that activated sludge treatment is feasible under thermophilic conditions but relatively long hydraulic retention times are required. In **Chapter 8**, an attempt is made to develop a new type of bioreactor with high concentrations of biomass in granular form, aiming at higher conversion rates and thus a more compact reactor system.

Chapter 9, presents a general discussion of the obtained results.

2

TEMPERATURE EFFECTS ON THE OXYGEN TRANSFER RATE BETWEEN 20 - 55 °C

ABSTRACT

The influence of temperature on the oxygen transfer rate (OTR) was studied in a bubble column. Aeration took place in three different liquids: tap water, anaerobically pretreated paper process water and thermophilic sludge grown on a mineral medium and volatile fatty acids as carbon source. The OTR was measured in a temperature range of 20-55 ^{0}C in case of tap- and process water. The OTR in the thermophilic sludge was determined at 55 ^{0}C. The OTR remained constant over the specified temperature range in case of tap water and showed a slight increase in case of process water. The constant OTR in case of tap water was due to the counteracting effect of an increased overall oxygen transfer coefficient versus the decreased oxygen saturation concentration at higher temperatures. At 55 ^{0}C the OTR in the thermophilic sludge was comparable to both other liquids at this temperature.

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Vogelaar, J.C.T. (2000) Author's reply to comments made by Urban and Gulliver on the above mentioned paper. Water Res. 34(13), 3486.

NOMENCLATURE

θ	theta factor, typical value approximately 1.024 (-)
a	interfacial surface area (m^2m^{-3})
c	dissolved oxygen concentration in the bulk liquid (mg O_2 l^{-1})
c_e	apparent oxygen saturation concentration, (mg O_2 l^{-1}).
c_s	saturation concentration of dissolved oxygen at the specified temperature, pressure and salinity (mg O_2 l^{-1}).
d_b	bubble diameter (m)
D_{ol}	oxygen diffusion coefficient in the liquid phase (m^2 s^{-1})
K_l	oxygen transfer coefficient (m h^{-1})
$K_la(T)$	overall oxygen transfer coefficient at temperature T (h^{-1})
OC	oxygenation capacity (mg O_2 l^{-1} h^{-1}).
OTR	oxygen transfer rate (mg O_2 l^{-1} h^{-1})
R	gas constant (=8.314) (J $mole^{-1}$ K^{-1})
r_{act}	actual respiration rate of the biomass (mg O_2 l^{-1} h^{-1})
t	time (s)
T	temperature (^{0}C in eq 2., K in eq. 5.)
τ_p	response time of the oxygen probe (s)
v_{bs}	bubble rise velocity relative to the liquid (m s^{-1})

1. INTRODUCTION

The paper and board industry is putting a lot of effort in closing the water systems in the production line resulting in the so-called zero discharge paper mills. Operation of these zero-discharge mills was shown to be possible in the board industry but requires an in-line treatment system to prevent bad smells in the end-products. Recently, Habets and Knelissen (1997) presented a sequenced anaerobic-aerobic treatment system as the most cost-effective option for in-line treatment of process water from a paper mill using recycled wastepaper as raw material. A disadvantage of their set-up is the required cooling of the process water to mesophilic conditions prior to biological treatment and subsequent heating afterwards. The process water needs to be reheated since the optimal temperature for paper production is approximately 55 ^{0}C. The current research project aims at a complete anaerobic-aerobic treatment under thermophilic conditions (50-55 ^{0}C) of paper process water in a closed cycle mill. Regarding the post treatment step, it is believed that thermophilic aerobic treatment may be limited by

a poor oxygen transfer due to the lower oxygen saturation concentrations (c_s) at higher temperatures. The goal of this study is to determine the OTR under mesophilic and thermophilic conditions in tap water and process water. The oxygen transfer rate is influenced by several factors. Stenstrom and Gilbert (1981) mention the following:

- air flow rate
- bubble diameter
- temperature
- viscosity
- basin geometry (affects contact time between gas and liquid)
- wastewater composition (salts, surfactants, biomass)

Equation 1 (Lewis and Whitman, 1924) describes the rate of oxygen transfer from a gas to a liquid phase.

$$\frac{dc}{dt} = OTR = K_l a(c_s - c) \qquad (1)$$

The oxygenation capacity (OC) of the aeration system is calculated as the OTR assuming a zero oxygen concentration in the bulk liquid.

Relation between OTR and temperature

Increasing temperatures result in a lower oxygen solubility leading to a smaller driving force (c_s-c) and hence to a lower OTR. However, the diffusion rate of oxygen increases with increasing temperatures while the liquid viscosity and surface tension decrease. These effects result in an increased $K_l a$ value that might offset the smaller driving force. An overview of the oxygen solubility data from various sources, with and without correction for the vapor pressure are presented in Table 1.

Table 1. Oxygen solubility data (mg l^{-1}) in aqueous solutions at $p=p_0$.

Temp ^0C	APHA (1995)[a]	Perry and Green (1984)[b]	Boogerd *et al.* (1990)[c]	Measured, this study[d]
20	9.09	9.43	9.09	9.2
30	7.56	7.98	7.55	7.43
40	6.41	7.11	6.41	6.5
50		6.50	5.50	
55	5.1		5.07	5.15
60		6.10	4.67	
70		5.82	3.84	
80		5.65	2.88	

[a]APHA (1995): demineralized water, vapor pressure corrected.

[b]Perry and Green (1984): demineralized water, no vapor pressure correction.

[c]Boogerd *et al*. (1990): 5 mM sulphuric acid, vapor pressure corrected.

[d]Tap water, vapor pressure corrected.

At higher temperatures the OTR was often estimated by use of the theta factor (θ). The theta factor is defined by equation (2) and can be used to estimate $K_l a$ values at higher temperatures (T) in case the value at 20 ^{0}C is known.

$$K_l a(T) = K_l a_{20} \theta^{T-20} \quad (2)$$

However, very different values were found for this factor under well defined experimental conditions. Stenstrom and Gilbert (1981) note that the use of this factor is inadequate to predict oxygen transfer at higher temperatures.

Bewtra *et al*. (1970) measured the OTR in a 40 m^3 aeration tank and found a constant OTR over a temperature range of 10-30 ^{0}C. Boogerd *et al*. (1990) found that the OTR showed only minor changes in a temperature range between 15 and 70 ^{0}C in a mechanically mixed fermentor while in an air-mixed fermentor the transfer capacity for O_2 decreased slowly but steadily.

2. MATERIALS AND METHODS

The experiments were performed in three different liquids: tap water, anaerobically pretreated paper process water and thermophilic sludge. The OTR was determined in a temperature range of 20 - 55 ^{0}C in case of tap water and process water while the OTR in the sludge was measured at 55 ^{0}C. The experimental set-up is presented in Table 2.

A cylindrical bubble column with an effective volume of 3 liters was aerated at different flow rates, i.e., 0.15, 0.3, 0.45 and 0.56 vvm (volume air volume liquid^{-1} min^{-1}). The column was temperature controlled. Mixing took place by aeration and a magnetic stirring device. Oxygen was measured with an autoclavable Mettler Toledo Inpro 6000 oxygen probe (silicon membrane) connected to a Knick 7302-2 oxygen meter. In all three experiments the $K_l a$ values were determined in duplicate with the "start-up dynamic method" as described by Linek *et al*. (1993). In experiment 2 the process water was aerated for a short period to oxidize possible interfering compounds like sulphides before the $K_l a$ determination took place.

The thermophilic sludge in experiment 3 was grown on a mineral medium (Schlegel, 1993) with volatile fatty acids as organic substrates. The thermophilic sludge was substrate depleted to assure a constant respiration rate during the $K_l a$ determination. The respiration rate (r_{act}) was measured by switching off the aeration while stirring was maintained. The oxygen saturation concentration (c_s) in the thermophilic sludge was estimated by eq. 3 which is similar to eq.1 when a steady state during aeration is reached. c_e is the apparent oxygen saturation concentration observed in the experiment with thermophilic sludge.

$$r_{act} = K_l a(c_s - c_e) \quad (3)$$

The use of an autoclavable stainless steel oxygen probe can lead to additional measurement difficulties because of the electrode response time. The probe response time, τ_p is defined as the time needed to record 63% of a stepwise change. The response time (τ_p) of this electrode is larger than the conventional oxygen probes that are used under moderate temperature conditions. A large τ_p can lead to an inaccurate calculation of the $K_l a$ if the dissolved oxygen concentration changes rapidly in time. Several correction programs were designed in the past to compensate for the dynamic behavior of the probe (Aiba *et al*. 1984; Koizumi and Aiba, 1984). Van 't Riet (1979) states that it is possible to measure $K_l a$ values without additional correction programs if eq. 4 is met.

$$\tau_p \leq \frac{1}{3k_l a}. \quad (4)$$

The above mentioned restriction was always met in this research and therefore no additional correction programs were used.

Table 2. Experimental set-up.

	liquid	temperature ^{0}C
exp. 1	tap water	20
		30
		40
		55
exp. 2	process water	20
		55
exp. 3	thermophilic sludge	55

3. RESULTS AND DISCUSSION

The results of the aeration experiments in tap water are presented in Fig. 1. The $K_l a$ increased as expected while c_s decreased in the same extent. Standard errors were less than 1 %.

The effect of the air flow rate on the $K_l a$ is shown in Fig. 1B. The $K_l a$ values and hence the OC are linearly related to the air flow rate within the specified range. Apparently, the bubble characteristics remained unchanged in this flow range. The $K_l a$ values in the sludge and the process water did not differ significantly from the values found for tap water.

The c_s values that were measured (exp. 1 and 2) or estimated (exp. 3) are presented in Table 3. These saturation data were used to calculate the oxygenation capacity (OC) of the aeration system in the different media at the various temperatures (Fig. 2A). The OC in the tap water remained constant in the temperature range of 20-55 ^{0}C while the OC in the process water increased. The OC in the

thermophilic sludge at 55 ^{0}C was comparable to the OC in the process water and slightly lower compared to tap water. This was caused by a lower oxygen saturation concentration in the thermophilic sludge compared to tap water.

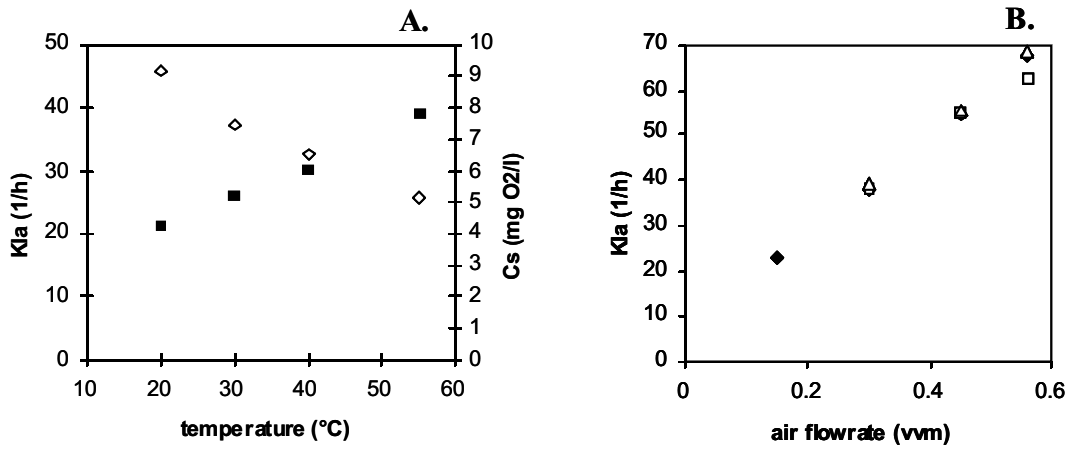

Fig. 1 A: C_s (◇) and K_la (■) values measured in tap water with a constant air flow rate of 0.3 vvm.
Fig. 1B. K_la values determined at 55 ^{0}C in tap water (◆), process water (□) and thermophilic sludge (△) at air flow rates between 0.15 - 0.56 vvm.

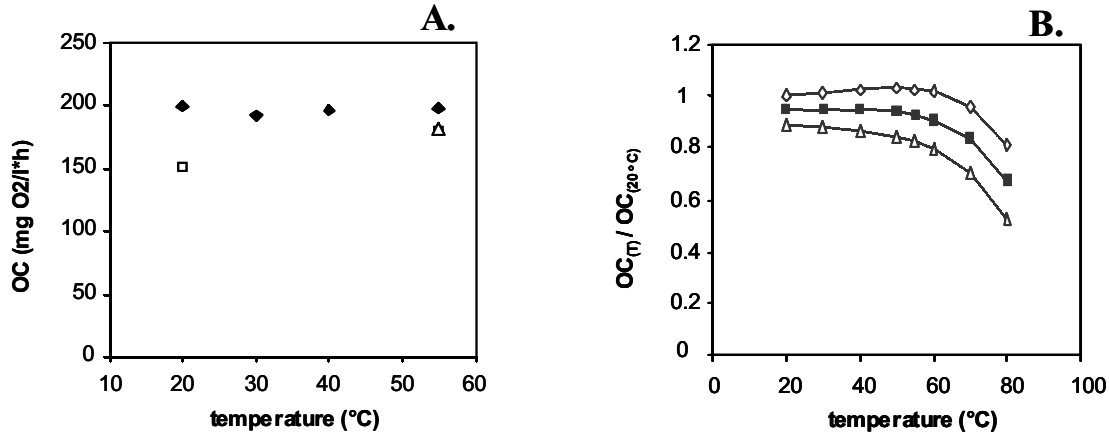

Fig. 2. A: Measured OC in tap water (◆) , process water (□) and thermophilic sludge (△) versus temperature. Air flow rate 0.3 vvm.
B: Estimated oxygenation capacity (OC) according to Urban and Gulliver (2000) as a function of temperature compared to a reference temperature of 20 °C. (◇) Residual oxygen concentration 0 mg/l, (■) residual oxygen concentration 0.5 mg/l, (△)residual oxygen concentration 1 mg/l.

Table 3. Measured c_s (mg l^{-1}) and K_la (h^{-1}) values in tap water and process water and standard deviations. c_s in thermophilic sludge was estimated by eq. 3. Air flow rate 0.3 vvm

Temp. ^0C	c_s tapwater	K_la tapwater	c_s process water	K_la process water	c_s thermophilic sludge	K_la thermophilic sludge
20	9.19	22.4 ± 0.4	8.7	17.4 ± 0.5		
30	7.43	26.0 ± 0.1				
40	6.5	30.6 ± 0.2				
55	5.15	38.8 ± 1.5	4.8	37.4 ± 1.6	4.55	39.7 ± 1.8

Comparable results were found by other researchers. Smith and Skidmore (1990) determined gas holdups and K_la values in a concentric tube airlift reactor at 30, 50 and 72 ^0C. Calculation of the OTR with their K_la data and oxygen saturation concentrations from Boogerd *et al*. (1990) results in a slightly increasing oxygen transfer rate with increasing temperatures. Krahe *et al.* (1996) also found increased oxygen transfer rates with increasing temperatures in a mechanically mixed fermentor. They determined the K_la values experimentally and used oxygen solubility data from Perry and Green (1984) to calculate the OTR. However, the solubility data from Perry and Green (1984) are not corrected for the increasing water vapor pressure at higher temperatures and overestimate the actual oxygen saturation concentrations. Boogerd *et al*. (1990) warn for this effect since it results in an overestimation of the OTR, especially at higher temperatures.

The present results are to some extent predictable by the general mass transfer theories. The increased temperature will lead to a higher diffusion rate for oxygen (D_{ol}) in the liquid phase due to the direct temperature effect and indirect by the lower liquid viscosity. This results in a higher oxygen transfer coefficient (K_l) value thus having a positive effect on the OTR. With regard to the diffusion rate for oxygen, Wise and Houghton (1966) give the following relation :

$$D_{ol} = 4.2 \cdot 10^{-6} \exp(-18 \cdot 10^3 / RT) \tag{5}$$

For bubbles larger than $2.5 \cdot 10^{-3}$ (m) the bubble surface is always mobile and K_l can be calculated using the Higbie model (Heijnen and Van 't Riet, 1984) with v_{bs} as the bubble rise velocity and d_b the bubble diameter:

$$K_l = \left(\frac{4 D_l v_{bs}}{\pi d_b} \right)^{0.5} \tag{6}$$

Visual inspection showed that the bubble diameter in this research was always larger than 2.5 (mm). This is in accordance with the theory for tap water. In a coalescing medium such as tap water, the

bubbles generated from a porous disc/stone are approximately $(2\text{-}4)\cdot10^{-3}$ (m) and tend to coalesce to an ultimate bubble diameter of $6\cdot10^{-3}$ (m) (Heijnen and Van 't Riet, 1984).

The oxygen saturation concentration decreases 44% with a temperature increase from 20 till 55 ^{0}C as measured in this research. Equations (5) and (6) predict a 48% increase in K_l in the same temperature range, partially counteracting the lower saturation values. However, possible changes in interfacial surface area have not been taken into account yet.

It is not expected that the bubble diameter will change with temperature but a lower liquid viscosity will most probably result in a higher bubble rise velocity. This leads to a lower gas hold-up and hence a smaller interfacial surface area. According to Stokes law, the terminal bubble rise velocity in case of rigid spheres (small bubbles) is inversely proportional to the liquid viscosity. Deckwer *et al*. (1982) found no effect of liquid viscosity on the gas hold-up with larger bubbles (1 cm). So, in the worst case, when Stokes law applies a 50 % decrease in the interfacial surface area can be expected with a temperature increase from 20 till 55 ^{0}C. The most optimal case predicts no temperature effect on the interfacial surface area.

A more comprehensive model to predict the overall oxygen transfer coefficient (K_la) as a function of temperature was developed by Urban and Gulliver (2000). It incorporates changes in the bubble rise velocity (v_{bs}) and the bubble diameter (d_b). This led to a much better fit of the current experimental data to the model when compared to the Higbie approximation. Using the indexing theory developed by Gulliver *et al.* (1990), an estimation of the oxygenation capacity of a bubble column was made as a function of temperature (Fig. 2B). It shows that until 60 °C, no significant change in the oxygenation capacity of the aeration system occurs, especially in case the dissolved oxygen concentration in the liquid is allowed to be very low (nearly zero). At temperatures beyond 60 °C, the OC drops rapidly which is mainly due to the increased water vapor pressure at these temperatures.

In general it can be concluded that until 55 °C, the oxygen transfer rate is only minorly affected by the liquid temperature. This effect might be slightly positive, negative or none at all (this research) depending on the bubble diameter and the reactor type. However, in the current research, the OC was calculated assuming a zero oxygen concentration in the liquid since it is defined as such. This situation is not desired in conventional aerobic wastewater treatment systems in which the dissolved oxygen concentration is usually maintained at 1 or 2 (mg l^{-1}). Thus, the residual oxygen concentration becomes increasingly important with increasing temperatures since its proportion to the saturation concentration increases with temperature. In an actual wastewater treatment system, designed to maintain a certain dissolved oxygen concentration, a decrease in the oxygenation capacity is thus to be expected with an increase in temperature.

4. CONCLUSIONS

The present research has shown that in tap water, at higher temperatures, the decreasing oxygen saturation concentration (c_s) was completely offset by the increased overall oxygen transfer coefficient (K_la). This results in a constant oxygen transfer rate in tap water within the temperature range of 20-55 ^{0}C. The temperature effect on the separate variables K_l and A has not been revealed yet.

The slightly lower OTR values in the process water and the thermophilic sludge compared to tap water are caused by the lower oxygen saturation concentrations in these liquids. The K_la values in these liquids do not differ significantly from the values found for tap water.

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3

KINETICS OF MESOPHILIC AND THERMOPHILIC AEROBIC BIOMASS GROWN ON ACETATE

ABSTRACT

Kinetics describing growth and decay of mesophilic (30 °C) and thermophilic (55 °C) aerobic biomass were determined in continuous and batch experiments by using respirometry. Biomass was cultivated on a single soluble substrate (acetate) and a mineral medium. The intrinsic maximum growth rate at 55 °C was 0.71 ± 0.11 (h^{-1}) which is a factor 1.5 higher than the intrinsic μ_{max} at 30 °C (0.49 ± 0.11 h^{-1}).). The biomass decay rates increased from 0.004 - 0.008 (h^{-1}) at 30 °C to 0.016-0.018 (h^{-1}) at 55 °C. Theoretical biomass yields were similar at 30 and 55 °C: 0.5 (g biomass COD · g acetate COD^{-1}). The observed biomass yields decreased under both temperature conditions as a function of the sludge retention time. Under thermophilic conditions, this effect was more pronounced due to the higher decay rates, resulting in a smaller biomass production at 55 °C as compared to 30 °C. The disbalance in the effect of temperature on growth and decay is that with increasing sludge ages, the maximum volumetric conversion capacity of a thermophilic reactor system will be smaller than a mesophilic analogue as the decrease in biomass content is not fully compensated by a higher specific conversion rate. This only holds true when both reactors are operated at the same organic loading rate. However, a higher loading rate can be imposed on a thermophilic bioreactor in case the sludge production is allowed to be similar to a mesophilic system and in this sense biokinetic advantages can be expected from thermophilic bioreactors.

Vogelaar, J.C.T., A. Klapwijk., H. Temmink and J.B. van Lier, accepted for publication in J. Indus. Microbiol. Biotechnol.

NOMENCLATURE

COD	Chemical Oxygen Demand (mg O_2 l^{-1})
CSTR	continuously stirred tank reactor
D	dilution rate (h^{-1})
D_{cr}	critical dilution rate (h^{-1})
DOC	dissolved organic carbon (mg l^{-1})
HRT	hydraulic retention time (h)
k_d	first order decay rate (h^{-1})
K_s	monod constant (mg COD l^{-1})
MBR	membrane bioreactor
OUR	Oxygen Uptake Rate (=respiration rate) (mg O_2 l^{-1} h^{-1})
S	readily biodegradable substrate (mg COD l^{-1})
X_h	heterotrophic biomass concentration (mg biomass COD l^{-1})
Y	theoretical yield (g biomass COD g substrate COD^{-1})
Y_{obs}	observed yield (g biomass COD g substrate COD^{-1})
θ = SRT	Sludge Retention Time (h)
μ	growth rate (h^{-1})
μ_{max}	maximum growth rate (h^{-1})

1. INTRODUCTION

Thermophilic wastewater treatment has gained increasing interest in recent years. This is mainly due to the growing tendency for industrial water system closure and the subsequent need to purify hot wastewater streams. Often mentioned advantages of thermophilic treatment are the higher conversion rates and a lower sludge production. Concerning anaerobic conversions at elevated temperatures, data are available that support the higher conversion rates (Buhr and Andrews, 1977; van Lier, 1996). However, kinetic data for thermophilic aerobic conversions are scarce, to some extent contradictory and not always reliable in case complex wastewaters were used in the studies. Table 1 presents an overview of all kinetic data concerning thermophilic aerobic wastewater treatment systems published to this date. The expected trend of higher conversion rates and lower biomass yields with increasing temperatures is not very clear when analyzing these data.

Based on a literature review, Lapara and Alleman (1999) mention 3 to 10 times higher rates in thermophilic treatment systems compared to their mesophilic analogous. Based on a more recent experimental study they state that biokinetic advantages are not to be expected at higher temperatures

(Lapara *et al*., 2000a,b). The lower yields have so far only been confirmed by Bérubé and Hall (2000a). Reasons for these discrepancies are related to: 1. the complex nature of the wastewaters that were used in the experiments, 2. the applied experimental set-up, and 3. experiments have only been performed under thermophilic conditions making a direct comparison with mesophilic kinetics impossible (Sürücü *et al*., 1976; Couillard *et al*., 1989; Block and Wiesmann, 1991, 1992; Bérubé and Hall, 2000a; LaPara *et al*., 2000b).

Table 1. Overview of kinetic data of thermophilic suspended growth systems.

substrate	Reactor	T (°C)	μ_{max} (h^{-1})	k_d (h^{-1})	Y [(1)]	K_s (mg COD l^{-1})	q [(2)]	Ref.
glucose	CSTR	58	0.22	0.02	0.34	740	0.65	1
Paper mill wastewater [(3)]	CSTR	20 53	0.13 0.14	0.06 0.02	0.34 0.6	60 890	0.21 0.23	2
Slaughterhouse wastewater	CSTR	45 52 58	0.24 0.25 0.42	0.022 0.013 0.033	0.35 0.3 0.32	46 30` 992	0.69 0.83 1.31	3
acetate	CSTR	60	0.51 4.5	0.3	0.41	2.4 1400	1.24	4
synthetic [(4)]	MBR	55 60 65 70	≅ 0.35 [(6)] ≅ 0.38 ≅ 0.2 ≅ 0.1				≅ 0.65 ≅ 0.85 ≅ 0.45 ≅ 0.2	5
Synthetic [(5)]	CSTR	55	0≅.22	0.002 ± 0.004	0.32 ± 0.035 [(5)]	660 ± 320	0.69	6
Synthetic [(5)]	batch	25 35 45 55 65	≅ 0.29 [(6)] ≅ 0.37 ≅ 0.4 ≅ 0.3 ≅ 0.22		≅ 0.45[(7)] ≅ 0.58 ≅ 0.45 ≅ 0.35		0.63 0.6 0.7 0.63	7

Note: [(1)] Presented yield values are theoretical yields, expressed as (g biomass VSS g substrate COD^{-1}).

[(2)] Metabolic quotient (q), expressed as (g COD g biomass VSS^{-1} h^{-1})

[(3)] Wastewater from a paper mill producing liner from recycled corrugated boxes.

[(4)] Synthetic kraft pulp mill condensate containing methanol.

[(5)] Synthetic medium containing lactose and gelatin as energy and carbon source.

[(6)] approximate values, obtained from a graph in the respective publications.

[(7)] Yield in mg TSS g substrate COD^{-1}.

References: 1: Sürücü *et al.* (1976), 2: Jackson (1983), 3: Couillard *et al.* (1989), 4: Block and Wiesmann (1991,1992), 5: Bérubé and Hall (2000a), 6: Lapara *et al*. (2000b), 7: Lapara *et al*. (2000a)

The complex wastewaters as applied in the researches of Jackson (1983) which was a paper mill wastewater, and Couillard *et al.* (1989), a slaughterhouse wastewater, most likely contained a non-biodegradable fraction. Effluent (non-biodegradable) COD was regarded as biodegradable substrate,

resulting in an overestimation of the Monod constants (Table 1). Inert COD can also be produced from biomass decay and substrate metabolism as was probably the case in experiments conducted by Sürücü *et al.* (1976) and LaPara *et al*. (2000b). Regarding the experimental set-up, estimations of the maximum growth rates and Monod constants from continuous reactor experiments without cell recycle is a difficult task since generally, Monod constants of dispersed cultures are very low (several mg COD l^{-1}). This means that over a wide range of sludge retention times, effluent substrate concentrations are very low; around the detection limit of the analytical equipment, making it difficult to obtain accurate estimates of the maximum growth rate and Monod constant. This also results in a poor correlation between the data and the model as was also observed in these studies (Sürücü *et al*., 1976; Jackson, 1983; Couillard *et al.*, 1989; LaPara *et al*., 2000b). The growth rates obtained in the batch experiments by LaPara *et al*. (2000a) are somewhat disturbed by the non-simultaneous degradation of both synthetic substrates at 55 °C while under mesophilic conditions, both substrates were oxidized simultaneously. If a single substrate would have been used, then a higher thermophilic maximum growth rate might have been obtained.

Many studies have also been performed on pure cultures of thermophiles (Kuhn *et al*., 1980; Cometta *et al*., 1982; Sonnleitner *et al.,* 1982; Brooke *et al.,* 1989; Becker *et al*., 1997). Most microbiological studies do report higher growth rates, approximately a factor two higher that mesophilic analogues but kinetic data vary to a large extent. Cometta *et al*. (1982) have shown that the maximum growth rate of thermophiles very much depends on the medium composition, pH, temperature and type of reactor vessel. A complex medium, containing peptone and meat extract yields higher growth rates than a mineral medium and chemostat cultures yield higher growth rates as compared to batch cultures as these are easily oxygen or substrate limited. In addition to higher growth rates, also higher maintenance requirements are reported (Kuhn *et al*., 1980; Cometta *et al.,* 1982; Sonnleitner *et al*., 1982). However, Brooke *et al.* (1989) and Becker and Märkl (2000) report similar maintenance requirements of thermotolerant and thermophilic *Bacilli* when compared to literature data describing kinetics of mesophiles. Although results are to some extent contradictory, it appears that in general the maintenance requirements increase as a function of temperature. Konings *et al.* (1992) suggest that at extreme temperatures, the high permeability of the cell mebrane to protons is one of the causes for the high maintenance requirements. In addition, it should be mentioned that extremely high growth rates for thermophiles can be found but these are obtained at the expense of the substrate affinity, resulting in a very high Monod constant. For instance Cometta *et al*. (1982) and Sonnleitner *et al*. (1982) found values of 3.5 (h^{-1}) for *Thermus* species. This was also confirmed by Block and Wiesmann (1991, 1992) who found two types of microorganisms in a CSTR: *Bacillus stearothermophilus* growing at a very high growth rate (μ_{max} 4.5 h^{-1}) and a low substrate affinity (K_s 1400 mg DOC l^{-1}) and another *bacillus* like species growing at a moderate rate (μ_{max} 0.51 h^{-1}) and a high substrate affinity (K_s 2.4 mg DOC l^{-1}).

According to Grady *et al*. (1996) kinetic parameter estimates can vary considerably for three main reasons: 1. culture history, 2. parameter identifiably and 3. the nature of the essay to employed to measure the parameters. They propose to use a new nomenclature when describing bacterial kinetics with "intrinsic" parameters meaning "belonging to the essential nature of a thing" and "extant" for currently existing parameters. Appropriate estimates of the intrinsic maximum growth rates are made in experiments in which the initial amount of substrate over the initial amount of biomass (S_0/X_0) exceeds 20 (on a COD basis) since this value is large enough to allow the bacteria to have unrestricted growth and it also results in a good parameter identifiably (Grady *et al*., 1996). In these experiments the bacterial population at the end of the experiment can even be completely different compared to the seed sludge in case a more rapidly growing bacterial species would develop during the test. True extant parameters are measured in case the S_0/X_0 ratio is less than 0.025 (Grady *et al*., 1996).
The aim of this research was to estimate intrinsic kinetic parameters of mesophilic and thermophilic biomass assuming that differences in kinetics are the sole result of the difference in temperature. Biomass was cultivated at 30 and 55 °C on a single soluble substrate (acetate) and a mineral medium. Experimental data were fitted to a model (Grady and Lim, 1980) and the estimated kinetic parameters were evaluated in the light of literature data.

2. MATERIALS AND METHODS

2.1 Model description

The model proposed by Grady and Lim (1980) assumes that substrate can only be removed by growth, i.e.- there is no substrate production from decay and respiration is associated with both growth and decay. This means that heterotrophic biomass oxidizes itself for maintenance purposes. We assume that the biomass is completely biodegradable although in practice some part is turned into inert material (and is thus not oxidized). The model looks as follows:

biomass growth: $$\frac{dX_h}{dt} = (\mu - k_d)X_h \quad (1)$$

substrate removal : $$\frac{dS}{dt} = -\frac{\mu}{Y}X_h \quad (2)$$

Monod equation: $$\mu = \mu_{\max}\frac{S_s}{K_s + S_s} \quad (3)$$

Oxygen Uptake Rate: $$OUR = \left(\frac{1-Y}{Y}\right)\mu X_h + k_d X_h \quad (4)$$

2.2 Experimental set-up

Two continuously fed bioreactors without biomass recycle were operated at 30 and 55 °C. Both systems were fed with acetate and a nutrient solution and were operated at 13 and 43 h hydraulic retention time (HRT, which is in this set-up equal to the sludge retention time, SRT). Sludge cultivated in the continuous reactors was used for batch experiments. Three types of batch experiments were performed, depending on the ratio of substrate over biomass:

1. growth experiments to determine the intrinsic maximum growth rate, at a high initial substrate/biomass ratio > 20
2. decay experiments to determine the decay rate, at a high initial biomass concentration and with no additional substrate added.
3. yield experiments to determine the theoretical yield and the Monod constant at a low initial substrate/biomass ratio <0.1

An overview of the experimental set-up is depicted in Table 2. Batch experiments were mainly performed with sludge cultivated at 13 hours sludge retention time (SRT). Only the yield experiments were conducted with sludge grown at a 13 and 43 hours SRT.

The continuous reactor experiments were run both for sludge cultivation and to determine the observed biomass yield at both 13 and 43 hours SRT. Furthermore, the extant maximum biomass growth rate (Grady *et al*., 1996) at 13 (h) SRT was determined in a wash-out experiment described by Pirt (1975).

Table 2. Experimental set-up.

Type of experiment	Estimated parameter	S_0/X_0	No. of experiments 30 °C	55 °C
No. 1 (growth)	μ_{max}	30 - 40	4	8
No. 2 (decay)	k_d	-	4	4
No. 3 (yield)	Y - K_s	0.05 - 0.1	6	6
Continuous wash-out	μ_{max}	-	3	3

2.3 Parameter estimation - Continuous reactors

The *observed biomass yield (Y_{obs})* at both SRTs was obtained directly from a COD balance over the reactors, assuming all effluent COD excluding acetate to be biomass COD. The *extant maximum growth rate (μ_{max})* was estimated from a wash-out experiment (Pirt, 1975). Increasing the reactor dilution rate beyond the critical dilution rate results in a biomass wash-out. From the rate at which the biomass was washed out and the imposed dilution rate, the maximum growth rate of the biomass at that time instant could be calculated.

2.4 Parameter estimation - Batch experiments

The *intrinsic maximum growth rate (μ_{max})* -minus decay rate was estimated from the exponential increase in the OUR (Oxygen Uptake Rate) in batch exp. 1. The model differential equations were solved analytically and the simulated OUR was fitted to the measured OUR by minimizing the sum of the squared errors. The *decay rate (k_d)* was obtained from the exponential decrease in the OUR in batch exp. 2, assuming the growth rate to be zero. The *theoretical biomass yield* (Y) was estimated from batch exp. 3 in which a known (small) amount of substrate was added to (a high concentration of) biomass. Y was calculated from the balance of the cumulative oxygen uptake due to substrate conversion and the amount of substrate added (biomass production is substrate conversion minus the oxygen uptake). The cumulative oxygen uptake is obtained after integration of the respiration rate over time after subtraction of the endogenous respiration rate. The initial endogenous OUR was estimated from a period of (a relatively) stable OUR before substrate addition. Possible changes in the endogenous OUR due to growth of biomass by the substrate additions and due to continuous decay (as estimated in the decay experiments) were accounted for. The Monod constant (K_s) was estimated from batch exp. 3. From the course of the respiration rate, the known initial amount of substrate and the calculated yield, the substrate concentration at each time instant could be calculated. The *Monod constant* was estimated as the substrate concentration at time (t) where the respiration rate was half the maximal respiration rate due to substrate depletion.

2.5 Reactor description

Two 2.5 liter temperature controlled continuous reactors were operated at 30 and 55 °C (Fig. 1). The reactor temperature was maintained by circulating water through the water jackets of the reactors. Both reactors were continuously fed with a non-sterilized synthetic influent. No biomass inoculum was used and temperature adapted biomass developed spontaneously as a result of the non-sterile operating conditions. Wall growth was controlled by daily reactor cleaning. The pH was controlled at 7.2. Mixing took place by aeration with pressurized air and by a magnetic stirring device at the reactor bottom. At 55 °C, dissolved oxygen (DO) was measured using a Mettler Toledo inpro 6000 probe, at 30 °C, DO was measured using a WTW OXY 191. In both reactors, DO values were always maintained above 3 (mg O_2 l^{-1}). Evaporation via the exhaust air was negligible due to a condenser on top of the reactor. Both reactors were operated at 13 and 43 (h) HRT. The 4 liter batch reactor was a temperature and pH controlled glass vessel connected to a respirometer. Evaporation in the batch vessel was negligible by cooling of the exhaust air and aeration took place with pressurized air.

2.6 Oxygen uptake rate (OUR) measurements

Batch respirometric experiments were performed using a modified version of a Manotherm RA 1000 respirometer (Spanjers *et al*., 1994). Measurement of the respiration rate under thermophilic

conditions took place by exchanging the conventional WTW DO probe for a Mettler Toledo Inpro 6000 probe with a silicon membrane. This electrode was connected to a Knick process Unit 7302-2 and the amplified DO signal (4-20 mA) was connected to the RA 1000 motherboard. Measured DO values were logged into a computer. The respiration rate was calculated from these data using the analytical method as described in Spanjers (1993). Every two minutes a new respiration data point was obtained.

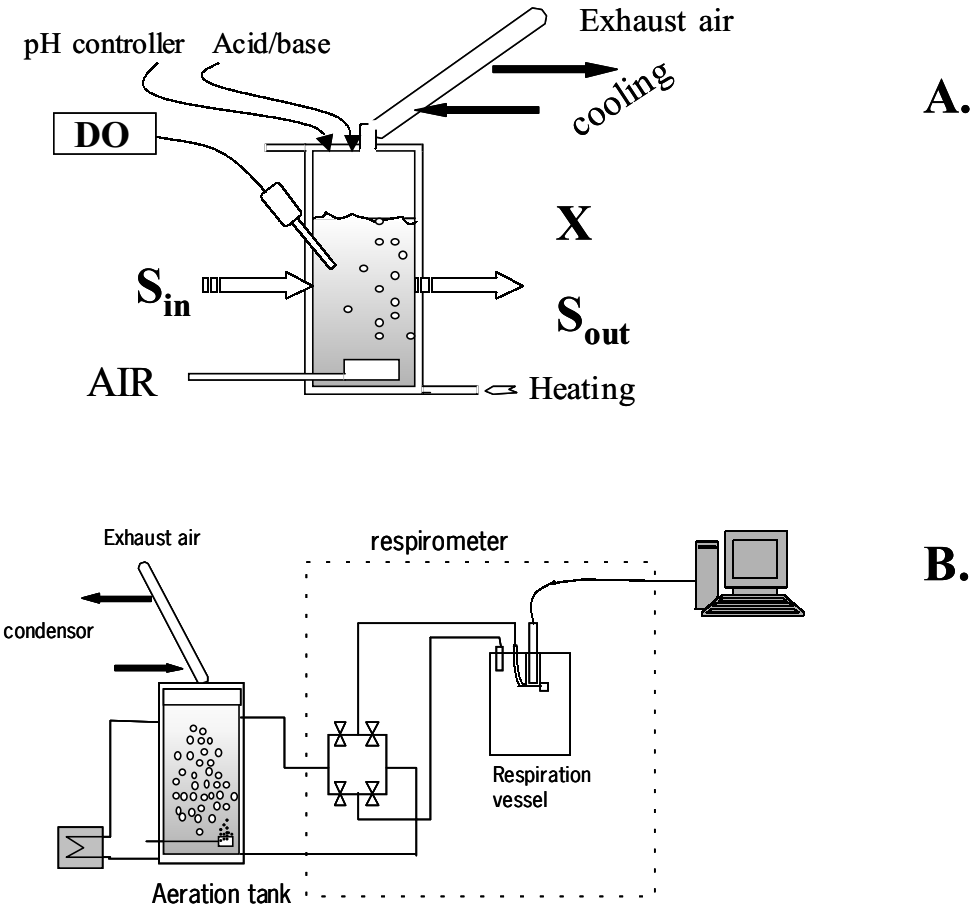

Fig. 1a: Continuously fed reactors without sludge retention.

Fig. 1b: Batch vessel connected to respirometer.

2.7 Reactor medium

Reactor influent consisted of un-neutralized acetic acid (2.6 g COD l^{-1}), NH_4Cl (480 mg l^{-1}), K_2HPO_4 (140mg l^{-1}), $MgSO_4 \cdot 7H_2O$ (50 mg l^{-1}), $FeSO_4 \cdot 7H_2O$ (2.5 mg l^{-1}), $CaCl_2 \cdot 2 H_2O$ (2.5 mg l^{-1}) and 25 µl trace element solution per liter as described by Zehnder *et al.* (1980).

2.8 Analytical methods

Mesophilic and thermophilic biomass was determined as Chemical Oxygen Demand (COD), according to APHA (1995). COD was measured over two wastewater fractions, total and soluble. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The optical density (OD600) was measured on a Milton Roy Spectronic 601 spectrophotometer at 600 nm. Acetate was measured on a Hewlett Packard GC, model 5890 A. The GC was equipped with a 2 m × 2 mm glass column, packed with Supleco port (100-120 mesh) coated with 10% fluorad FC431. Carrier gas was nitrogen saturated with formic acid.

3. RESULTS

3.1 Continuous reactor experiments

Both continuous reactors received the same influent COD (2600 mg COD l^{-1}). Effluent acetate concentrations decreased slightly as a function of the SRT and were on average 6 ± 3 and 4 ± 1 (mg COD l^{-1}) at 13 and 43 (h) SRT at 30 °C. Effluent acetate concentrations of the thermophilic reactor were similar: 6 ± 3 and 4 ± 0 (mg COD l^{-1}) at 13 and 43 (h) SRT respectively.

Effluent COD concentrations, excluding acetate, for both reactors are listed in Table 3. These data thus only comprise biomass COD and slowly biodegradable soluble COD originating from biomass decay and microbial metabolism: so called soluble microbial products (SMP). At both sludge retention times, effluent COD levels for both the soluble and the total fraction were lower at 55 °C as compared to 30 °C. Observed biomass yields as calculated from these data decreased as a function of the SRT. This effect was most pronounced at 55 °C resulting in a significantly lower observed yield at 55 °C (Y_{obs} =0.28 mg biomass COD mg substrate COD^{-1}) than at 30 °C (Y_{obs} =0.39) both at 43 (h) SRT. Theoretical and observed yields are listed in Table 4, all expressed as (g biomass COD g substrate COD^{-1}). These yield values were calculated under the assumption that all COD excluding acetate was biomass COD since it was defined as such by the model. However, when taking SMP production into account, lower observed biomass yields are obtained: 0.39 ± 0.04 and 0.35 ± 0.04 at 13 and 43 h HRT at 30 °C and 0.37 ± 0.02 and 0.26 ± 0.01 at 55 °C respectively.

In both continuous reactors, three biomass wash-out experiments have been performed. The HRT was suddenly lowered from 13 (h) to 1.54 (h) at 30 °C (D=0.65 h^{-1}) and to 1.03 (h) at 55 °C (D=0.98 h^{-1}). The decrease in the reactor biomass content in time (measured as OD600) is depicted in Fig. 2. In both cases a lag phase occurred for 15 min after which the biomass content dropped as expected according to the model. Estimated extant maximum growth rates are 0.18 ± 0.05 (h^{-1}) at 30 °C and 0.33 ± 0.07 (h^{-1}) at 55 °C (Table 4). The maximum growth rate rate was approximately double at 55 °C as compared to 30 °C but the absolute values were rather low.

Table 3. Average effluent COD concentrations minus the amount of substrate in the effluent, ± standard deviations. Numbers in between brackets represent the number of measurements.

SRT (h)	30 °C reactor		55 °C reactor	
	total COD	soluble COD	total COD	soluble COD
13	1191 ± 89 (6)	107 ± 22 (6)	1000 ± 57 (6)	62 ± 36 (6)
43	997 ± 120 (7)	63 ± 23 (7)	727 ± 49 (4)	39 ± 7 (4)

Table 4: Kinetic constants of mesophilic and thermophilic biomass obtained from batch and continuous experiments. μ_{max} and k_d expressed as (h^{-1}), K_s expressed as (mg COD l^{-1}), Y and Y_{obs} expressed as (mg biomass COD mg acetate COD^{-1})

		Mesophilic (30 °C)	Thermophilic (55 °C)
μ_{max} - k_d	batch exp. 1	0.48 ± 0.1	0.69 ± 0.09
k_d	batch exp. 2	0.1 ± 0.02	0.2 ± 0.03
k_d	continuous exp.	0.004 - 0.008	0.016-0.018
μ_{max} -k_d	wash-out exp.	0.18 ± 0.05	0.33 ± 0.07
K_s	batch exp. 3	9 ± 2	3 ± 2
Y		0.50 ± 0.04	0.49 ± 0.09
Y_{obs}	13 h HRT	0.42 ± 0.04	0.39 ± 0.05
Y_{obs}	43 h HRT	0.38 ± 0.02	0.28 ± 0.01

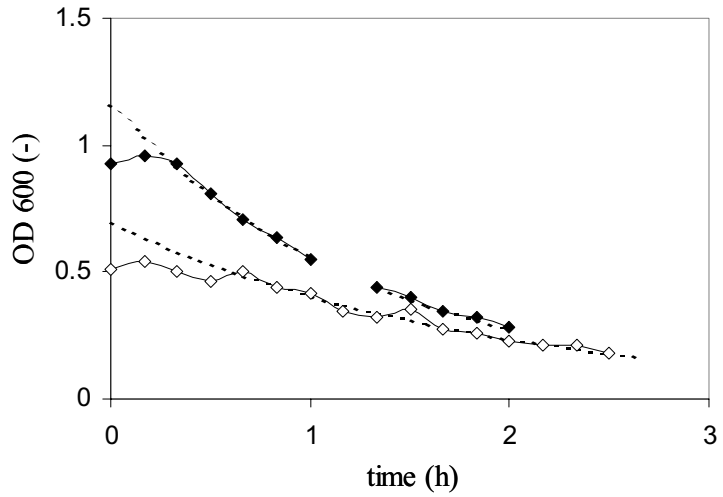

Fig. 2. Biomass wash-out, measured as Optical Density (OD 600) during a temporary increase in the reactor dilution rate. Open diamonds: 30°C, closed diamonds: 55 °C. Interrupted line represents the model fit to the data.

3.2 Batch experiment 1 - intrinsic maximum growth rate

In total, 4 mesophilic and 8 thermophilic growth experiments were carried out. Fig. 3 presents two representative growth curves under both temperature conditions. In both cases, the OUR increased

exponentially in the growth phase and dropped sharply when substrate was depleted. Intrinsic maximum growth (minus decay rates), obtained directly from these data were respectively 0.48 ± 0.1 (h^{-1}) and 0.69 ± 0.09 (h^{-1}) at 30 and 55 °C. The model could be fitted accurately to the experimental data in each growth experiment. The growth rate was higher under thermophilic conditions (approximately 50%), but not as much as expected beforehand.

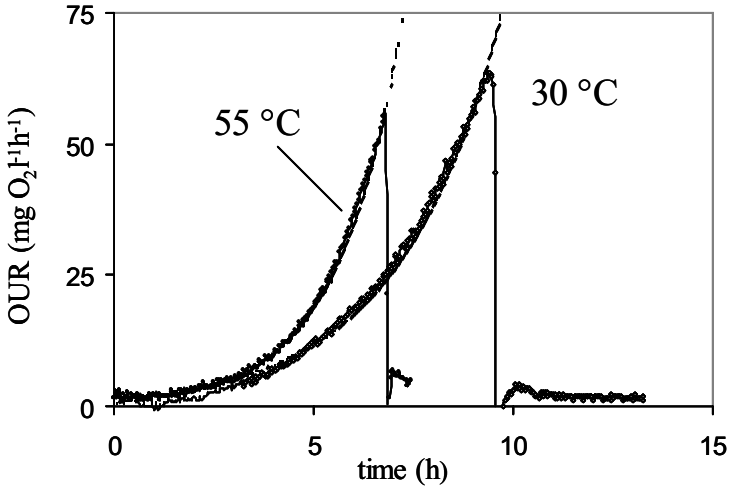

Fig. 3: Two representative curves showing the increase in the OUR in time in a growth experiment (batch exp. No. 1). Data points: diamonds: 30 °C, circles: 55 °C. Model fit: dotted line.

3.3 Batch experiment 2- decay rate

In the decay experiments (Fig. 4), the measured OUR decreased exponentially in time. Under both temperature conditions, 4 experiments were conducted, all giving a reasonably good fit of the data to the model. The rate at which the OUR dropped at 55°C was double as compared to 30 °C, resulting in first order decay constants of respectively 0.2 ± 0.03 (h^{-1}) and 0.1 ± 0.02 (h^{-1})

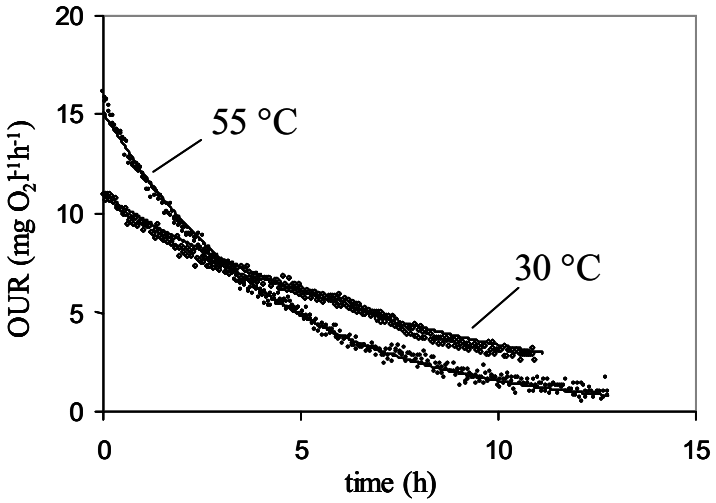

Fig. 4. Exponential decrease in the OUR during a decay experiment (batch exp. No. 2). Datapoints: diamonds: 30 °C, circles: 55 °C. Model fit: uninterrupted line through the data.

3.4 Batch experiment 3 - theoretical yield and Monod constant

Fig. 5 shows the effect of additions of equal amounts of acetate (and nutrients) to mesophilic (30 °C) and thermophilic (55 °C) sludge, both cultivated at 13 (h) SRT. Similar experiments were also conducted with sludge cultivated at 43 (h)SRT (respirograms not shown). The sludge concentrations in the 30 and 55 °C experiments were similar, 560 and 490 (mg biomass COD l^{-1}) respectively. Calculated theoretical yield factors over 4 experiments were 0.50 ± 0.04 at 30 °C and 0.49 ± 0.09 (mg biomass COD mg substrate COD^{-1}) at 55 °C. At 55 °C, slightly higher yields were obtained with biomass cultivated at 13 (h) HRT than with 43 (h) HRT biomass while at 30 °C, it was the other way around.

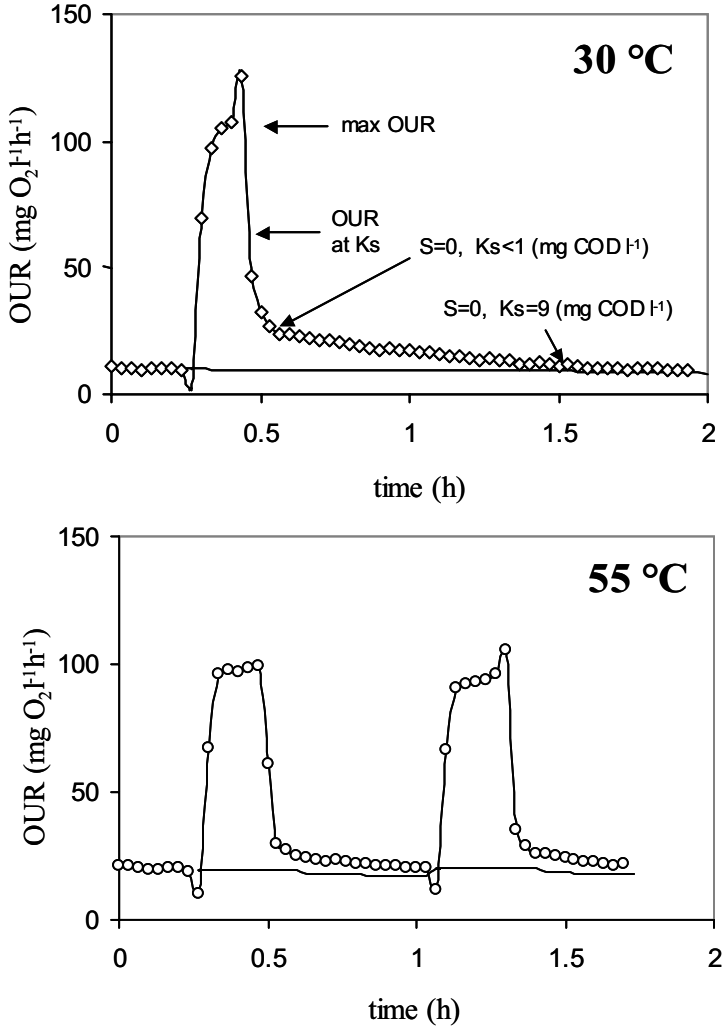

Fig. 5: Course of the OUR during equal additions of acetate to mesophilic and thermophilic biomass (batch exp. No. 3). Open diamonds: 30 °C, open circles: 55 °C. Uninterrupted line; endogenous OUR taking growth and decay into account.

Monod constants estimated from the calculated substrate concentrations during the yield experiment (batch exp. 3) were 9 ± 2 and 3 ± 2 (mg COD l^{-1}) at 30 and 55 °C, respectively. The higher values at

30 °C were due to the more pronounced tailing in the respirograms after the first sharp peak. When it is assumed that substrate concentrations are already zero at the point of inflection in the respirogram, then Monod constants less than 1 (mg COD l^{-1}) are obtained for both temperature conditions. This assumption can be made since generally microorganisms produce storage products that are subsequently oxidized after substrate depletion, causing the tailing in the respirogram (Majone *et al.*,1999)

The maximum obtained OURs due to substrate conversion are listed in Table 5 while the endogenous rates are listed in Table 6. The measured maximum OURs are calculated per gram biomass COD and per liter of continuous reactor. The maximum OUR per gram biomass COD was slightly higher at 55 °C than at 30 °C, respectively 213 ± 12 (mg O_2 g biomass COD^{-1} h^{-1}) at 55 °C and 194 ± 8 (mg O_2 g biomass COD^{-1} h^{-1}) at 30 °C (at 13 h SRT). However, volumetric OURs per liter reactor were lower at 55 °C compared to 30 °C due to a lower biomass concentration in the cultivation vessel (although both reactors received the same influent COD under continuous operation). This effect became more pronounced with increasing SRTs, i.e. the maximum OUR per liter reactor dropped more rapidly as a function of the SRT under thermophilic conditions than under mesophilic conditions. At 43 h SRT, maximum volumetric OURs were 136 ± 5 (mg O_2 l^{-1} h^{-1}) at 30 °C and 111 ± 14 (mg O_2 l^{-1} h^{-1}) at 55 °C. Endogenous respiration rates at 55 °C were twice as high as the rate at 30 °C.

Table 5. Maximum OUR of mesophilic and thermophilic sludge cultivated at 13 and 43 (h) SRT (substrate respiration plus endogenous respiration). Rates per liter reactor are the estimated maximum rates in the continuous reactors used for biomass cultivation.

	max OUR 30°C		max OUR 55 °C	
HRT (h)	per liter reactor (mg O_2 l^{-1} h^{-1})	per gram biomass (mg O_2 g biomass COD^{-1} h^{-1})	per liter reactor (mg O_2 l^{-1} h^{-1})	per gram biomass (mg O_2 g biomass COD^{-1} h^{-1})
13	213 ± 3	194 ± 8	206 ± 13	213 ± 12
43	136 ± 5	133 ± 6	111 ± 14	157 ± 20

Table 6. Endogenous OURs of mesophilic and thermophilic sludge cultivated at 13 and 43 h SRT. Rates per litre reactor are the estimated endogenous rates in the continuous reactors.

	endogenous OUR 30°C		endogenous OUR 55 °C	
HRT (h)	per litre reactor (mg O_2 l^{-1} h^{-1})	per gram biomass (mg O_2 g biomass COD^{-1} h^{-1})	per litre reactor (mg O_2 l^{-1} h^{-1})	per gram biomass (mg O_2 g biomass COD^{-1} h^{-1})
13	22 ± 3	18 ± 4	39 ± 0.3	41 ± 0.2
43	18 ± 2	18 ± 2	20 ± 2	28 ± 3

4. DISCUSSION

4.1 Effluent soluble COD and Monod

In both reactor effluents, acetate concentrations were very low. This is well in accordance with the low Monod constants (less than 9 mg COD l^{-1}) that were estimated from the batch experiments. Most likely, Monod constants for both sludges are in the order of 1 (mg COD l^{-1}) since the estimations of 9 (mg COD l^{-1}) were made by neglecting possible storage product oxidation. However, both estimations were that low that in all cases very low effluent substrate concentrations are to be expected. Higher Monod constants have been reported in various researches (Sürücü *et al.*, 1976, Jackson, 1983; Couillard *et al*., 1989; LaPara *et al*., 2000b) but these were confounded by the presence of an un-biodegradable wastewater fraction (Jackson, 1983; Couillard *et al*., 1989) or by SMP that were regarded as non-biodegraded substrate (Sürücü *et al.*, 1976; LaPara *et al*., 2000b). Generally, Monod constants are extremely low (Pirt, 1975) and the current study shows that this is also the case for thermophilic microorganisms.

In both reactor effluents, a large part of the soluble COD could not be identified as acetate. These unidentified soluble compounds (SMP) originate from biomass decay by cell lyses or arise as a by-product from substrate metabolism (Barker and Stuckey, 1999). This effect is not incorporated in the model that we used. The effluent soluble COD was however not completely inert since the COD concentrations decreased as a function of the sludge age. Possibly, the effluent soluble COD (originating from the biomass) was completely biodegradable when a sufficiently long sludge age is imposed. These observations are of interest since in other studies (LaPara *et al.,* 2001b; Chapter 4,5,6) differences in COD removal were observed between mesophilic and thermophilic conditions for treatment of complex wastewaters and so far it is uncertain whether this was caused by a higher production of inert by-products under thermophilic conditions, or whether thermophilic biomass is unable to convert the same variety of compounds as the mesophilic biomass is capable of. Based on these results, the latter explanation seems most likely.

4.2 Decay rates

Based on the measured biomass concentrations in the continuous experiments, an estimation of the theoretical yield and decay rate can be made. The relationship between the observed yield and the theoretical yield (Y) as a function of the decay rate and the SRT (θ) is given by eq. 5 (for a continuous reactor without biomass recycle) :

$$Y_{obs} = \frac{Y}{1 + k_d \theta} \qquad (5)$$

Estimated parameters, based on the measured biomass concentrations were: Y=0.44 and k_d=0.004 (h^{-1}) at 30 °C and Y=0.47 and k_d= 0.016 at 55 °C. When assuming a theoretical yield of 0.5 as obtained

from the batch experiments and calculating solely the decay rate from these data yields k_d values of 0.008 (h^{-1}) at 30 °C and 0.018 (h^{-1}) at 55 °C. These calculations show that the theoretical yields from the batch and continuous experiments were well in accordance, especially at 55 °C. In this study, the reactor biomass contents was measured at 2 dilution rates and although these present multiple measurements (Table 3), the accuracy of the decay estimation would improve in case more measurements had been performed at different dilution rates.

The estimated decay rates from these observations were approximately a factor 10 lower than the decay rates found in the batch experiments. The reason for this is that in the batch experiments, the OUR did not drop as a result of actual decay, reducing the weight of the biomass, but most likely a transition in the metabolic activity of the biomass took place towards starvation conditions. This process is called stringent response (Mason *et al*., 1986). Under low growth conditions, metabolic and maintenance processes are slowed down, resulting in a lower measured OUR. The biomass content however does not decrease significantly. This is also clear from Fig. 4: the cumulative oxygen uptake underneath the curves is approximately 50 (mg O_2 l^{-1} h^{-1}) while an initial amount of 500 (mg biomass COD l^{-1}) was used. This means that during the batch experiment, only 10% of the biomass was actually oxidized while the OUR dropped to almost zero. The decay rates obtained from the continuous experiments are therefore considered to be more reliable in describing the decay process as these were obtained from measurements of the actual biomass content. The decay rate obtained at 55 °C was well in accordance with literature data (Sürücü *et al.*, 1976; Jackson, 1983) while LaPara *et al*. (2000b) found a tenfold lower value. Literature data concerning pure cultures of microorganisms mostly report higher maintenance requirements under thermophilic conditions (Cometta *et al*., 1982; Kuhn *et al*., 1980; Sonnleitner *et al.,* 1982) although some did not (Brooke *et al*., 1989; Becker and Märkl, 2000) Based on the current results and literature data, we believe that maintenance requirements are higher under thermophilic conditions as compared to mesophilic conditions but to what extent remains subject to some speculation. A two-fold increase as reported by this research and by Konopka *et al.* (1999) to an approximate 10 fold increase have been reported (LaPara and Alleman (1999). Most evidence however points to an approximate doubling of the decay rate with a temperature increase from 30 to 55 °C.

4.3 Theoretical and Observed yield

From a theoretical perspective, the theoretical yields are expected to be similar under both temperature conditions. Based on thermodynamic considerations, the theoretical yield only depends on the balance between the Gibbs energy release from the oxidation of acetate with oxygen and the building of new biomass from acetate and inorganic nutrients (Heijnen, 1999). Assuming a similar biomass composition under both temperature conditions (this is valid when comparing moderate thermophiles and mesophiles: Sundaram, 1986) will result in a similar theoretical yield for both types

of biomass. Calculation of the theoretical biomass yield according to Heijnen (1999) gives a value of 0.48 (g biomass COD g substrate COD^{-1}). Both measured values (0.50 and 0.49 at 30 and 55 °C respectively) correspond quite well with the theoretical value and therefore theoretical yield factors are regarded as similar for mesophilic and thermophilic aerobic biomass.

The observed yield was found to be lower under thermophilic conditions as compared to mesophilic conditions due to the higher maintenance requirements. The difference became larger with an increasing sludge retention time. Comparison of the absolute yield values with literature data was cumbersome since different units were used in various researches. Mostly, no specific trend could be observed between the theoretical yield and growth temperature (see Table 1) with the exception of Bérubé and Hall (2000a) who also found a decrease in the observed yield as a function of temperature.

4.4 Maximum growth rates

Comparison of the maximum growth rates obtained in the batch and in the continuous wash-out experiments shows a significant difference in their absolute values (Table 4) although the relative differences between 30 and 55 °C were quite similar. This clearly shows the difference between intrinsic and extant μ_{max} values. The extant μ_{max} was lower as a result of the low biomass growth rate in the continuous reactors (0.077 h^{-1}) having its effect on the physiological state of the bacteria (relatively low levels of proteins, RNA, DNA). The biomass could not change its physiological state instantaneously into a state of maximum growth and therefore a lower extant μ_{max} was found when compared to the intrinsic μ_{max} (Grady *et al*., 1996).

Combining the intrinsic (μ_{max}-k_d) from the batch experiments with the estimated decay rates from the continuous experiments leads to μ_{max}=0.49 ± 0.11 (h^{-1}) and k_d=0.004 - 0.008 (h^{-1}) at 30 °C and μ_{max}=0.71 ± 0.11 (h^{-1}) and k_d=0.016 - 0.018 (h^{-1}) at 55 °C. This is a 50% increase in the maximum growth rate and an approximate doubling of the decay rate with temperature. Reported μ_{max} values for thermophilic wastewater treatment processes vary between 0.1 and 0.5 (h^{-1}) as shown in Table 1. For pure cultures of microorganisms μ_{max} values of (1.0 h^{-1}) have been reported (Becker *et al*., 1997) but extremely high growth rates of 3.5 (h^{-1}) could only be obtained under typical conditions with incomplete substrate utilization (Cometta *et al*., 1982; Sonnleitner *et al*., 1982) or by growth on a complex medium (Kuhn *et al.,* 1980). The intrinsic μ_{max} values obtained in this study correspond well with the reported literature data and given the reproducibility of the results, are regarded as accurate estimates of the intrinsic μ_{max} for the specified substrate and temperatures.

4.5 Model simulations

In order to estimate the consequences for thermophilic wastewater treatment processes, model simulations were made with the obtained kinetic data. Reactor biomass concentrations and maximum volumetric substrate removal rates were estimated as a function of the sludge retention time (SRT) for

a reactor with and without biomass recycle (Fig. 6). Kinetic parameters used in the simulations are: μ_{max}=0.48 (h^{-1}), k_d=0.008 (h^{-1}), Y=0.5 (g biomass COD g substrate COD^{-1}) and K_s=9 (mg COD l^{-1}) at 30 °C and μ_{max}=0.71, k_d=0.018, Y=0.5 and K_s= 9 at 55 °C. For both reactor configurations, reactor influent COD was 2600 (mg COD l^{-1}), the HRT of the reactors with sludge retention was 12 (h) for both temperatures.

The simulations show that with low SRTs, due to the higher μ_{max} also higher volumetric conversion rates can be obtained at 55 °C as compared to 30 °C. However, the biomass concentration will be lower in the thermophilic reactor as compared to the mesophilic reactor, especially at higher sludge ages, without being fully compensated by the 50% increase in the maximum growth rate. Therefore, maximum volumetric conversion rates are expected to be lower in a thermophilic bioreactor when compared to a mesophilic analogue provided that both systems receive the same organic loading rate.

In these simulations, the intrinsic μ_{max} values were used whereas the wash-out experiment has already shown that extant maximum growth rates decrease as a function of the SRT. The maximum substrate removal rates are thus likely to be lower than the estimations of Fig. 6. However, the same trend: a lower conversion capacity at 55 °C than at 30 °C at long SRTs is expected. This was confirmed in the yield experiments (Fig. 5) with acetate additions to biomass grown at 13 (h) and 43 (h) SRT. The maximum volumetric OUR in the 55 °C batch experiment was smaller than the OUR in the 30 °C experiment, especially with biomass cultivated at 43 (h)SRT.

In practice however, the low biomass concentration in a thermophilic bioreactor with sludge retention will be increased by increasing the loading rate on the system. In this sense biokinetic advantages are to be expected: the same wastewater flow can be treated in a thermophilic bioreactor of approximately half the size of a mesophilic analogue with a similar sludge production. Note that these estimations only hold for wastewaters that are completely biodegradable. Other requisites are a sufficient oxygenation capacity and sludge settleability which both can become limiting factors (LaPara and Alleman, 1999). To confirm these findings for wastewater treatment applications, additional experiments should be performed with biomass cultivated at higher SRTs and reliable data concerning actual decay rates are still scarce.

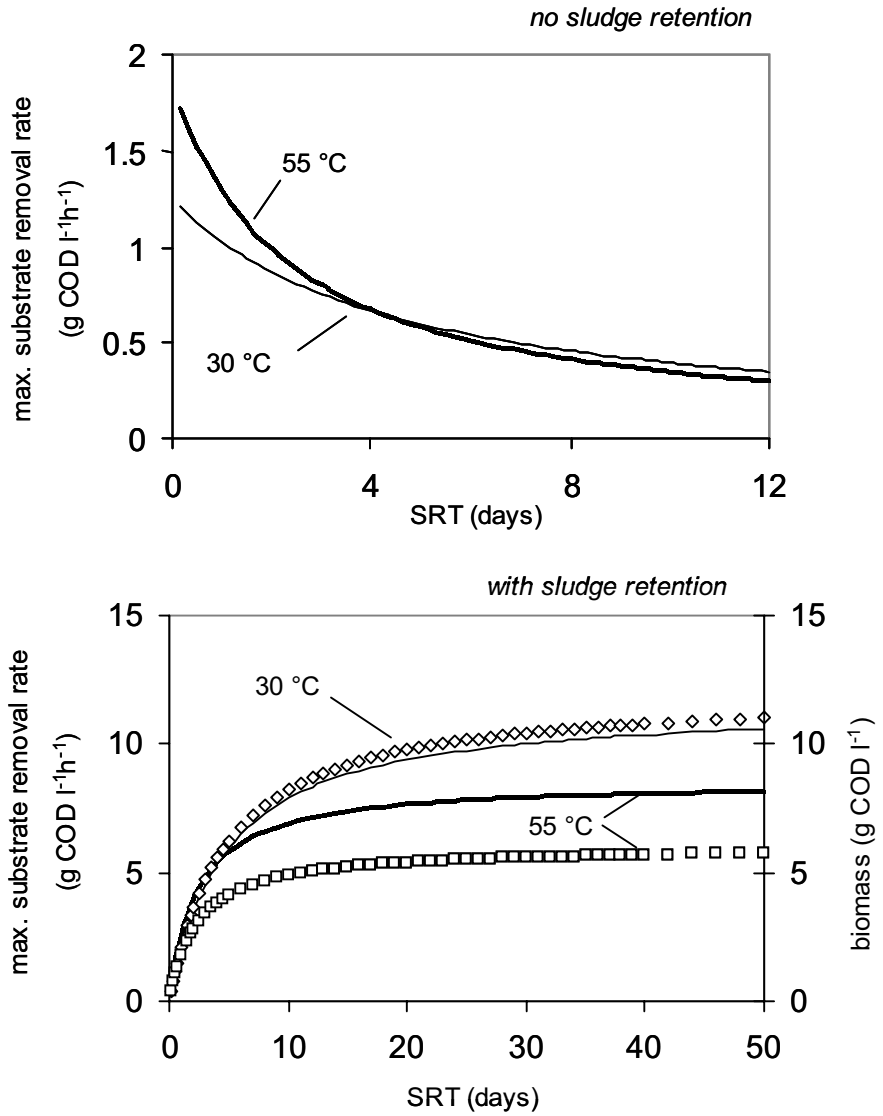

Fig.6: Model predictions for the maximum substrate conversion rate as a function of the SRT, at 30 and 55 °C, in reactors with and without sludge retention. Reactors with sludge retention are operated at the same hydraulic retention time.
Thin drawn line: max. substrate conversion rate 30 °C, thick drawn line: max. substrate conversion rate 55 °C.
Open diamonds: biomass concentration 30 °C, open squares: biomass concentration 55 °C.

5. CONCLUSIONS

Intrinsic maximum growth rates increase with approximately 50% while decay rates are doubled with a temperature increase from 30 to 55 °C. Theoretical biomass yields are similar under both temperature conditions while observed biomass yields are lower at 55 °C than at 30 °C, especially at higher sludge retention times. A higher organic loading rate can be imposed on a thermophilic bioreactor with sludge recycle in case sludge production is allowed to be similar to a mesophilic reactor.

4

SLUDGE AND WASTEWATER CHARACTERIZATION USING BATCH EXPERIMENTS

ABSTRACT

Anaerobic pretreated paper process water was characterized in terms of readily biodegradable, slowly biodegradable, very slowly biodegradable and inert wastewater fractions under mesophilic and thermophilic conditions. The anaerobic pretreated paper process water contains a relatively high amount of slowly biodegradable components and few easily biodegradable components as indicated by the ratio of short term BOD over the BOD5. Wastewater readily biodegradable COD, determined as short term BOD, was almost similar when measured under both temperature conditions. Fractions of slowly biodegradable COD and inert COD of the same wastewater were found to depend on the type of biomass involved in the test. Thermophilic aerobic biomass was not able to degrade the wastewater to the same extent as the mesophilic biomass resulting in higher apparent inert COD levels. Furthermore, wastewater colloidal COD does not flocculate under thermophilic conditions and is thus not removed from the liquid phase.

Vogelaar,J.C.T., A. Klapwijk, J.B. van Lier and G. Lettinga, accepted for publication in Biodegradation.

ABBREVIATIONS

ASM 1	Activated Sludge Model No. 1
BOD	biochemical oxygen demand (mg O_2 l^{-1})
BOD-st	short term BOD (mg O_2 l^{-1})
COD	Chemical Oxygen Demand (mg COD l^{-1})
f_p	fraction of inert COD in heterotrophic biomass (-)
S_s	readily biodegradable substrate (mg COD l^{-1})
S_I	initially present inert soluble COD (mg COD l^{-1})
S_p	soluble inerts generated from biomass decay (mg COD l^{-1})
X_p	particulate inerts generated from biomass decay (mg COD l^{-1})
X_s	slowly biodegradable substrate of particulate nature (mg COD l^{-1})
X_I	initially present inert particulate COD (mg COD l^{-1})
SMP	soluble microbial products
VFA	Volatile fatty acids (mg COD l^{-1})

1. INTRODUCTION

Thermophilic biological wastewater treatment has gained increasing interest in recent years. Reasons for this are, amongst others, the increasing system closure in pulp and paper mills resulting in higher process water temperatures and the subsequent need to treat these process waters under thermophilic conditions (LaPara and Alleman, 1999).

Process water from the paper and board industry, using recycled wastepaper as raw material, contains high concentrations of easily biodegradable substrates: fatty acids and lactate (Habets and Knelissen, 1997). This type of wastewater can easily be treated anaerobically, however, aerobic post treatment is required to remove residual BOD and sulphide from the anaerobic effluent. After the post treatment step, the water can be re-used in the process. This research focuses on the thermophilic aerobic post treatment of anaerobically pretreated paper process water (hereafter named wastewater).

The aim of this chapter was to characterize the wastewater in terms of readily, slowly and very slowly biodegradable fractions under both mesophilic and thermophilic conditions. Furthermore, the amount and origin of inert wastewater COD and the fate of sulphide was assessed under both temperature conditions. Characterization took place by three types of batch experiments, differing mainly in the time scale of the experiments. The results from this characterization study, combined with other research using continuous reactor experiments can be used to evaluate the feasibility of thermophilic aerobic post treatment of anaerobic effluents.

2. MATERIALS AND METHODS

2.1 Wastewater description

Anaerobically pretreated paper process water was obtained from a zero effluent mill producing fluting and liner from recycled wastepaper (Habets and Knelissen, 1997). The wastewater contained less than 10 (mg VFA-COD l^{-1}) and approximately 50 (mg l^{-1}) sulphide. Wastewater pH was 7 ± 0.2, nutrients were supplied at the mill in a ratio of COD:N:P of 100:2.6:0.4.

2.2 Mesophilic and thermophilic sludge

Mesophilic and thermophilic seed sludge for the batch experiments was obtained from two lab-scale activated sludge reactors operating under semi-steady state conditions at 30 and 55 °C (Chapter 5). Both reactors had a sufficiently long temperature adaptation for the sludge to acclimatize to the specific temperature conditions. Both systems were operated at a 20 days sludge retention time (SRT).

2.3 Batch respirometric experiments - short term BOD

Readily biodegradable BOD was determined by measuring the short term BOD of the wastewater with mesophilic and thermophilic sludge (Spanjers *et al*., 1994). The readily biodegradable BOD is defined as the oxygen consumption of activated sludge caused by readily biodegradable substrate. Batch respirometric experiments were performed with a modified version of a Manotherm RA 1000 respirometer connected to a temperature controlled 4 liter batch vessel as described in Chapter 3.

2.4 BOD5 tests

BOD-5 experiments of fractionated wastewater samples were used as a measure for the slowly biodegradable substrates. Experiments were conducted using WTW Oxitops®. 300 ml Schott bottles were filled with 40 ml wastewater and seeded with 0.25 ml thermophilic or mesophilic sludge from the continuous activated sludge reactors. Four different wastewater fractions were used: raw, paper filtered, membrane filtered (Whatman GF/F) and a centrifuged particulate fraction of the wastewater re-suspended in a nutrient medium. Sulphide was removed in advance from all the samples by stripping the solution with nitrogen gas. A 100 % sulphide removal was verified experimentally. All samples were taken from one batch of wastewater collected at the mill. The contents of the bottles were mixed by magnetic stirrers. Incubation took place at 30 or 55 °C, three bottles were used for each temperature. The decrease in the headspace pressure due to oxygen consumption was measured every 20 minutes for 5 days. Carbon dioxide was fixed using sodium hydroxide pellets. Oxygen consumption was calculated using the gas law and Henry's law and taking the water vapor pressure at 30 and at 55 °C into account. Before and after the experiment, the COD of 4 different fractions (total,

suspended, colloidal, soluble) was measured to establish a COD balance. Nitrification was inhibited by addition of allylthiourea (ATU, 5 mg l^{-1}).

2.5 Biodegradability tests

The amount of inert wastewater COD was assessed by batch biodegradation experiments at 30 and 55 °C after Germirli *et al*. (1993). At both temperatures, two biodegradation tests were performed, one with the raw sample and one with a membrane filtered fraction of the sample (Whatman GF/F). From each vessel, total and soluble COD was measured over time. These data enable one to estimate the amount of initially present soluble and particulate inert COD (S_I, X_I) and soluble and particulate inerts generated from biomass decay (S_p and X_p). Inoculation took place with 0.5 ml mesophilic or thermophilic sludge from the continuous activated sludge reactors. Water losses due to evaporation were corrected for.

2.6 Analytical methods

Chemical Oxygen Demand (COD) was determined according to APHA (1995). COD was measured over four wastewater fractions: total, suspended, colloidal and soluble. Paper filtration (Schleicher and Schuell 595½ folded paper filters, pore size 4.5 μm was used to distinguish between the total and the suspended fraction. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The colloidal fraction was calculated as the difference between the soluble fraction and the paper filtered fraction. Mixed liquor total- and volatile suspended solids were measured according to the Dutch normalized standards (NEN 6621). Sulphate and thiosulphate were determined on a Dionex ionchromatograph with an anion exchange column (Dionex, IonPac AS17) using a hydroxide gradient. Eluent was made on-line with an EG-40 eluent generator using deionized water as carrier. Ions were detected using suppressed conductivity. The presence or absence of sulphite was determined using Merckoquant sulphite test strips (Merck, Germany). Sulphide was determined according to Trüper and Schlegel (1964).

3. RESULTS

3.1 Batch respirometric experiments - short term BOD

In total 10 BOD-st measurements were performed, 5 with mesophilic sludge and 5 with thermophilic sludge. Fig. 1 shows two representative respirograms of these tests. The short term BOD of the wastewater was calculated as the area under the curve after substrate addition. Correction took place for the endogenous respiration rate (the grey line in Fig.1). The short term BOD was in both cases very low (average values 89 ± 34 and 96 ± 34 mg O_2 l^{-1} at 30 and 55 °C) and the results were

susceptible to a large variation. Additional BOD-st experiments were performed with wastewater containing no sulphide due to preliminary stripping of the solution with nitrogen gas. In these respirograms there was no sharp peak as was found in the foregoing with sulphide.

Additions of a pure sulphide solution to the sludge (Fig. 2) gave only a sharp peak with little or no tailing. No sulphate, thiosulphate nor sulphite formation was detected during the experiment. The first peak in the wastewater respirograms could thus be identified as the biological oxidation of sulphide into elemental sulphur.

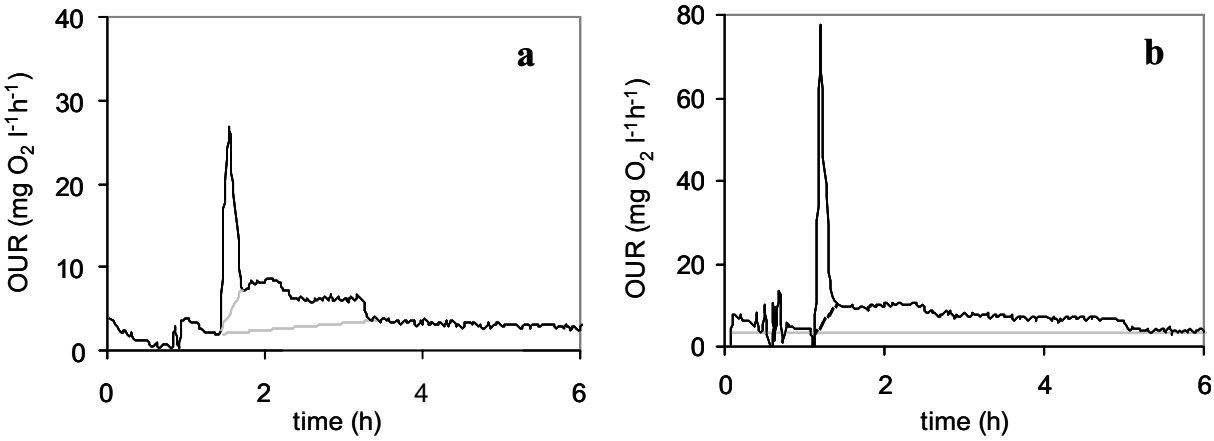

Fig. 1. Wastewater short term BOD of: a: T=30 °C, mesophilic sludge, b: T=55°C, thermophilic sludge.

The relative amount of sulphide oxidation as a percentage of the total BOD-st was calculated by comparing the areas under the first sharp peak and the area under the shoulder after this peak. Oxidation of sulphide accounted for approximately 26% of the BOD-st at 30°C while at 55 °C this figure was 33%.

The tailing after the initial sharp peak was due the oxidation of other readily biodegradable matter. These components could not be identified. The maximum specific oxidation rate of the sludge was calculated as the height of the first shoulder after the sulphide peak, divided by the MLVSS concentration in the batch vessel. The maximum rates for the mesophilic and thermophilic sludge were low and did not differ much (Table 1).

Table 1. Wastewater short term BOD and standard deviations at 30 and 55 °C. Average values over all experiments are presented as well as specific data extracted from Fig. 1.

sludge	*MLVSS* $[g \cdot l^{-1}]$	*BOD-st* $[mg\ O_2\ l^{-1}]$	*% S-oxidation to BOD-st*	*r O_2-max* $[mg\ O_2 \cdot l^{-1} \cdot h^{-1}]$	*BOD-5* $[mg\ O_2\ l^{-1}]$	*% BOD-st to BOD-5*
Mesophilic	0.8	95	26	8		
average	0.9 ± 0.2	89 ± 34			614 ± 35	15
Thermophilic	1.2	146	33	10		
average	0.9 ± 0.1	96 ± 34			522 ± 28	28

Additions of a pure solution of sulphide were not only made to clean water but also to sludge in order to asses the fate of sulphide present in the wastewater when entering the aerobic treatment system (Fig. 2). The first two peaks in the respirogram (Fig 2a) represent the addition of 150 and 300 ml sulphide solution (150 mg S^{2-} l^{-1}) to aerated demineralized water. After the second peak, mesophilic or thermophilic sludge was added (1 g MLSS l^{-1} in the batch vessel) and two more additions (150 and 300 ml) of the same sulphide solution were made. Addition of sulphide to aerated demineralized water did result in an oxygen uptake but no sulphate nor thiosulphate nor sulphite could be detected. Instead, the solution changed from transparent into milky white. Most probably, elemental sulphur was formed. The measured oxygen consumption upon addition of sulphide to demineralized water and to activated sludge was also in accordance with the partial oxidation of sulphide into elemental sulphur.

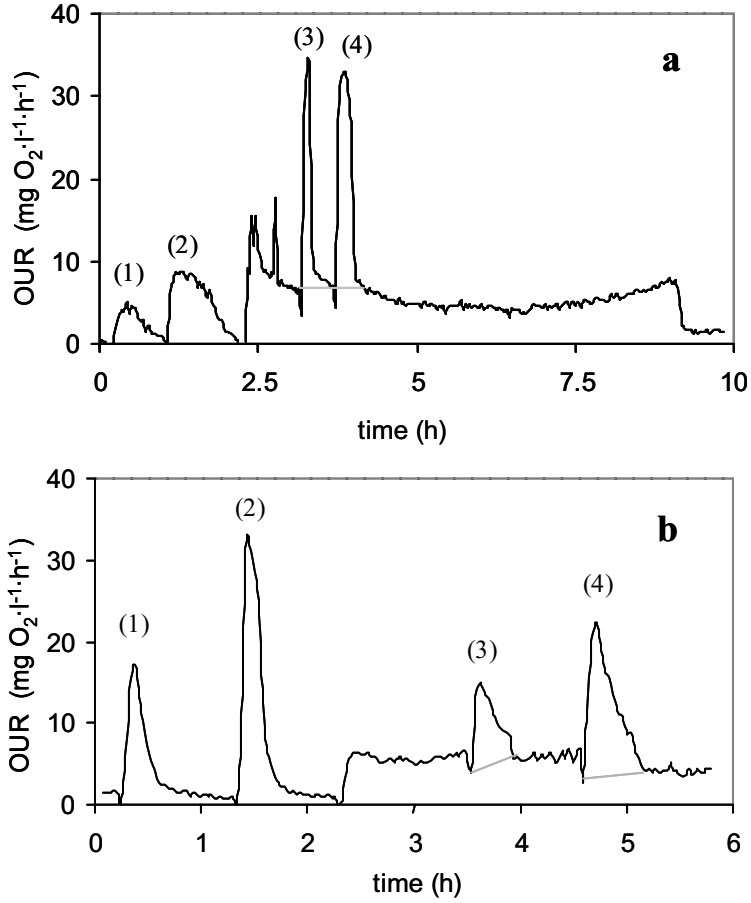

Fig. 2. Sulphide additions (150 mg l^{-1}) to: a: T=30 °C, demineralized water and mesophilic sludge, b: T=55 °C, demineralized water and thermophilic sludge.

Increasing the sulphide dosage to demineralized water led to an increased oxygen uptake rate (Fig. 2 a,b; first two peaks). Additions of sulphide to activated sludge under both temperature conditions (Fig. 2 a,b; last two peaks) did not result in a production of sulphate either just as when adding sulphide to

demineralized water. However, monitoring the influent and effluents of both lab-scale activated sludge reactors for sulphide and sulphate showed that under continuous reactor operation a complete oxidation of sulphide took place to sulphate.

In the presence of sludge, under mesophilic conditions, a higher sulphide dosage did not increase the reaction rate as the maximum OUR remained almost similar (Fig. 2a). Presence of active biomass strongly increased the oxidation rate of sulphide into elemental sulphur and/or sulphate from 8 till approximately 32 mg O_2 $l^{-1}h^{-1}$ when comparing the second and fourth sulphide addition. At 55 °C, the presence of biomass did not increase the reaction rate. The abiotic reaction also proceeded much more rapid at this temperature (Fig. 2b).

3.2 BOD5

Results of the BOD5 experiments with wastewater from the same sample as in the short term BOD experiments are incorporated in Table 1. Comparing the data of the short term BOD and the BOD5 experiment shows that the anaerobic effluent contained little readily biodegradable matter compared to the amount of COD that is oxidized in a 5 days BOD test. Furthermore, the BOD5 differed significantly for both temperature conditions. In this research, the amount of COD oxidized in a BOD5 test is regarded as the sum of readily biodegradable and slowly biodegradable COD.

Fig. 3 and 4 show a series of BOD experiments with different wastewater fractions. This particular batch of wastewater contained a higher amount of easily biodegradable substrate as the anaerobic pretreatment system was overloaded at that moment. Therefore the data of this experiment can not be compared directly to data of the other batch experiments. Fig. 3 shows the amount of oxygen present in the BOD bottles as a function of time. At 55 °C, the initial oxygen consumption rate was higher compared to 30 °C but then leveled off much quicker compared to the 30 °C bottles. Ultimately, the total amount of oxygen consumed over a 5 days period was lower under thermophilic conditions as compared to mesophilic conditions.

Fig. 4 presents the distribution of different COD fractions before and after a 5 days BOD test with different wastewater fractions. A COD balance was established over the bottles. The average deficit in the balance was 8 % with a maximum of 19 %. Three main observations can be made from Fig. 4: 1. There was a distinct difference in removal of soluble COD between both temperature conditions (Fig. 4 a,b,c). 2. The batch test with the membrane filtered wastewater showed a slight production of colloidal COD both at 30 and at 55 °C that was not significantly different. 3. At 30°C, all of the colloidal COD present in the wastewater was absorbed to the sludge and partly hydrolyzed and oxidized while at 55 °C a large colloidal fraction remained unaffected (Fig. 4d). The difference in COD removal in Fig. 4d was approximately similar to the amount of colloidal COD that remained in the liquid at 55 °C while it was removed at 30 °C. The rate at which colloidal and suspended COD

was oxidized in the test was lower under thermophilic conditions compared to mesophilic conditions, as can be seen from the decrease in the oxygen concentration in time (Fig. 3d).

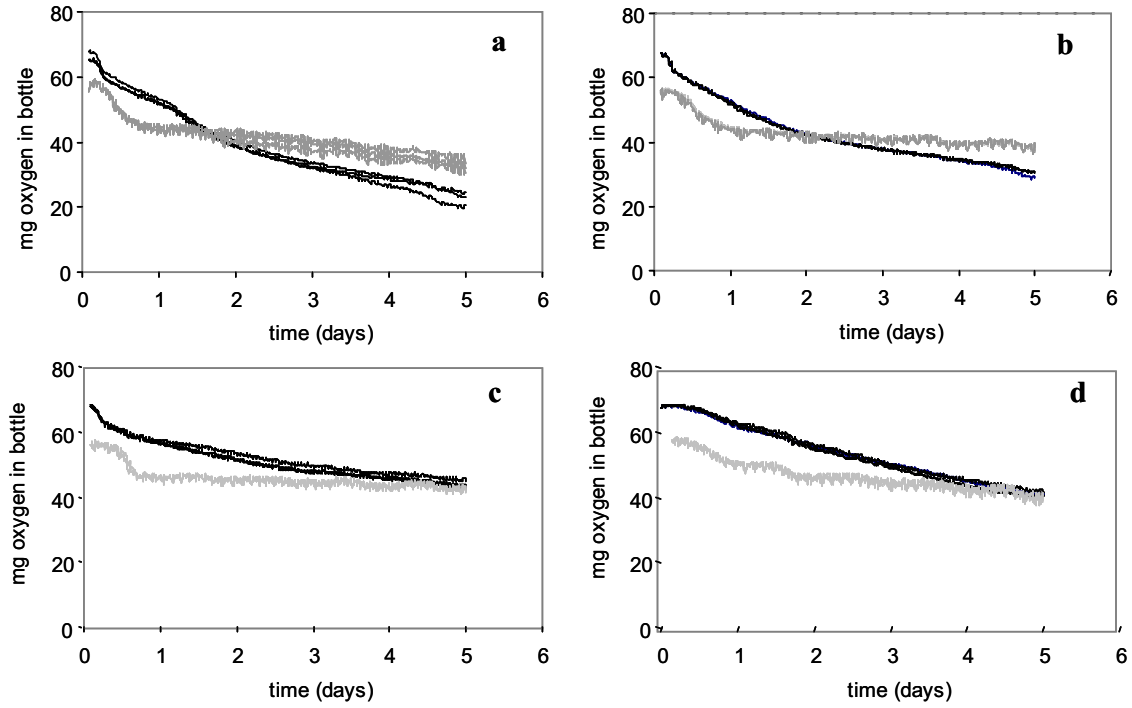

Fig.3. Amount of oxygen present in the BOD bottles over time. a: raw wastewater, b: paper filtered , c: membrane filtered, d: resuspended centrifuged fraction. (—) T=30 °C, (—) T=55 °C.

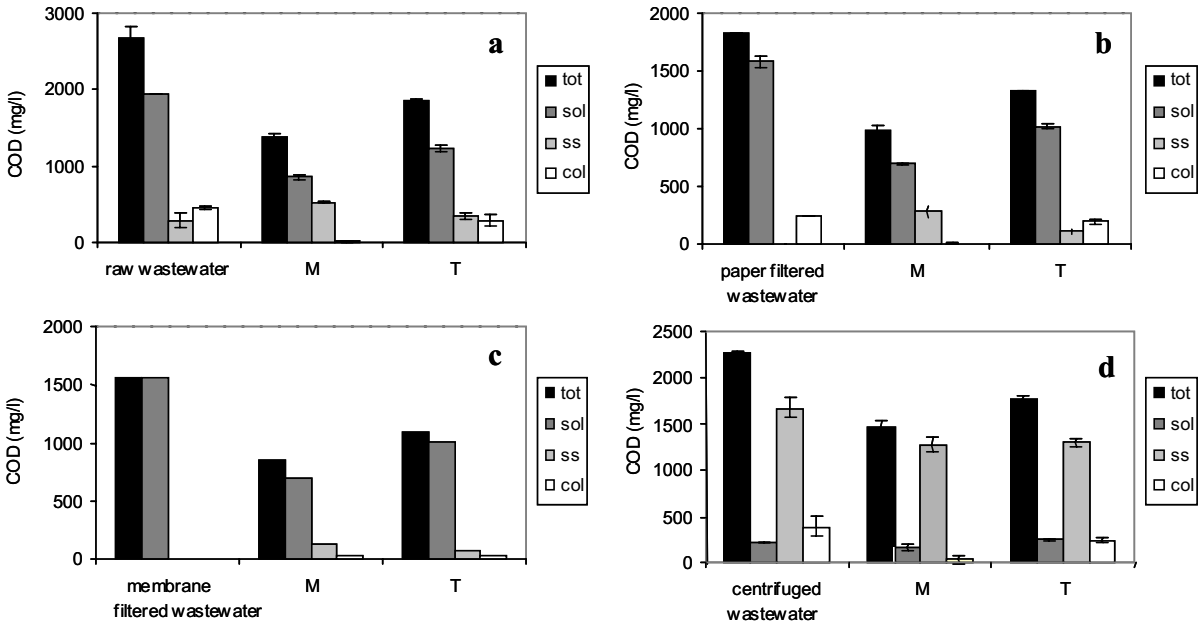

Fig.4. Distribution of COD fractions before and after a 5 day biodegradation (BOD) test. a: raw wastewater b: paper filtered fraction, c: membrane filtered fraction, d: resuspended centrifuged fraction.

3.3 Biodegradability test

The course of the wastewater COD in time in the biodegradability test is shown in Fig. 5. After 83 days, at 55 °C, the level of remaining COD was 686 (mg COD l^{-1}) while at 30 °C, the remaining COD level was much lower (455 mg COD l^{-1}). At that point in the test, the temperature in the thermophilic vessel was lowered to 30 °C and the suspension was re-inoculated with mesophilic sludge. The COD levels then again dropped and the remaining COD in both batches became almost similar after 180 days (500 and 480 mg COD l^{-1} at 30 and 55 °C respectively). Application of the calculation method as proposed by Germirli *et al*. (1993) indicated only a minor production of inert soluble and particulate matter, especially when compared to the initially present amount (Table 2).

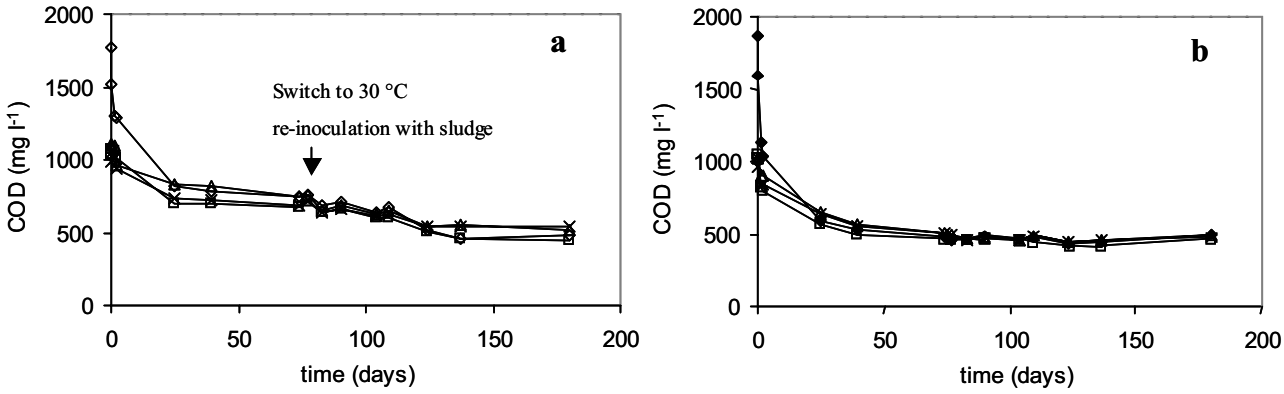

Fig. 5. a: Thermophilic biodegradation of the wastewater, subsequently followed by mesophilic degradation (and re-inoculation with mesophilic sludge). b: Mesophilic biodegradation.
Batch 1, COD_{tot} (♦); Batch 1, COD_{sol} (□); Batch 2, COD_{tot} (Δ); Batch 2, COD_{sol} (×).

Table 2: Wastewater characterization in terms of biodegradable and non-biodegradable fractions according to the biodegradability test after 83 days.

Wastewater fraction (mg COD l^{-1})	symbol	mesophilic	thermophilic
Initially present total COD	$C_{T,0}$	1868	1768
Initially present soluble COD	$C_{s,0}$	1044	1035
Initially present particulate COD	$C_{p,0}$	824	733
Initially present soluble inert COD	S_I	492	636
Produced soluble inert COD	$S_{p,I}$	-29	4
Initially present particulate inert COD	X_I	17	16
Produced particulate inert COD	$X_{p,I}$	21	30

Fig. 6 depicts two diagrams showing the relative distributions of the different wastewater fractions for both temperatures. Average short term BOD values are converted into readily biodegradable COD by assuming a yield factor of 0.5 for both sludges (Chapter 3). The BOD5 is converted into the sum of the slowly biodegradable COD and the readily biodegradable COD by assuming a yield factor of 0.3.

Inert COD is regarded as the nonbiodegradable fraction after 83 days of biodegradation (Fig. 5) and the very slowly biodegradable fraction is calculated from the balance of the total wastewater COD and the before mentioned COD fractions. All COD fractions were obtained from experiments with wastewater that was obtained under a normal operation of the anaerobic pretreatment. The diagram shows that under thermophilic conditions, the inert COD fraction was higher and slowly and very slowly biodegradable COD fractions were smaller as compared to mesophilic conditions.

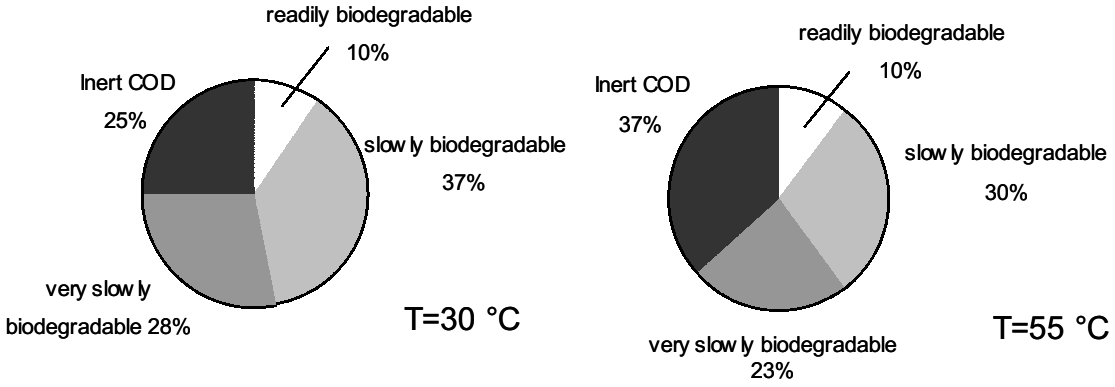

Fig.6: Wastewater fractions at 30 and 55 °C.

4. DISCUSSION

4.1 Batch respirometric experiments - short term BOD

Short term BOD values were in both cases very low and susceptible to a relatively high variation. The higher variation was probably due to the dynamic response when sulphide was added to the sludge and to the low respiration rates after the sulphide peak, increasing the relative errors in the measurements. The low value of the short term BOD indicates a well functioning of the anaerobic pretreatment as is also reflected in the low volatile fatty acid (VFA) concentrations (< 10 mg COD l^{-1}). VFA could only account for 10% of the short term BOD (the part that is not due to sulphide oxidation) indicating that the wastewater also contained some other readily biodegradable components besides VFA.

The maximum respiration rates obtained upon the oxidation of readily biodegradable COD were almost similar and in both cases very low (Table 1). Maximum growth rates of thermophilic bacteria on a simple substrate are higher compared to mesophilic bacteria (Chapter 3) but a different ratio of active biomass over the total amount of biomass can suppress the intrinsic rate difference leading to a similar maximum respiration rate as observed here.

The results of Fig. 2a,b concerning the oxidation of sulphide show that a competition took place between an abiotic and a biotic reaction. Steudel (2000) reports that metal ions can catalyze the

chemical oxidation of sulfide to elemental sulphur following overall reaction (1). Trace amounts of metals like iron could be present in the reaction vessel, catalyzing the chemical oxidation.

$$2\ HS^- + O_2 \rightarrow \tfrac{1}{4}\ S_8 + 2\ OH^- \quad (1)$$

The increase in the reaction rates with an increasing sulphide dosage supports this hypothesis since the chemical reaction as described above increases in rate as the concentrations of the reactants increase. Also, the abiotic reaction rate increases as expected with temperature and from the rate increase an approximate doubling of the reaction rates for each 10 °C can be calculated. These results show that as temperature increases, the importance of abiotic reactions increases significantly.

Interestingly, in the mesophilic experiment, an increase in the sulphide dosage to the activated sludge did not increase the reaction rate thus apparently under these conditions, the amount of biocatalyst was controlling the conversion rate. Addition of sulphide to both mesophilic and thermophilic sludge did not result in a significant sulphate production (measured directly after the respiration rates dropped after the sulphide additions in Fig. 2a,b) while under continuous reactor operation, for both systems, virtually all influent sulphide could be found back in the effluent as sulphate. Probably, at 30 °C, sulphide is partially oxidized by biological means and stored as elemental sulphur granules inside the cells that can later on be oxidized into sulphate. It is known that *Thiothrix spp*. are able to store elemental sulphur in granules inside the cells as an energy source (Nielsen *et al*., 2000). Under thermophilic conditions, the first partial oxidation takes place to some extent by abiotic means, and elemental sulphur in the liquid phase or inside the cells is later on oxidized into sulphate.

Somewhat similar results were obtained by Bérubé and Hall (2000b) who studied the removal of hydrogensulfide from an evaporator condensate in a thermophilic aerobic membrane bioreactor operating at 60 °C. All hydrogensulfide was removed from the mixed liquor but the authors could not distinguish between the biological and the abiotic reaction. They suspect, based on their results, that the abiotic reaction contributes significantly to the overall sulfide removal due to the high temperature conditions. Stripping of hydrogen sulfide with the off-gas was probably negligible since no sulfide smell was ever detected.

4.2 BOD5 tests

The BOD5 experiments with raw, paper filtered and membrane filtered wastewater showed a higher initial degradation rate under thermophilic conditions compared to the mesophilic bottles owing to the higher biomass growth rate at 55 °C. The rapid leveling off suggests that the wastewater fractions that can be degraded at 30 °C are to a much lesser extent biodegradable at 55 °C.

In contrast to these results, the rate of oxygen uptake in the bottles containing only suspended and colloidal COD was higher at 30 °C compared to 55 °C (Fig. 3d). Since these bottles contained virtually no soluble COD, oxygen consumption could only take place after hydrolysis of suspended and colloidal material. Normally, higher hydrolysis rates are expected under thermophilic conditions.

Two possible explanations are suggested: 1. possibly not the full diversity of enzymes was available at 55 °C since the biomass might simply not be able to synthesize these enzymes or synthesis takes place after a certain lag phase. LaPara *et al*. (2000a) found that gelatin and α-lactose were biodegraded at 55 °C but not simultaneously in contrast to bioconversion at 25°C. At 55 °C, lactose was only converted after nearly all gelatin was removed. Similar results were obtained by Kosseva *et al*. (2001) who also found a non-simultaneous degradation of protein and other carbonaceous compounds from Stilton whey at 65 °C.

A second explanation is the location of the exo-enzymes and the substrates. Frølund *et al.* (1995) found that most activated sludge exo-enzymes are located in the activated sludge floc matrix. In the mesophilic bottles, wastewater colloidal material flocculated and became adsorbed to the sludge flocs since colloidal COD was removed from the liquid phase at 30 °C. The substrate thus comes in close contact with the enzymes and hydrolysis can take place effectively. In the thermophilic bottles, wastewater colloidals did not flocculate for unknown reasons so far and are thus less susceptible to direct enzymatic degradation unless enzymes are released into the bulk solution.

The non flocculating behavior of the colloidal fraction under thermophilic conditions is of utmost importance for practical applications since under continuous operation, influent colloidal COD would wash through the reactor system, deteriorating the effluent quality. This was indeed found to be the case under continuous reactor operation (Chapter 5). In case the influent contains virtually no colloidal material, the thermophilic effluent was also found to be clear. This in accordance with the results of the BOD test with a membrane filtered wastewater fraction (Fig 3,4c): hardly any colloidal COD was produced and the liquid remained transparent.

4.3 Biodegradability test

After 83 days of biodegradation, different inert COD levels remained in the batch vessels. Most likely, part of these inerts consist of lignin like components as these are hardly biodegradable under anaerobic conditions (Franta *et al*., 1994; Kortekaas, 1998).

The difference in residual COD concentrations at 30 and 55 °C could be due to two reasons: 1. thermophilic bacteria are not able to degrade the same range of compounds as the mesophilic biomass does and 2. thermophilic biomass produces more soluble (and non-soluble) microbial products (SMP) compared to mesophilic biomass. Other possible causes such as higher threshold concentrations and a minimum substrate concentration required for growth are not applicable under these circumstances.

Production of SMP by biomass can take place via decay processes (modeled in Activated Sludge Model No. 1 as cryptic growth) and by substrate metabolism itself (Barker and Stuckey, 1999). SMP are mostly complex molecules that are still to a large extent biodegradable. Significant production of SMP from substrate metabolism mainly occurs when sludge is heavily overloaded resulting in an excretion of complex intermediates that can later on still be degraded to a large extent (as observed by

Schiener *et al*., 1998 in a heavily loaded anaerobic baffled reactor). SMP might be produced during the initial stage of the batch test but can still be converted during the long time course of the experiment.

Production of inert material from biomass decay is modeled in ASM1 by assuming an inert fraction of heterotrophic biomass that is non-biodegradable As biomass decays, 92% of the biomass is assumed to be returned into biodegradable substrate for new biomass growth and 8% (the fraction f_p) remains as inert particulates (Henze *et al*, 1987). This cycle of growth and decay continues until all biomass is converted and eventually, 21% of the initial amount of heterotrophic biomass remains as non biodegradable particulates while the rest is oxidized. The question now is whether thermophilic bacteria could have such a different cell composition, with a larger non-biodegradable fraction as compared to mesophilic bacteria, explaining the difference in remaining inert COD levels in the biodegradability test.

This hypothesis was validated by conducting two batch biodegradation experiments at 30 and 55 °C with a fully biodegradable substrate (glucose) supplemented with mineral salts. No inoculum was used and biomass developed spontaneously, presumably by microorganisms present in the pressurized air. Residual COD after the test could only arise from production of inerts from biomass decay. The remaining COD levels were almost similar and calculated f_p values were 5.7% at 30 °C and 4.4 % at 55 °C after 35 days of biodegradation. In case the difference in residual COD levels in the biodegradation test with wastewater (Fig. 5, day 83) are explained with a difference in inert COD production from biomass decay, this would imply that 18% of a thermophilic bacterium needs to consist of inert material compared to the 8% value for a mesophilic microorganism. This seems very unlikely given the results of the biodegradation test with glucose and since moderate thermophiles and mesophiles are almost similar in cell composition (Sundaram, 1986). Furthermore, these results were confirmed by continuous reactor experiments at 30 and 55 °C with acetate as sole carbon source giving similar residual effluent soluble COD levels (not comprising any acetate), as described in Chapter 3.

Differences in remaining inert COD levels between mesophilic and thermophilic treatment were also found by other researchers (Tripathi, 2000; LaPara *et al*., 2001b). LaPara *et al.* (2001b) explain their results by production of SMP, containing a higher recalcitrant fraction at 55 °C as compared to 30 °C. They came to this conclusion from batch experiments with lactose and gelatin as sole carbon source giving a higher residual COD level at 55 °C compared to 25 °C (LaPara *et al*., 2000a). Furthermore, subsequent treatment of a pharmaceutical wastewater at 55 and at 30 °C did not yield the same COD removal as 30 °C treatment alone (LaPara *et al*., 2001b). The major difference between their work and the current findings is the time scale of the experimental work: 25 to 48 hours in their experiments compared to 35-85 days in the current research. In the glucose biodegradation experiment as described above, residual COD levels at 55 ° remained higher compared to the 30 °C batch vessel for

a 20 days period after which they dropped to a similar level during the end of the experiment. It thus seems plausible that thermophilic biomass produces more SMP, but given a sufficient time period, these can still be degraded. Summarizing: the difference in remaining COD levels in Fig. 5 is most likely the result of the inability of the thermophilic biomass to degrade the whole range of components that can be degraded under mesophilic conditions.
In Fig. 6 the wastewater characterization is summarized by fractionating the total COD into readily-, slowly-, very slowly biodegradable and inert COD. Based on the current results and these diagrams, it is proposed that readily biodegradable COD, comprising simple soluble substrates, is to a similar extent biodegradable under both temperature conditions but as substrates become more complex in nature, their biodegradability decreases to a larger extent under thermophilic conditions as compared to mesophilic conditions.

5. CONCLUSIONS

The anaerobic pretreated paper process water has a relatively high COD concentration but contains few easily biodegradable components. Fractions of the anaerobic effluent, characterized in terms of readily, slowly or very slowly biodegradable are not solely dependent on their inherent nature but also the type of microbial community degrading them. Colloidal wastewater COD is removed from the liquid phase by a flocculation process under mesophilic conditions. Under thermophilic conditions, the colloidal fraction remains almost completely stable in the water phase and will be washed out in a continuous reactor system.

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5

MESOPHILIC AND THERMOPHILIC ACTIVATED SLUDGE POST TREATMENT OF PAPER MILL PROCESS WATER

ABSTRACT

The feasibility of thermophilic activated sludge post treatment was studied using anaerobic effluent of a paper mill. Two lab-scale plug flow activated sludge reactors were run in parallel for 6 months; a thermophilic reactor at 55 °C and a reference reactor at 30°C. Both reactors were operated simultaneously at 20, 15 and 10 days SRT. The effects of temperature and SRT on sludge settleability and COD removal efficiencies of different wastewater fractions were investigated. Total COD removal percentages over the whole experimental period were 58 ± 5 % at 30 °C and 48 ± 10 % at 55 C°. The effect of the SRT on the total COD removal was negligible. Differences in total COD removal between both systems were due to a lesser removal of soluble and colloidal COD at 55 °C compared to the reference system. At 30 °C, colloidal COD removal percentages were 65 ± 25 %, 75 ± 17 % and 86 ± 22 % at 20, 15 and 10 days SRT. At 55 °C these percentages were 48 ± 34, 40 ± 28 and 70 ± 25 %, respectively. The effluent concentrations of colloidal COD in both systems were related to the influent concentration of colloidal material. The thermophilic sludge was not able to retain influent colloidal material as well as the mesophilic sludge causing a higher thermophilic effluent turbidity. Sludge settling properties were excellent in both reactor systems. These were not temperature nor SRT dependent but were rather caused by extensive calcium carbonate precipitation in the aeration tanks creating a very dense sludge. For application in the board industry, a thermophilic in line treatment system seems feasible. The higher effluent turbidity is most likely offset by the energy gains of treatment under thermophilic conditions.

Based on: Vogelaar, J.C.T., E. Bouwhuis,, A. Klapwijk,, H. Spanjers and van Lier, J.B (2002) Mesophilic and thermophilic activated sludge post treatment of paper mill process water. Water Res. 36, 1869-1879.

1. INTRODUCTION

In recent years a lot of effort is paid to water system closure in paper and board mills. This has led to reduced water demands and thus lesser effluent discharges, sometimes resulting in so-called zero-effluent mills. Zülpich Papier, a recycled wastepaper mill producing board and liner is operating totally effluent free for more then 20 years now. Initially no attention was paid to the accumulation of polluting compounds in the closed water cycle. Extension of the production capacity and bad odours in the product and the surroundings of the mill, caused by these pollutants led to the implementation of an in line process water treatment system. The most cost effective wastewater treatment system for this type of paper mill turned out to be a combination of anaerobic and aerobic biological treatment (Habets and Knelissen, 1997). At present, biological treatment of the process water takes place at 35 °C and the water is subsequently reheated to 55 °C to be re-used in the process. This system is operating satisfactory for several years now. A point of improvement, however, would be to purify the water at 55 °C to avoid energy losses due to cooling and reheating of the process water. The current research project aims at a complete anaerobic-aerobic treatment under thermophilic conditions (50-55 °C) of paper process water in a closed cycle mill.

The aim of this chapter was to study the feasibility of activated sludge as a suitable post treatment system for anaerobically pretreated paper process water under thermophilic conditions. Attention was paid to sludge settling properties, effluent turbidity and COD removal efficiencies under mesophilic and thermophilic conditions. Effluent turbidity was quantified as the amount of colloidal COD in the effluent.

Two identical plug flow activated sludge reactors were studied under continuous operation for more than 6 months. Operating temperatures were 30 and 55 °C, anaerobic pretreated paper process water was used as the influent. The HRT was kept constant at 12 hours, the SRT was lowered from 20 to 15 and 10 days.

2. MATERIALS AND METHODS

2.1 Lab-scale bioreactors

Two selfsame 4 liter stainless steel activated sludge reactors were used. Both reactors were divided in 5 compartments, each compartment containing 800 ml of mixed liquor. A plug flow reactor type was chosen since it results in a substrate gradient over the system. This can have a beneficial effect on the sludge settleability. Both the activated sludge reactors and the settlers were temperature controlled by a 2 cm water jacket surrounding the inner tanks. The jackets were connected to two thermostats, Julabo E5. Each compartment was aerated with pressurized air sparged via an aeration stone. The DO

in the aeration tank was maintained above 2 (mg l^{-1}) in all cases. The DO in the settler varied between 0.9 and 1.3 (mg l^{-1}). The settler volume was 2 liters. Both settlers were equipped with a scraper device with a rotation speed of 0.2 rpm.

The daily influent of the reactors was stored in a refrigerator (8 °C). Solids were kept in suspension by a mixing device installed on top of the refrigerator. The influent was pumped in the first compartment of the reactor whereas the return sludge was pumped into the second compartment. The first compartment thus functioned as an aerated tank. This set up was chosen since the reactor influent contained approximately 500 (mg l^{-1}) calcium that precipitated mainly in the first compartment. The flow of return sludge was practically equal to the influent flow. Excess sludge was wasted daily in equal amounts from the aerated compartments to control the SRT. A schematic set-up of the system is depicted in Figure 1.

Evaporation was extensive in the thermophilic reactor. Therefore additional demineralized water was pumped in the thermophilic reactor to account for part of the water losses. Evaporation percentages were calculated by measuring influent and effluent volumes daily for both reactors. All measured parameters were corrected for the evaporation.

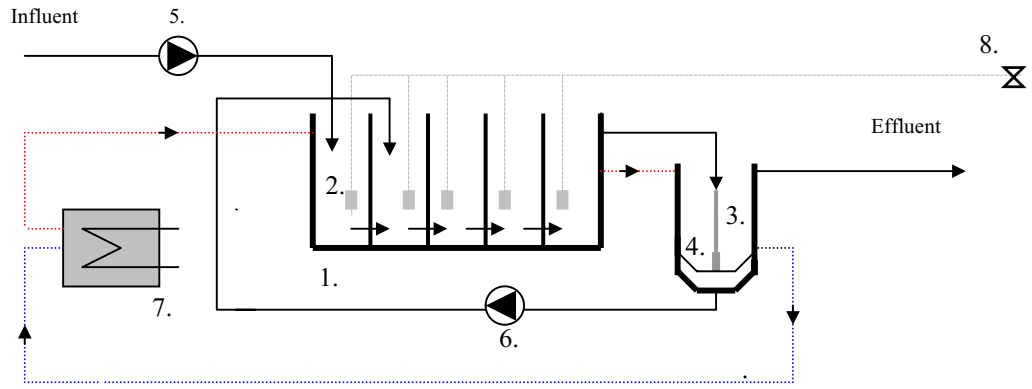

Fig. 1. Schematic set-up of the activated sludge system.

1. plug flow reactor; 2. aeration stone; 3. settler; 4. scraper device; 5. influent pump; 6. recycle pump; 7. temperature-controlled bath 8. Pressurized air

2.2 Influent and seed sludge

The influent of both activated sludge reactors was obtained every three weeks from the in- line process water treatment plant of Zülpich Papier GmbH. The paper process water was pre-treated in two UASB reactors operating at 35 °C. It was assumed that the effluent of an anaerobic mesophilic reactor would be reasonably good approximation of a thermophilic anaerobic effluent. Prior to the anaerobic treatment the process water was led in a small pre-acidification tank and nutrients were

supplied in a ratio of COD:N:P; 100: 2.6:0.4. No additional nutrients were supplied. The sludge was checked for a possible nitrogen deficiency by a respirometric method as described by Ning *et al.* (2000) and showed not to be nitrogen deficient both at 30 and at 55 °C. The anaerobic pretreated paper process water (hereafter named "wastewater") was stored in jerry cans in a temperature controlled room at 4 °C. During three weeks of storage no significant COD reduction could be detected. The influent vessel in the refrigerator was filled daily with wastewater. The influent was sampled daily at the same time from the influent vessel after half the volume was pumped into the reactors. Table 1 presents the typical composition of the reactor influent.

Mesophilic seed sludge was obtained from the activated sludge plant of Zülpich Papier. Seed sludge for the thermophilic reactor was obtained from a pilot plant in Oude Pekela, The Netherlands. The pilot plant was treating a similar process water of a small paper mill, operating at temperatures fluctuating between 35 °C and 50 °C. Both lab-scale reactors were operated directly at their set point temperatures. An acclimatization period of two months was imposed for both systems to obtain a steady state operation (three times a 20 days SRT). Reactor sampling was irregular during this period, therefore these results are not shown.

Table 1. Characteristics of the anaerobically pretreated paper process water of Zülpich. COD and VFA in mg COD·l^{-1}, BOD-5 in mg O_2 ·l^{-1}, cations and anions in mg·l^{-1}. Duplicate samples. [1]. S.D. is standard dev.

Analyses	Value ± S.D[1].
COD_{tot}	2113 ± 48
COD_{ss}	394 ± 15
COD_{col}	391 ± 42
COD_{sol}	1328 ± 74
VFA	9 ± 0.1
BOD-5 (at 30°C)	615 ± 20
Ca^{2+}	398 ± 22
S^{2-}	51 ± 4
Cl^{-}	439 ± 3
pH	7 ±0.2

2.3 Analytical methods

Chemical oxygen demand (COD) and sludge volume index (SVI) were determined according to APHA (1995). Mixed liquor total- and volatile suspended solids, (MLTSS/MLVSS) were determined according to the Dutch normalized standards (NEN 6621). Chemical oxygen demand was measured over four wastewater fractions: total, suspended, colloidal and soluble. Paper filtration (Schleicher and Schuell 595½ folded paper filters, pore size 4.5 µm was used to distinguish between the total and the suspended fraction. The soluble fraction was obtained by centrifuging the paper filtered samples in an IAC Micromax at 10.000 rpm for 10 min. The colloidal fraction was calculated as the difference between the soluble fraction and the paper filtered fraction. Sulphide (S^{2-}) was measured according to

Trüper and Schlegel (1964). Volatile fatty acids (VFA) of pre centrifuged samples were measured on a Hewlett Packard GC, model 5890 A. The GC was equipped with a 2 m × 2 mm glass column, packed with Supleco port (100-120 mesh) coated with 10% fluorad FC431. Carrier gas was nitrogen saturated with formic acid. Calcium (Ca^{2+}) was determined by an atomic absorption spectrometer (Model AA975, Varian, Springvale, Australia) according to NEN 6446 using a 422.7 nm wavelength lamp. Lanthaan chloride solution was used to dilute the samples. The amount of lignin was estimated by measuring the absorption of UV at 280 nm (Rintala and Lepistö, 1992). The samples were centrifuged (5 min IEC Micromax, 10,000 rpm) and UV measurement took place on a Milton Roy Spectronic 601 Spectrophotometer. The COD of the lignin like substances could be estimated by using Beer's law with an absorbidity coefficient of 22.3 L g^{-1}·cm^{-1} (Sierra-Alvarez *et al.*, 1991) and a chemical oxygen demand conversion factor of 1.9 mg COD·mg $lignin^{-1}$. Sulphate and Chloride (SO_4^{2-}, Cl^-) were measured with a HPLC using a Vydac-anion exchange column. Potasium-biphtalate was used as eluens, the anions were detected with a Waters 431 conductivity detector.

3. RESULTS AND DISCUSSION

3.1 COD removal

The reactors were operated for five months under semi-steady state conditions at three different sludge ages. The set point sludge ages were 20, 15 and 10 days respectively. After correction of the SRT for the solids leaving the reactor with the effluent, the exact figures were 19.9, 14.9 and 9.9 days at 30 °C and 19.7, 14.8 and 9.8 days at 55 °C, respectively.

The average applied sludge loading rate for total COD was approximately 0.64 (g COD g $MLVSS^{-1}$ day^{-1}) for the thermophilic reactor and 0.49 (g COD g $MLVSS^{-1}$ day^{-1}) for the mesophilic reactor over the first 80 days. Based on biodegradable COD, these were 0.44 and 0.35 (g COD g $MLVSS^{-1}$ day^{-1}), respectively. After day 80 the average loading rate was higher due to lower MLVSS concentrations in both reactors. These were on average 2.3 and 1.5 (g COD g $MLVSS^{-1}$ day^{-1}) based on total COD for the thermophilic and the mesophilic reactor. Based on biodegradable COD, these figures were 1.5 and 1.3 (g COD g $MLVSS^{-1}$ day^{-1}), respectively.

The results of the reactor studies in terms of COD removal are depicted in Figure 2. Effluent samples were composite samples over a 12 hour period. Influent samples were grab samples. High concentrations of influent particulate matter made it difficult to obtain representative samples, resulting in high fluctuations in the measured influent COD. Therefore, it seemed more appropriate to present the average values for the influent total- and suspended COD for each batch of wastewater.

The removal efficiencies for total COD in the mesophilic reactor were 59 ± 6 , 57 ± 5 and 60 ± 2 % at SRTs of 20, 15 and 10 days, respectively. For the thermophilic reactor the percentages are 48 ± 10, 43

± 10 and 56 ± 3 %, respectively. Table 2 shows the average removal percentages for both systems at the different SRTs. The difference in the total COD removal efficiency was mainly caused by the differences in removal of colloidal matter and soluble COD. Suspended matter was removed in both reactors for almost 100%, probably due to physical entrapment in the sludge. Effluent colloidal COD levels were distinctly higher in the thermophilic reactor compared to the 30 °C reactor leading to a turbid effluent. Visual inspection showed that the mesophilic effluent was not always completely clear but was significantly less turbid than the 55 °C effluent. Statistical analysis of the mesophilic and thermophilic removal efficiencies was done using a Wilcoxon one sample test (Berry and Lindgren, 1990) using a 95% confidence interval. For the course of the entire experimental period, differences between mesophilic and thermophilic COD removal percentages for the total, suspended, colloidal and soluble fraction were all statistically significant at the 95% confidence level. For each separate SRT a statistically significant difference in removal efficiency was found in all cases except for the removal of suspended COD at the 15 and 10 days SRT and colloidal and soluble COD at the 20 days SRT. Relevant statistical parameters are presented in Table 3.

Table 2. Mean values and standard deviation of the COD removal percentages of both plug flow reactors under steady state conditions.

	Removal percentage (%) mesophilic reactor			Removal percentage (%) thermophilic reactor		
SRT	20	15	10	20	15	10
total	59±6	57±5	60±2	48±10	43±10	56±3
suspended	99±6	96±5	100±2	89±13	95±14	99±2
colloidal	65±25	75±17	86±22	48±34	40±28	70±25
soluble	26±10	29±5	33±5	14±8	18±7	27±5

Table 3. Observed significance levels (=critical level) for a Wilcoxon one sample test comparing removal percentages of the mesophilic and thermophilic reactor for different COD fractions and experimental periods.

[a] marked critical levels are significant at a 95% confidence level

[b] bracketed figures indicate the number of observations

Observed significance (%)	Total period (36) [b]	SRT 20 (14) [b]	SRT 15 (16) [b]	SRT 10 (6) [b]
Total COD	< 0.04 [a]	0.4 [a]	0.08 [a]	2 [a]
Suspended COD	0.3 [a]	0.7 [a]	33.7	>10
Colloidal COD	0.02 [a]	9.1 [a]	0.18 [a]	10
Soluble COD	0.04 [a]	6	0.36 [a]	2 [a]

The increase in the thermophilic effluent COD at day 75 was due to a technical failure of a thermostat. The temperature dropped to 20 °C for at least one day. This resulted in a large wash-out of suspended solids as is depicted in Figure 2b. The reactor needed approximately two weeks to recover from the temperature shock (Fig. 2a and b).

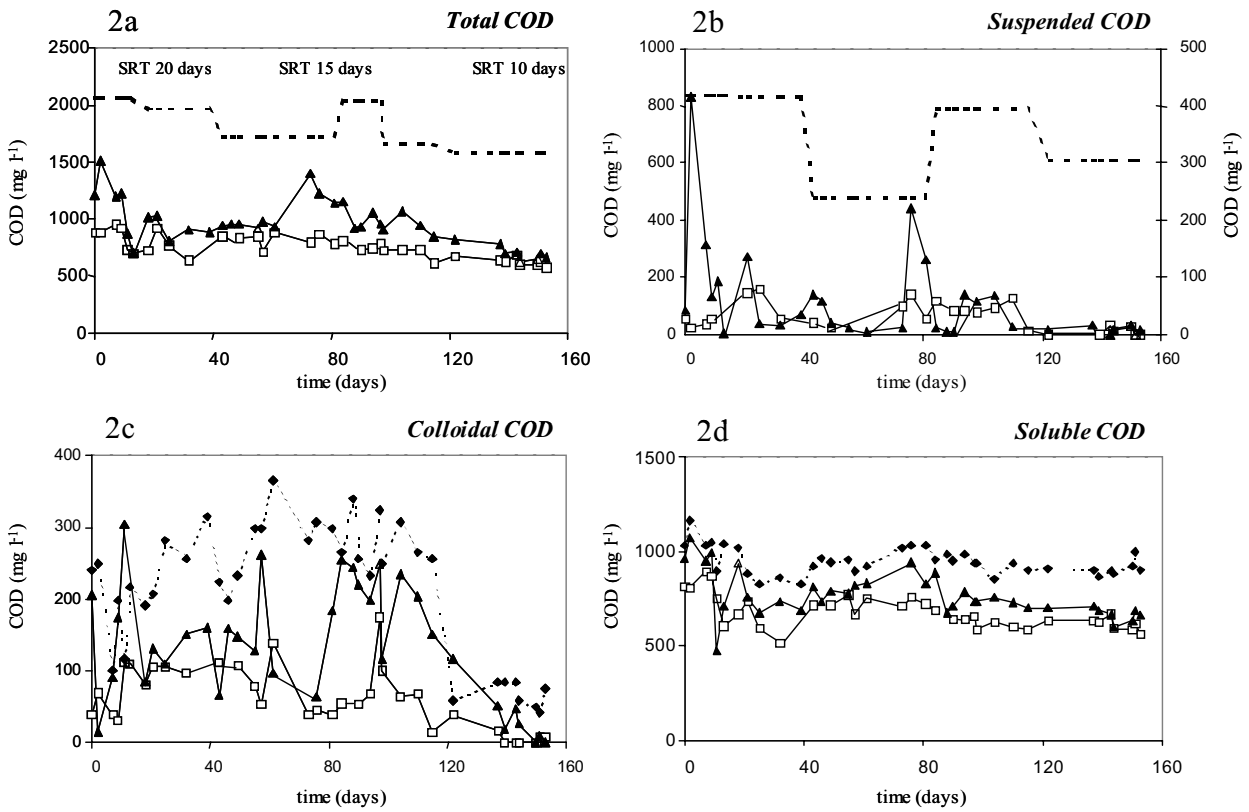

Fig. 2. COD concentrations of : (♦) Influent actual data points, (--) influent average total or suspended COD, (□) mesophilic effluent, (▲) thermophilic effluent. The suspended effluent COD is plotted on the secondary Y-axis.

During the course of the experiment, the removal percentages as a function of the SRT were rather constant for the mesophilic reactor. The thermophilic reactor showed some variation in removal of colloidal and soluble COD when the SRT was changed from 15 to 10 days (Table 2). However, it is not completely clear whether this can be attributed to the lower SRT or to a simultaneous change in influent composition. The SRT reduction from 20 to 15 days did not have any pronounced effect on the removal efficiencies. Barr *et al.* (1996) found no significant effect of the SRT on removal percentages either.

The change in influent composition was attributed to an altered paper mill operation leading to a large reduction in the amount of colloidal material in the process water (Fig 2c, day 120). This resulted in a completely clear effluent in both reactors. Apparently, the thermophilic effluent turbidity was caused by influent particles that were not effectively retained by the thermophilic sludge. Removal of colloidal COD in the mesophilic reactor was to a much lesser extent affected by the influent colloidal COD concentration. The observed phenomenon might explain why Barr *et al.* (1996) found a clear effluent at 50 °C while Tripathi and Allen (1999) did not. Both authors used bleach kraft mill effluent (BKME) as reactor feed but in Barrs research the influent was pre-settled. No data concerning the

influent colloidal material were given in both researches. It seems plausible that a part of the influent colloidal COD was removed by the settling process, leading to a clear effluent in Barrs case.

In Figure 3, the influent colloidal COD is plotted versus the effluent colloidal COD of the thermophilic reactor *minus* the effluent colloidal COD of the mesophilic reactor. The graph shows that with an increasing concentration of colloidal COD, the difference in effluent quality of both reactors becomes larger. A Spearman rank correlation test (Conover, 1980) was applied to test for a positive correlation between influent colloidal COD and the difference in effluent quality with regard to colloidal material. A significant positive correlation was found at a 95% confidence level (Spearmans rankcorrelation coefficient r_s: 0.35; 29 observations).

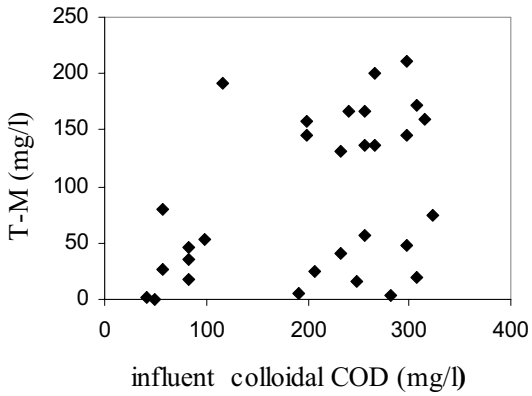

Fig. 3. Influent colloidal COD plotted versus thermophilic effluent colloidal COD minus mesophilic colloidal effluent COD (T-M), paired samples, units in mg COD l^{-1}.

The reduced capacity of the thermophilic sludge to remove colloidal COD is not yet fully understood. So far, several explanations for this phenomenon can be thought of. LaPara and Alleman (1999) notice that under thermophilic conditions, protozoa and other life forms are absent. Protozoa are known to consume free swimming bacteria and small colloidal particles. In our present research, microscopic examination evidenced only a very low number of protozoa in the thermophilic reactor while the mesophilic system was characterized by a large number of these organisms.

Secondly, the cohesion of activated sludge flocs and their ability to entrap colloidal particles is governed by several types of interactions. These are: 1. DLVO type of interactions (Zita and Hermansson, 1994), 2. bridging by negatively charged EPS and/or by polyvalent cations such as Ca^{2+} of Fe^{3+} (Keiding and Nielsen, 1997) and 3. Hydrophobic interactions (Block, 2001). Hydrophobic interactions (more precise: the hydrophobic effect) is mainly an entropic phenomenon. Water molecules, when faced with non-polar molecules or hydrophobic domains re-orientate in such a way that they can participate in H-bond formation more or less as in bulk water (Israelachvili, 1992). This is entropically very unfavorable since it disrupts the existing water structure and imposes a new and

more ordered structure on the surrounding water molecules. The net result is a clotting of hydrophobic domains and/or hydrophobic molecules in order to minimize the entropy loss of the water molecules. At higher temperatures, due to the increased randomization of water, structure formation around a non-polar molecule plays a less important role resulting in a lower hydrophobic interaction (Lyklema, 1991). Electrostatic interactions, (DLVO interactions and polymer bridging) are only weakly temperature dependent in the temperature range of 30-55 °C (Lyklema, 1995).

Liao *et al.* (2001) presented a recent study in which sludge surface properties were combined with sludge flocculation, settleability and effluent suspended solids. They found that the effluent suspended solids concentration was strongly inversely correlated with sludge hydrophobicity. Zita and Hermansson (1997) have shown that an increasing cell surface hydrophobicity of bacterial cells leads to a stronger adsorption to activated sludge flocs. These findings, combined with the temperature dependency of the hydrophobic interaction is a strong clue towards a feasible explanation for the observed thermophilic effluent turbidity.

Regarding the role of EPS (extracellular polymeric substances), Liao *et al.* (2001) state that sludge surface properties such as surface charge and hydrophobicity and the composition of EPS rather than the quantity of EPS affect the sludge bioflocculation. So far, little is known concerning EPS quantities and composition of thermophilic activated sludge. Schmidt and Ahring (1994) found for anaerobic granular sludge that the amount of EPS, especially the protein content was lower under thermophilic than under mesophilic conditions. Tripathi (2000) extracted EPS from thermophilic activated sludge and found no statistically significant trend of the total amount of extracted EPS as a function of temperature. There was an increasing trend in protein content with increasing temperature, although not statistically significant.

3.2 Soluble COD

With regard to the removal of soluble COD, the mesophilic reactor showed a better performance throughout the entire experimental period. The average soluble COD removal efficiencies were 26 ± 10, 29 ± 5 and 33± 5 % at 30 °C at 20, 15 and 10 days SRT, respectively. At 55 °C these percentages were 14 ± 8, 18 ± 7 and 27 ± 5 %, respectively. It should be noted that these samples were corrected for the evaporation taking place in the reactors. UV measurements indicated that the wastewater contained approximately 200 mg lignin COD l^{-1}. At 30 °C 16 ± 8 % lignin COD removal took place, at 55 °C, 12 ± 7 % lignin COD was removed. Removal of lignin like components is generally expected to be low. Jahren (1999) found comparable results when treating whitewater from a thermo-mechanical pulping mill at 55 °C with an aerobic biofilm process.

In order to get some idea of the validity of the results for full-scale application, the experimental data of the mesophilic lab-scale reactor were compared to the full-scale mesophilic reactor at the mill site (Fig. 4). The hydraulic retention times in both systems were equal, MLVSS concentrations were

almost similar (5.7 g MLVSS l^{-1} in Zülpich versus 6.5 g MLVSS l^{-1} in the lab reactor). The full-scale plant was operated continuously at a 20 days SRT. The data from the lab-scale reactor presented in Figure 4 comprise the first 100 days of operation of the reactors as depicted in Figure 2a. The lab-scale reactors were thus operated at a 20 and a 15 days SRT. In the lab-scale reactor bubble aeration was applied while surface aeration was used at the full-scale plant. Analyzing the data of both systems showed that the differences in COD removal at 30 °C were marginal (Fig. 4). The lab-scale results obtained at 55 °C, therefore, seem a good estimate of what can be expected at full-scale with this type of wastewater at 55°C.

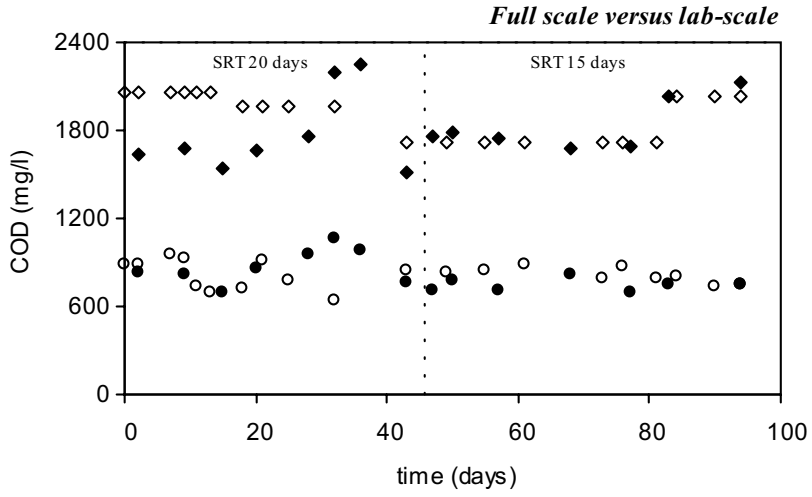

Fig. 4. Comparison based on total COD removal between the full-scale mesophilic reactor in Zülpich and the mesophilic lab-scale reactor. The SRT in the full-scale system was 20 days over the whole period, the lab-scale reactor was operated at 20 days SRT for the first 50 days and 15 days SRT from day 50-100. Influent lab-scale reactor (◊), influent full scale reactor (♦), effluent lab-scale reactor (○), effluent full-scale reactor (●).

3.3 Sludge properties

During the entire experiment the sludge settling properties for both reactors have been excellent. This, however, was the result of the extensive calcium precipitation that took place in the aeration tanks and was not so much related to temperature effects or the plug flow type of reactor as applied in this particular case.

During the pulping process in the mill, calcium is dissolved from the waste paper as described by Habets and Knelissen (1997). The anaerobic effluent contains high concentrations of bicarbonate keeping the calcium in solution. In the aeration tank, CO_2 is stripped from the water leading to a shift in the chemical equilibrium (1), leading to an extensive calcium carbonate precipitation.

$$2\ HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

At 30 °C, 93±1 % of the influent calcium precipitated in the aeration tanks versus 96±2 % at 55 °C. The higher removal percentage at 55 °C is most probably due to a lower calcium carbonate solubility at higher temperatures. The extensive calcium carbonate precipitation resulted in extremely high ash percentages in the sludge: 71±7 % at 30°C and 77±6 % at 55°C. This led to high settling rates and extremely low SVI values: 21± 8 (ml g $MLVSS^{-1}$) in the mesophilic reactor and 12 ± 8 (ml g $MLVSS^{-1}$) in the thermophilic reactor. Some researchers take advantage of this phenomenon by dosing talc (3 $MgO \cdot 4SiO_2 \cdot H_2O$) to the aeration basin in order to increase the floc weight and thus to improve the settleability (Claus *et al*., 1998).

The MLVSS concentrations of the sludge are depicted in Figure 5. At the 15 days SRT, the MLVSS concentration dropped. A combination of three possible causes can be thought of: 1. more excess sludge wastage at 15 days SRT compared to 20 days SRT, 2. a lower influent colloidal COD concentration and 3. higher levels of effluent suspended solids during day 80-110 (Fig. 2b).

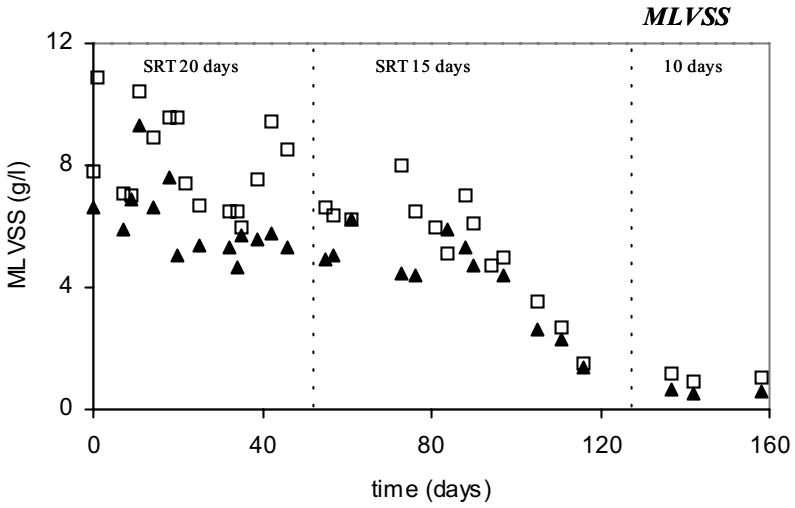

Fig 5 . MLVSS concentrations in the mesophilic (□) and thermophilic (▲) reactor.

MLVSS concentrations were slightly higher in the mesophilic reactor. Sludge production was almost similar in both systems; 0.18 ± 0.09 (g MLVSS g COD^{-1} removed) at 30 °C and 0.21 ± 0.11 (g MLVSS g COD^{-1} removed) at 55 °C over the whole experimental period. Various authors mention a lower sludge production with increasing temperatures because of the higher biomass decay rates (Sürücü *et al*., 1976; Couillard *et al*., 1989). However, the present results show no significant difference in the observed sludge production. This is due to the fact that a large part of the sludge consisted of entrapped inert or slowly hydrolysable solids while only a very small fraction of the sludge consisted of active biomass. Couillard *et al*. (1989) and Sürücü *et al*. (1976) used more easily- and fully biodegradable substrates as influent. Their sludge therefore consisted solely of biomass (dead and alive) and EPS components lacking any entrapped influent solids.

3.4 Practical implications

For application in the board industry, the thermophilic effluent turbidity is a disadvantage, however not very critical. Treated process water to be re-used in the pulping of recycled wastepaper is allowed to contain relatively high levels of suspended solids. For other in-mill applications, higher water quality demands prevail. In such cases an additional treatment step is necessary. Another strategy is to use different wet-end additives on the paper machine in order to retain more colloidal material in the end product and thus to remove colloidal particles from the process water. The decrease in colloidal COD in the process water at day 120 (Fig. 2C) has shown the effectiveness of this strategy. Energy gains by treating the process water at 55 ° instead of 35 °C in a closed cycle mill are in the order of 250 MJ per ton of produced board (assuming the purification of 3 m^3 of process water per ton of board produced). So far, thermophilic in-line treatment seems a feasible and cost effective option for application in the board industry.

4. CONCLUSIONS

- The effect of the SRT on the reactor performance, in terms of total COD removal, was negligible. Removal of soluble and colloidal COD at 55 °C was affected by the SRT and/or the influent composition.
- The mesophilic activated sludge system showed a higher removal of total COD as compared to the thermophilic reactor.
- The difference in COD removal between mesophilic and thermophilic treatment was caused by a diminished removal of soluble and colloidal COD in the thermophilic reactor.
- Colloidal COD removal in both reactor systems was related to the concentration of influent colloidal material. The mesophilic sludge was better able to remove and retain colloidal particles from the influent as compared to the thermophilic sludge.
- Thermophilic effluent turbidity was related to the amount of influent colloidal material. A clear thermophilic effluent could be obtained in case the influent contained only minor amounts of colloidal material.
- The sludge settling properties in both reactors were excellent, as reflected by the SVI values. In this research however, sludge settling was mainly determined by the calcium precipitation in the aeration tanks while temperature effects played only a very minor role.

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6

ASSESSMENT OF EFFLUENT TURBIDITY IN MESOPHILIC AND THERMOPHILIC ACTIVATED SLUDGE REACTORS - ORIGIN OF EFFLUENT COLLOIDAL MATERIAL

ABSTRACT

Two lab-scale plug flow activated sludge reactors were run in parallel for 4 months at 30 and 55 °C. Research focused on 1. COD removal, 2. effluent turbidity at both temperatures, 3. the origin of effluent colloidal material and 4. the possible role of protozoa upon the turbidity levels. Total COD removal percentages over the whole experimental period were 66 ± 7 % at 30 °C and 53 ± 11 % at 55 °C. Differences in total COD removal between both systems were due to a lesser removal of soluble and colloidal COD at 55 °C compared to the reference system. Thermophilic effluent turbidity was caused by a combination of influent colloidal particles that were not effectively retained in the sludge flocs and by erosion of thermophilic activated sludge itself, as shown by Denaturing Gel Gradient Electrophoresis profiles. DGGE analysis of PCR-amplified 16S rDNA fragments of mesophilic and thermophilic sludge differed from each other indicating that different microbial communities were present in both reactor systems. The effects of protozoal grazing upon the effluent turbidity of both reactors was negligible and could thus not account for the large observed turbidity differences.

Based on: Vogelaar, J.C.T., J.B. van Lier, A.. Klapwijk, M.C. de Vries, and G. Lettinga. (2002) Assessment of effluent turbidity in mesophilic and thermophilic activated sludge reactors - origin of effluent colloidal material. Appl. Microbiol. Biotechnol.59:105-111.

1. INTRODUCTION

Thermophilic wastewater treatment systems are gaining more and more interest due to increasing water system closure in for instance paper mills and higher process water temperatures (LaPara and Alleman, 1999). The application of thermophilic activated sludge is frequently hampered by a turbid effluent (Visvanathan and Nhien, 1995; Tripathi and Allen, 1999) and sludge settling problems (Tardiff and Hall, 1997; Tripathi and Allen, 1999). Sludge losses with the effluent result in an activity loss and a worsening of the effluent quality. However, Barr *et al.* (1996) reported a clear effluent and no settling difficulties when treating bleach kraft mill effluent at 51 °C. In Chapter 5 it was shown that a clear thermophilic effluent can be obtained in case the influent contains little colloidal material. This suggests that the effluent turbidity is caused by influent material that is not retained in the activated sludge flocs, in contrast to the mesophilic treatment.

The reason for the increased thermophilic effluent turbidity is still subject to speculation. LaPara and Alleman (1999) mention the absence of protozoa and other higher life forms as a possible cause. Protozoa are known to graze upon activated sludge flocs and feed upon free swimming bacteria (Ratsak *et al*., 1996). In Chapter 5, a possible decrease in the hydrophobic interaction with an increase in temperature is proposed as a possible cause. The hydrophobic interaction (or hydrophobic effect) is mainly an entropic effect and is of importance in the cohesion of activated sludge flocs (Block, 2001).

The current research aims to provide more insight in the nature of the colloidal material in the reactor effluent and to investigate the effect of protozoal grazing upon effluent turbidity levels. A comparative study is made between sludges and effluents of two selfsame lab-scale activated sludge reactors operated at 30 and 55°C. Colloidal material in the reactor effluent can originate from the activated sludge itself (eroded bacteria, exopolymeric substances) or from influent material that is not retained in the activated sludge flocs as suggested by previous results (Chapter 5).

Attempts were made to unveil the nature of the effluent colloidal material of both reactors by performing FISH (Fluorescent *In-Situ* Hybridization) and DGGE analyses (Denaturing Gradient Gel Electrophoresis) of 16 S rRNA gene (rDNA) fragments. The activated sludge reactor influent is the effluent of an anaerobic pre-treatment system (Upflow Anaerobic Sludge Blanket reactor) which contains a certain amount of Archaea (eroded methanogens) compared to Bacteria (for instance acidifiers). The activated sludge itself consists merely of Bacterial cells and some entrapped methanogens from the influent. Therefore, the ratio of Archaea/Bacteria is an indication of the origin of the micro-organisms in the reactor effluent as a high ratio indicates a strong resemblance with the influent and a low ratio indicates a higher similarity with the sludge. Comparing DGGE patterns of PCR amplified 16S rDNA fragments from the reactor influent, the reactor sludges and the reactor effluents provides information regarding the origin of the effluent microbial material as well.

2. MATERIALS AND METHODS

2.1 Lab-scale reactors

Continuous experiments were performed using two similar lab-scale activated sludge systems operating at 30 and 55 °C (Chapter 5). The aeration tank consists of 5 compartments of each 0.8 liters, connected in series. The influent was pumped in the first compartment while the return sludge was pumped in the second compartment. The return sludge flow was approximately equal to the influent flow. Both the reactors and the settlers were temperature controlled. The DO in the separate compartments was maintained above 2 (mg l^{-1}) in all cases. The Sludge Retention Time (SRT) and Hydraulic Retention Time (HRT) were kept constant at 10 days and 12 hours, respectively. COD removal of four different fractions was measured over time as a function of the temperature.

2.2 Wastewater and seed sludge

Anaerobic pretreated paper process water (hereafter named "wastewater") was used as reactor influent. Wastewater was obtained every three weeks from Kappa Zülpich Papier, a paper mill producing board and liner out of recycled wastepaper. The influent was stored at 4 °C, daily reactor influent was stored in a fridge. Mesophilic seed sludge was obtained from the full scale plant in Zülpich while thermophilic sludge was grown by acclimatizing the mesophilic sludge to the high temperature conditions for two months. During start-up, both reactors were operated directly at their set point temperatures. The effluents were corrected for the evaporation.

2.3 Effect of protozoa on effluent turbidity

After 115 days of steady state operation of the lab-scale reactors, the protozoal populations were selectively inhibited using Nystatin (Sigma N6261) and cycloheximide (Merck 2328) in a similar dosage as applied by Lee and Welander (1994). Effects of inhibitor addition upon effluent turbidity and protozoal counts were monitored under continuous reactor operation. Effluent turbidity was determined as the Optical Density (OD) at 600 nm. Protozoa were determined and counted daily according to Eikelboom (1999) by light microscopy (Olympus BH-2). The counts are relative values and represent the number of protozoa in 0.5 ml mixed liquor.

2.4 Analyses

Chemical Oxygen Demand (COD) was measured over four wastewater fractions, total, suspended, colloidal and soluble. Paper filtration (Schleicher and Schuell, 595 ½ folded paper filters, pore size 4.5 μm) was used to distinguish between the total and the suspended fraction. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The colloidal fraction was calculated as the difference between the soluble and the paper filtered fraction. COD of the four

different fractions was determined photometrically (APHA, 1995). Sulphide (S^{2-}) was determined according to Trüper and Schlegel (1964). pH was measured with a Knick 510 pH-mV meter. Volatile Fatty Acids (VFA) of pre-centrifuged samples were measured on a Hewlett Packard GC, model 5890A, equipped with a 2 m × 2 mm glass column, packed with Supleco port (100-120 mesh) coated with 10% fluorad FC431. Carrier gas was nitrogen saturated with formic acid. Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids (MLSS/MLVSS) were determined according to NEN 6621, Dutch Normalized standards (1988).

2.5 FISH

FISH analyses were performed according to a protocol described by Amann (1995). Cell fixation took place in 4% (w/v) formaldehyde, hybridization and washing temperatures were respectively 46 and 48 °C. Anaerobic effluent (= activated sludge reactor influent), mesophilic and thermophilic activated sludge and mesophilic and thermophilic effluent were hybridized with the EUB338 (Amann *et al*., 1990a) and ARCH915 (Amann *et al*., 1990b) probe and stained with 4,6-diamidino-2-phenylindole (DAPI). The EUB338 probe was labelled with fluorescein, the ARCH915 probe was Cy3 labelled. Stained cells were counted under a Leica DMR fluorescence microscope and the average ratio Archaea/Bacteria was calculated over 6 different counts.

2.6 DGGE

DGGE analyses were performed by Bioclear B.V. (Groningen, The Netherlands). Samples were fixed with ethanol, DNA was extracted according to Stephen *et al*. (1999). PCR reactions were performed using DGGE primer sets (Bacteria primers: EUB968F-GC-/EUB1401R and Archaea primers: ARC344F-GC/ARC915R). PCR conditions for the EUB primers were an initial cycle of 3 min at 94 °C, 35 cycles of 1 min at 94 °C followed by 1 min at 56 °C and 1 min at 72 °C and a last cycle of 5 min at 72 °C. PCR conditions for the ARC primer are described in Casamayor *et al.* (2000). Separation took place on a DGGE gel (9% acrylamide, 30-70% denaturant). Samples were run for 30 min at 200 V and 16 h at 110 V. The DGGE gel was analyzed using Molecular Analyst Software (version 1.5; Bio-Rad, Hercules, Calif.).

3. RESULTS

3.1 COD removal

The wastewater characteristics (reactor influent) are depicted in Table 1. A distinction is made between two time periods during which the lab scale reactor was operated. During period 1 (day 0-66), normal reactor operation took place while under period 2 (day 66-115), the anaerobic post

treatment system at the mill was overloaded resulting in a higher effluent COD concentration (the lab-scale reactor influent). This resulted also in a higher organic loading of the lab-scale reactor since the operational conditions were left unchanged.

Table 1. Average influent composition and standard deviations.
COD and VFA in [mg COD l^{-1}], S^{2-} in [mg l^{-1}].

analyses	Period 1 day 0 – 66	No. of measurements	Period 2 day 66 - 115	No. of measurements
COD_{tot} (1)	1674 ± 402	16	3022 ± 683	7
COD_{ss} (1)	531 ± 458	16	1213 ± 721	7
COD_{col} (1)	178 ± 72	16	449 ± 66	7
COD_{sol} (1)	867 ± 125	16	1360 ± 224	7
VFA	9 ± 2	2	N.D. (2)	-
S^{2-}	51 ± 4	4	48 ± 4	2
pH	7 ± 0.2	6	6.6 ± 0.3	4

(1) tot: total sample, ss: suspended fraction, col: colloidal fraction, sol: soluble fraction.

(2) N.D. is not determined.

Fig. 1 shows the results of the continuous reactor experiment in terms of COD removal. The graphs show that there is a significant difference in COD removal efficiencies when comparing the mesophilic and thermophilic reactors. Total COD removal percentages were 66 ± 7 % at 30 °C and 53 ± 11% at 55 °C over the whole experimental period. The difference in total COD removal was mainly caused by a higher removal of soluble and colloidal COD in the mesophilic reactor as compared to the thermophilic reactor (Table 1). Suspended matter was almost completely removed in both systems by physical entrapment in the sludge.

Table 2. Mean values and standard deviation of the COD removal percentages of both plug flow reactors under steady state conditions for both periods.

Wastewater	Period 1 (day 0-66)		Period 2 (day 66-115)	
fraction	30 ° reactor	55 °C reactor	30 °C reactor	55 °C reactor
total	64 ± 7	53 ± 7	70 ± 6	54 ± 16
suspended	99 ± 2	100 ± 7	89 ± 10	84 ± 34
colloidal	84 ± 25	51 ± 33	96 ± 6	39 ± 23
soluble	35 ± 6	21 ± 6	45 ± 3	28 ± 13

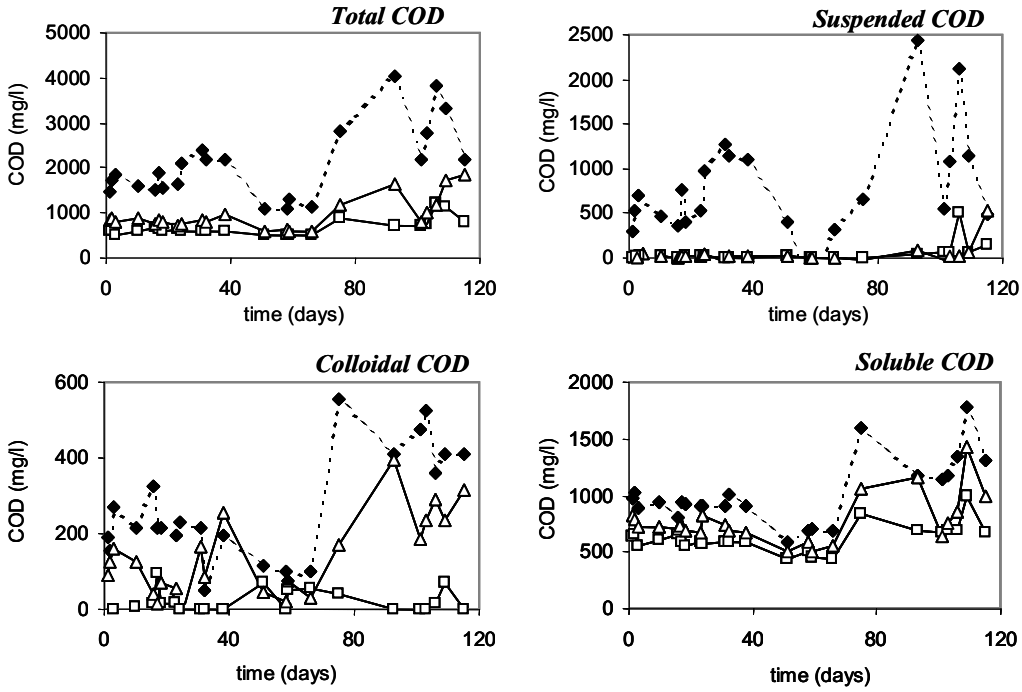

Fig. 1. COD concentrations of : Influent (♦), mesophilic effluent (□), thermophilic effluent (Δ). Period 1 is defined as day 0-66, period 2 is day 66-115.

Fig. 2 depicts the influent colloidal COD for both reactor systems plotted versus the effluent COD of the thermophilic reactor *minus* the effluent COD of the mesophilic reactor. The graph shows that with an increasing influent concentration of colloidal COD, the difference in effluent quality of both reactors becomes larger. A Spearman rank correlation test (Conover, 1980) was applied to test for a positive correlation between influent colloidal COD and the difference in effluent quality with regard to colloidal material. A significant positive correlation was found at a 95% confidence level (Spearmans rank correlation coefficient r_s: 0.58; 23 observations).

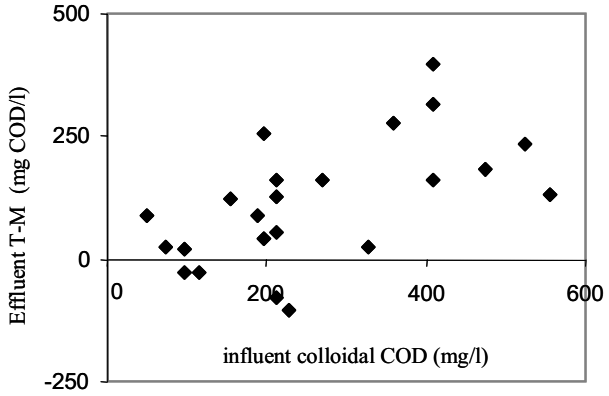

Fig. 2. Influent colloidal COD plotted versus thermophilic effluent colloidal COD *minus* mesophilic effluent colloidal COD. Units in mg COD l^{-1}.

3.2 Effects of protozoa on effluent turbidity

The effects of protozoal grazing upon effluent turbidity of both activated sludge systems is depicted in Fig. 3. The optical density and the protozoal numbers were counted during period 2 (day 75-140) of reactor operation. Addition of cycloheximide and nystatin mainly affected the population of matured protozoa in the mesophilic reactor (Fig 3a). However, no significant effect was observed upon mesophilic effluent turbidity (Fig. 3b).

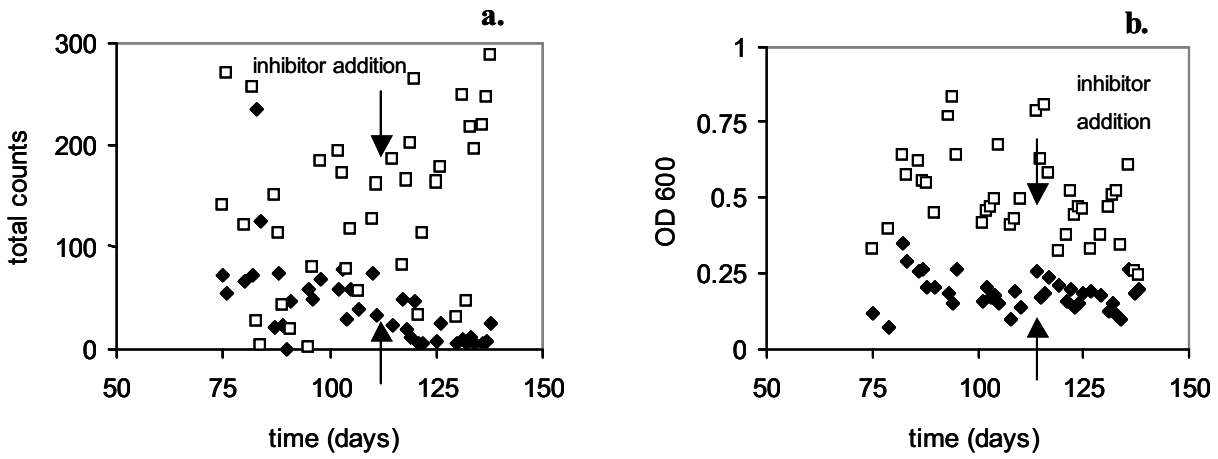

Fig. 3. Effect of eukaryotic inhibitor addition on:
a: Total counts of protozoa, sum of all reactor compartments. (♦) mesophilic reactor, (□) thermophilic reactor. b: effluent turbidity. (♦) mesophilic effluent, (□) thermophilic effluent.

In the mesophilic reactor, most eukaryotic organisms were *Vorticella sp*. Some *Epistylles sp.* and some nematodes were present as well. Most protozoa were attached to the activated sludge flocs and their numbers were positively correlated with the MLSS concentration (data not shown). The distribution of their numbers over the different reactor compartments was relatively constant (Fig. 4).

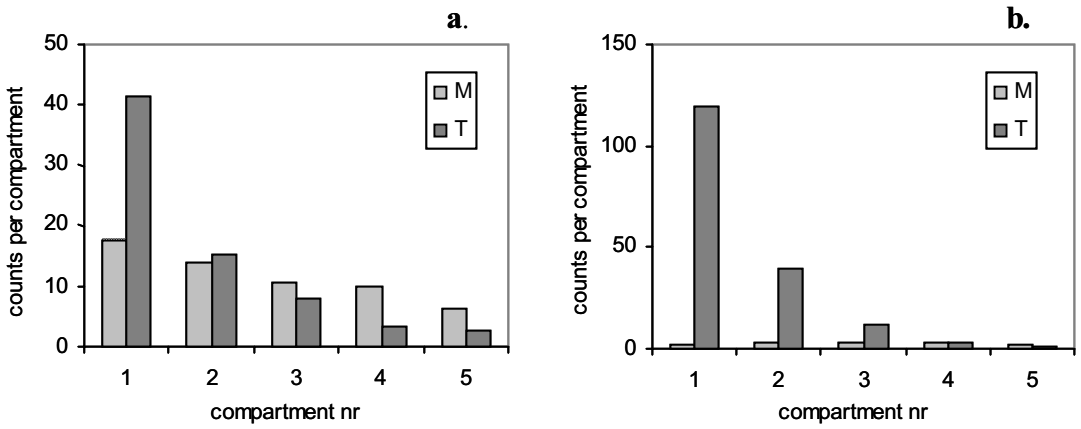

Fig. 4. Relative distribution of protozoa over the compartments. a: before addition of inhibitors (day 75-115), b: after addition of inhibitors (day 115-140). M: 30°C reactor, T: 55 °C reactor.

3.3 FISH and DGGE

FISH analyses showed that the ratio of Archaea over Bacteria in both reactor effluents was in accordance to the ratio as found in the influent (Table 3). In the sludge samples, the ratio was significantly lower. Furthermore, most of the influent colloidal material was of microbial origin since virtually all colloidals were stained with DAPI.

Table 3: Ratio Archaea over Bacteria in different samples. Six counts per sample.

	influent	Mesophilic sludge	Thermophilic sludge	Mesophilic effluent	Thermophilic effluent
ratio Archaea/Bacteria	0.23	0.02	0.01	0.36	0.47
stdev	0.15	0.04	0.02	0.17	0.16

The DGGE fingerprint of PCR amplified 16S rDNA of the wastewaters and sludges is presented in Fig. 5. The microbial diversity of the Archaea group in the samples was rather limited, 4 to 6 bands could be detected in the different lanes. The Bacterial diversity in the samples was larger, 13 bands could be detected in the influent and in the mesophilic sludge, 10 bands in the mesophilic effluent and the thermophilic sludge and 8 bands in the thermophilic effluent. The band patterns for the Bacterial primer were compared and correlated according to their position in the gel (Fig. 6). The dendogram shows that the bacterial populations in the thermophilic sludge and the thermophilic effluent were strongly correlated (67% correlation) and also showed some correlation (45%) with the influent. The mesophilic sludge was hardly correlated to the influent, the mesophilic effluent or the thermophilic sludge.

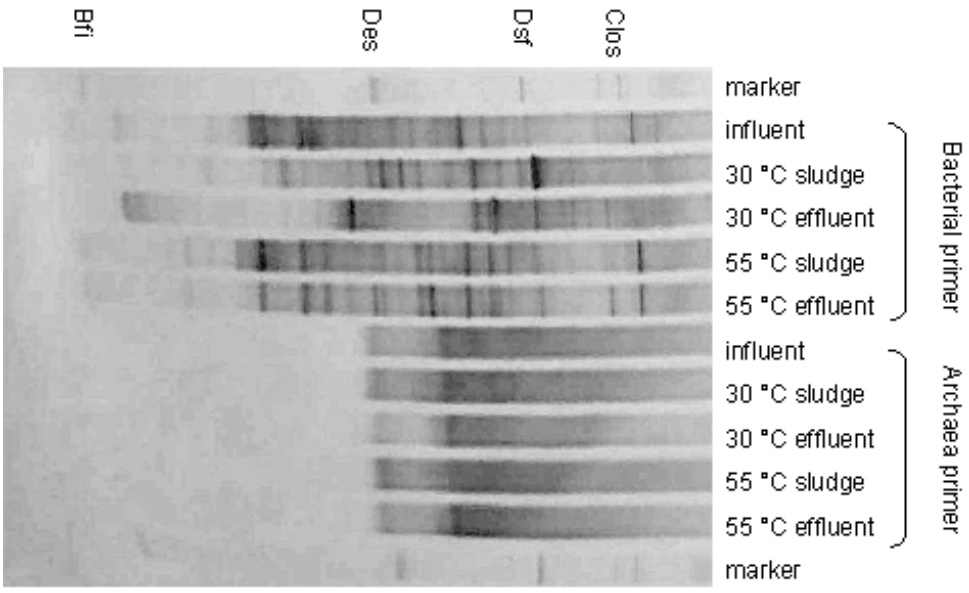

Fig. 5. DGGE profiles of PCR amplified 16S rDNA from the reactor influent, mesophilic and thermophilic sludge and both effluents.

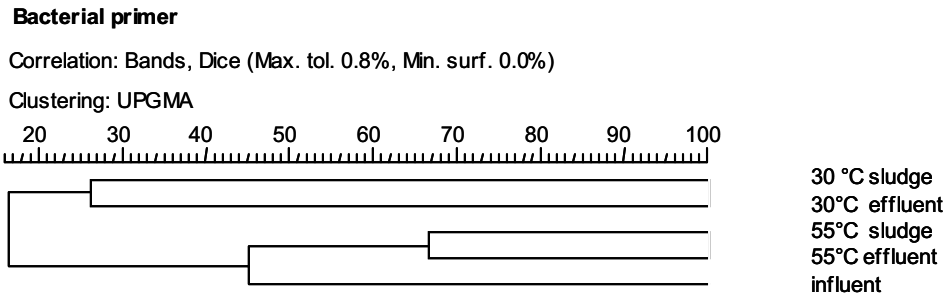

Fig. 6. Dendogram showing the correlation between the 16S rDNA fingerprints for the different samples (fingerprint pattern with the EUB999F-GC/EUB1416R primer).

4. DISCUSSION

COD removal percentages were slightly higher in period 2 compared to period 1. Under normal operation in period 1, the COD removal efficiency is limited by the amount of non-biodegradable material in the influent (Chapter 4,5). During period 2, the anaerobic effluent contained more easily biodegradable substrates due to the overloading, as was confirmed by respirometric assays (results not shown). These compounds are easily oxidized in the post treatment system resulting in a higher removal percentage when compared to the influent.

A marked difference between the mesophilic and the thermophilic reactor is that as soon as the loading rate of colloidal material increased, the difference in reactor performance between both systems became larger as well.

Effluent colloidal material in the thermophilic reactor can originate from the influent or from the sludge itself, by erosion of sludge flocs or from dispersed growing bacteria. The correlation between influent and effluent colloidal COD suggests that most of the effluent material arises directly from the influent. This would indicate that the thermophilic sludge is not able to retain (and possibly to oxidize) influent colloidal material as well as the mesophilic sludge does. This hypothesis was also confirmed by the FISH analyses since the ratio of Archaea over Bacteria in both effluents was more in accordance to the reactor influent as compared to the sludges.

However, the DGGE fingerprints showed that the thermophilic effluent was more closely related to the thermophilic sludge as compared to the influent. This indicates that the thermophilic effluent did not consist solely of influent colloidal material but also of thermophilic biomass that was growing dispersed or that was eroded from the sludge flocs. Furthermore, the fingerprint shows that under mesophilic conditions, the microbial populations of the influent, the sludge and the effluent were well segregated. Thus, at 30 °C, separation of influent material and effluent bacteria took place efficiently by the flocculation and final settling process while at 55 °C the separation was less efficient. A

diminished bioflocculation and biomass separation was observed before under thermophilic conditions by visual inspection (e.g. Tardiff and Hall, 1997; Tripathi and Allen, 1999) but not by application of DGGE analyses.

In Chapter 5 it was shown that a clear effluent can also be obtained under thermophilic conditions in case the influent contains little colloidal COD. This suggests that the thermophilic sludge can be separated effectively from the effluent which was not the case in the present research, as shown by DGGE analyses. In the current research, samples for FISH and DGGE analyses were taken at day 108 and 110 in the trial, under conditions of overloading of the anaerobic pretreatment. The reactor influent contained higher amounts of volatile fatty acids easily leading to dispersed bacterial growth and subsequent wash-out. In the previous research , the reactor influent contained hardly any easily biodegradable substrate making dispersed growth unlikely (Chapter 4).

Comparison of the DGGE band pattern of mesophilic and thermophilic activated sludge shows a difference in bacterial community structure since only a weak correlation (15%) was observed. These findings are confirming previous research by Tripathi and Allen (1999), Konopka *et al*. (1999) and LaPara *et al*. (2000a,c) who also found differences in microbial community structure as a function of temperature using a Biolog © technique and DGGE analyses respectively. Results of Konopka *et al*. (1999) and LaPara *et al*. (2000c) suggest that the bacterial species richness decreases with increasing reactor temperatures. This could not be confirmed in the present research but this is an interesting phenomenon that could possibly explain the differences in removal of soluble COD between mesophilic and thermophilic treatment (Chapter 4,5).

In contrast to the expectations, also in the thermophilic reactor large blooms of free swimming, newly developing protozoa occurred. Due to the high temperature conditions and the plug flow type of the reactor, their numbers decreased rapidly with an increasing residence time in the reactor system (Fig. 4). Presumably, protozoal cysts, already present in the wastewater, developed rapidly in the presence of oxygen and the high temperature conditions in the first reactor compartment and died rapidly as well due to the prolonged exposure to 55 °C. The presence of these blooms seemed to be related to the batch of wastewater collected at the mill. Since these protozoa did not become mature, no positive effect of their presence is expected upon effluent turbidity. The results in Fig. 1-colloidal COD and Fig. 3b clearly show the difference in effluent turbidity between mesophilic and thermophilic treatment, as was also described by Tardiff and Hall (1997) and Tripathi and Allen (1999). Addition of inhibitors did affect the protozoal population in the 30 °C reactor but hardly affected the effluent turbidity. Noteworthy is that the inhibitors mainly affected the matured protozoa that were attached to the sludge flocs in the 30 °C reactor. The blooms of newly developing protozoa in the 55 °C reactor still occurred. The effect of a stable population of matured protozoa upon the effluent turbidity was shown to be very small compared to the differences observed between 30 and 55 °C treatment and can thus not account for those differences.

5. CONCLUSIONS

Sludge separation in a thermophilic activated sludge reactor is not as efficient as in a mesophilic activated sludge system. Colloidal material in the thermophilic effluent originates from both the sludge as well as from the influent. COD removal in a thermophilic reactor is lower than in a mesophilic analogue and the differences become larger with an increasing loading rate of colloidals.

Acknowledgements:
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7

BIOSORPTION AND FLOCCULATION PROPERTIES OF MESOPHILIC AND THERMOPHILIC ACTIVATED SLUDGE

ABSTRACT

Thermophilic activated sludge treatment is often hampered by a turbid effluent. Reasons for this phenomenon are so far unknown. In this chapter, the hypothesis of the temperature dependency of the hydrophobic interaction as a possible cause for diminished thermophilic activated sludge flocculation is tested. Furthermore, biosorption capacities and sludge surface characteristics of mesophilic and thermophilic activated sludge were determined. Under anaerobic conditions, biosorption capacities of both sludges were found very low. Only in the presence of oxygen, with biologically active sludge, the differences in flocculation behavior become evident. Flocculation was shown only to occur with the combination of wastewater and viable mesophilic sludge at 30 °C, in the presence of oxygen. Flocculation did not occur in case the sludge was inactivated or when oxygen was absent. Thermophilic activated sludge hardly showed any flocculation, also under mesophilic conditions. Despite the differences in flocculation behavior, sludge hydrophobicity and sludge zeta potentials were almost similar. Using fixed angle reflectometry, the adsorption of wastewater particulates on different flat surfaces was monitored. Adsorption of wastewater components on a hydrophobic surface did not change as a function of temperature between 20-60 °C, invalidating the hydrophobic interaction hypothesis. Theoretical calculations using the DLVO (Derjaguin, Landau, Verweij and Overbeek) theory showed that flocculation is unlikely in all cases due to long range electrostatic forces. These calculations, combined with the fact that flocculation actually does take place at 30 °C, and the invalidation of the hydrophobic interaction, point in the direction of bacterial exo-polymers governing bridging flocculation. Polymer interactions are not included in the DLVO theory and may vary as a function of temperature.

Vogelaar,J.C.T., A. de Keizer, S. Spijker and G Lettinga. Submitted in a shortened version.

NOMENCLATURE

DD	initial degree of dispersion (%)
DF	dispersible fraction (%)
EPS	exo-polymeric substances
G	turbulent shear rate (s^{-1})
K_e	ratio of bacteria in octane phase over water phase (-)
K_{ss}	shear sensitivity constant (%)
OUR	oxygen uptake rate (mg O_2 l^{-1} h^{-1})
ε	dielectrical constant (C V^{-1} m^{-1})
κ	reciprocal thickness of the electrical double layer (m^{-1})
σ_0	surface charge density (C m^{-2})
ψ_0	surface potential (mV)
MLSS	mixed liquor suspended solids (g SS l^{-1})

1. INTRODUCTION

Thermophilic treatment of industrial wastewaters has gained increasing interest in recent years. Especially in the forest industry, due to the increasing water system closure and subsequent higher process water temperatures, there is a need for efficient thermophilic treatment systems (LaPara and Alleman, 1999). The research field is relatively new and fundamental knowledge concerning optimum levels of temperature, pH and dissolved oxygen are still lacking (LaPara and Alleman, 1999). Current literature shows that, amongst others, thermophilic aerobic wastewater treatment is characterized by at least two fundamental phenomena. These are:

1. A lower removal of complex soluble COD compared to mesophilic treatment (LaPara *et al*., 2001b; Chapter 4,5,6).
2. a higher effluent turbidity compared to mesophilic treatment (Tardiff and Hall 1997; Tripathi and Allen 1999; Chapter 5,6)

Thermophilic effluent turbidity is a frequently mentioned phenomenon. Only one research group (Barr *et al*., 1996) was able to obtain a clear effluent using pre-settled bleach kraft mill effluent as reactor influent. In all other investigations concerning thermophilic activated sludge treatment, turbid effluents are reported (Chapter 1).

Effluent colloidal material, causing the turbidity, originates from both the influent as well as from the sludge itself. Under mesophilic conditions, influent colloidals are effectively retained in the activated sludge while under thermophilic conditions, a part is washed through (Chapter 5). DGGE profiles of

PCR amplified 16S rDNA fragments of the thermophilic effluent showed that significant amounts of thermophilic bacteria were present in the effluent as well (Chapter 6). Apparently, bacteria are eroded from the sludge or dispersed growing bacteria are washed out with the effluent. In all cases; a poorer retention of influent colloidal material in the thermophilic sludge, release of cells from the sludge and/or dispersed growth of bacteria, point out that the intracellular bonding between thermophilic bacteria and other floc components is weak under thermophilic conditions, i.e. at least poorer as compared to mesophilic conditions.

So far, no fundamental research has been conducted on this phenomenon but several hypothesis have been proposed: the absence of protozoa at higher temperatures could be a reason for the higher effluent turbidity since protozoa are known to scavenge activated sludge flocs and feed on free living bacterial cells (Ratsak *et al.,* 1996; LaPara and Alleman, 1999). However, recently it was shown that the effects of protozoal grazing upon removal of colloidal COD was only marginal and could thus not account for the observed effluent turbidity (Chapter 6). Another hypothesis is a possible temperature dependency of the hydrophobic interaction (Chapter 5). The hydrophobic interaction is an important factor in activated sludge flocculation (Block, 2001) and may vary as a function of temperature between 0 and 100 °C (Tanford, 1973). Electrostatic interactions are known to be hardly temperature dependent (Lyklema, 1995).

The aim of this paper is to unveil the reason for the thermophilic effluent turbidity on a more fundamental level. This is done via several approaches: 1: the individual role of temperature, sludge type, sludge viability and presence or absence of oxygen on the flocculation process was evaluated in flocculation experiments. 2: biosorption experiments were performed with mesophilic and thermophilic activated sludge to determine the biosorption capacity of both sludge types under aerobic and anaerobic conditions with different wastewaters. 3: the temperature dependence of the hydrophobic interaction and the surface characteristics of the wastewater colloidals were determined using fixed angle reflectometry. 4: mesophilic and thermophilic activated sludge were characterized for their surface characteristics (zeta potential and hydrophobicity), the sensitivity towards shear forces and the sludge particle size distribution.

2. THEORETICAL BACKGROUND

2.1 Introduction

The cohesion of activated sludge flocs and their ability to retain colloidal particles is governed by several types of interactions. The following interactions are suggested in various researches: 1. DLVO (Derjaguin, Landau, Verweij and Overbeek) type of interactions (Zita and Hermansson, 1994), 2. bridging by negatively charged exopolymeric substances (EPS) and/or by polyvalent cations such as

Ca^{2+} or Fe^{3+} (Keiding and Nielsen, 1997; Sobeck and Higgins, 2002), 3. formation of alginate gels in the presence of calcium (Bruus *et al*., 1992) and 4. hydrophobic interactions (Block, 2001). The first three interactions are of electrostatic nature and are used to describe the role of divalent cations in activated sludge flocculation. Recently, it was shown that the bridging theory (2) best describes the role of divalent cations in flocculation of activated sludge (Sobeck and Higgins, 2002). However, for pure cultures of bacteria without fibrils and/or fimbrae, the DLVO theory was found to be valid in describing the initial steps of bacterial adhesion (Van Loosdrecht *et al*., 1989).

2.2 DLVO theory

The DLVO theory (Verwey and Overbeek, 1948) describes long range (a few nm) electrostatic and Van der Waals interactions. It can be used to describe the stability of lyophobic colloids, thus can predict whether flocculation actually occurs. However, it does not include short range interactions that determine the strength of the bonding.

The theory assumes that interactions between colloidal particles in aqueous solutions are determined by two types of forces: Van der Waals forces and electrostatic forces. Van der Waals forces result from interactions between atomic or molecular dipoles while electrostatic interactions arise from charge separation across the particle/solution interface and overlap of the electric double layers of two particles. At a relatively short distance, the attraction energy between two colloidal spheres resulting from the Van der Waals forces is approximately inversely proportional to the distance separating the spheres. The repulsive electrostatic forces between two spheres decreases exponentially with increasing distance between the spheres and depends amongst others upon the zeta potential of the particles and the double layer thickness (Kruyt, 1969). The overall stability of the system is governed by the sum of the attractive and repulsive forces.

In biological systems, most particles are negatively charged. The negative surface charge of the particle is the result of dissociation of end groups on the surface, such as amino ($-NH_2$), hydroxyl (-OH) and carboxyl groups (-COOH). As a result of the negative charge, oppositely charged ions are attracted. A layer of counter ions accumulates at the particle surface, partially neutralizing the surface charge. This layer is called the Stern layer. The potential at the edge of the Stern layer is the Stern potential which is approximately equal to the zeta potential. The zeta potential is the potential at the hydrodynamic plane of shear, which is the potential at the interface between a particle and its attached liquid and the surrounding bulk liquid. In practice, the zeta potential is the parameter that can be measured and which is also the variable quantity that is actually “felt” by other particles in solution. The unit for the zeta potential is milli Volts and expresses the amount of energy required to move 1 unit of charge from the bulk solution to the hydrodynamic plane of shear. Note that the zeta potential is not equal to the surface charge which is expressed as coulomb per square meter.

Due to the electric potential at the hydrodynamic plane of shear, a diffuse layer of positive and negative ions surrounds the particle (Fig. 1a). The concentration of counter ions gradually reduces until it reaches the concentration in the bulk liquid. Along with the reduction in counter ions, also the electric potential drops as a function of the distance from the particle. The rate at which the potential drops depends upon the concentration and charge of indifferent ions in the solution. A higher ionic strength shields the charged particles off from other particles in solution making them able to come in closer contact with each other (Fig. 1b). When these particles are able to come in close proximity of each other, attractive Van der Waals forces can dominate over the repulsive electrostatic forces and flocculation can occur in the secondary or primary minimum (Fig. 1c).

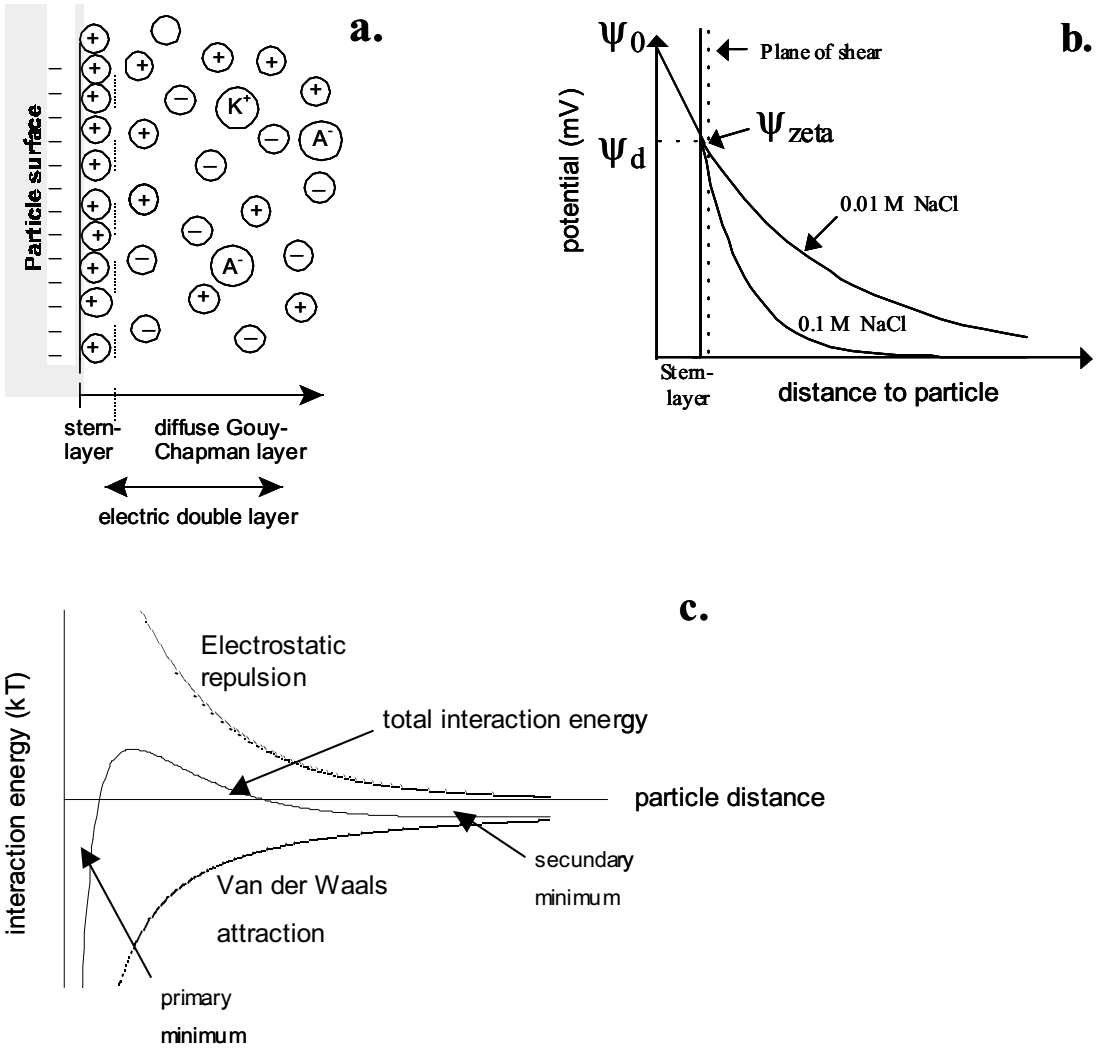

Fig.1a,b,c: a: diffuse Gouy Chapman layer.

b: decline of potential as a function of ionic strength and distance to a particle.

c: sum of repulsive and attractive forces as a function of the distance to a particle.

2.3 Hydrophobic interaction

The hydrophobic interaction is a short range interaction having effect over a distance of at maximum three layers of water molecules, in total approximately 1 nm. The hydrophobic interaction (or hydrophobic effect) is an entropic phenomenon. Water molecules, when faced with non-polar molecules or hydrophobic domains re-orientate in such a way that they can participate in H-bond formation more or less as in bulk water (Tanford, 1973). This is entropically very unfavorable since it disrupts the existing water structure and imposes a new and more ordered structure on the surrounding water molecules. The net result is a clotting of hydrophobic domains and/or hydrophobic molecules in order to minimize the entropy loss of the water molecules. At higher temperatures, due to the increased randomization of water, structure formation around a non-polar molecule plays a less important role and the unfavorable entropy gain becomes smaller. However, the decrease in entropy with increasing temperatures is accompanied by an increase in the enthalpic interaction resulting in only a weak temperature dependence of the Gibbs free energy of the hydrophobic interaction (entropy-enthalpy compensation effect). DLVO interactions (electrostatic interactions and London-Van der Waals interactions) are hardly temperature dependent between 30 and 55 °C at all (Lyklema, 1995).

2.4 Hydrophobic interactions in sludge flocculation

Hydrophobic interactions were shown to be important in activated sludge floc formation (Valin and Sutherland, 1982) and sludge hydrophobicity was positively correlated with sludge settleability (Urbain *et al*., 1993). Liao *et al.* (2001) found that sludge surface properties such as hydrophobicity, surface charge and EPS composition, rather than the absolute amounts of EPS determine bioflocculation. A more hydrophobic and less negatively charged sludge surface corresponded with lower levels of effluent suspended solids in continuous reactor experiments. Adsorption of dispersed bacterial cells to activated sludge was found to be stronger with an increasing cell surface hydrophobicity of the individual cells (Zita and Hermansson, 1997a) while about 80% of the free living bacteria in domestic wastewater (thus not attached to sludge flocs) were shown to have a hydrophilic surface (Zita and Hermansson, 1997b). Similar results were obtained by Olfson *et al.* (1998). Using FISH techniques, Nielsen *et al.* (2001) were able to locate *in situ* hydrophobic structures within activated sludge flocs. They showed, using CTC staining, that the most hydrophobic surfaces were produced by very active cells.

Research with pure cultures of various types of bacterial strains showed that the metabolic state of the bacteria influences their cell surface properties, although the results do not always seem consistent. In some cases, starvation conditions led to a decreased cell surface hydrophobicity (Wrangstadh *et al.*, 1986; Ljungh and Wadstrom, 1995; Jana *et al*., 2000) while in others it did not or opposite effects were observed (Ascensio *et al*., 1995; Castelanos *et al*., 2000; Watanabe *et al.*, 2000). However, in all

cases, a more hydrophobic surface led to a stronger adhesion of the bacteria to the respective carrier materials (an artificial hydrophobic surface, wound tissue, plant roots, activated sludge flocs).

2.5 Working hypothesis

It seems that bacteria can to some extent regulate their adhesion to certain surfaces depending on the environmental conditions. Combining the fact that cells may change their surface properties depending on their metabolic state and the fact that hydrophobic cells adsorb better to for instance activated sludge flocs leads to the hypothesis that supply of substrate and oxygen to activated sludge increases the cell surface hydrophobicity resulting in a stronger adsorption of the cells to the flocs and providing a better adsorption surface for colloidal particles. However, under thermophilic conditions, the Gibbs free energy gain due to the hydrophobic effect might be smaller resulting in a lower sorption of colloidals to the flocs and a weakening of the floc structure.

3. MATERIALS AND METHODS

3.1 Wastewater description

Anaerobic pretreated paper process water, as described in Chapter 4 was used in all experiments and is referred to as "wastewater". In some batch experiments, municipal wastewater, collected freshly from the Bennekom WWTP, The Netherlands was used as a reference wastewater, and is referred to as “sewage".

3.2 Activated sludge

Mesophilic and thermophilic activated sludge was obtained from two similar lab-scale reactors treating wastewater at 30 and 55 °C (Chapter 5). Both reactors were operated at 12 (h) hydraulic retention time and 10 days sludge retention time. Activated sludge from a low loaded (0.1 g COD g $MLSS^{-1}$ day^{-1}) completely mixed pilot scale activated sludge reactor treating sewage was used as a reference sludge. The system was under continuous aeration, nitrification took place but no denitrification nor bio-P removal.

3.3 Oxygen uptake rate (OUR) measurements

Batch respirometric experiments were performed as described in Chapter 3.

3.4 Analytical methods

Chemical Oxygen Demand (COD) was measured according to APHA (1995). COD was measured over four wastewater fractions: total, suspended, colloidal and soluble. Paper filtration (Schleicher and

Schuell 595½ folded paper filters, pore size 4.5 μm was used to distinguish between the total and the suspended fraction. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The colloidal fraction was calculated as the difference between the soluble fraction and the paper filtered fraction. It should be mentioned that in the wastewater fractionation, colloidals are regarded as particles in-between 0.6 and 4.5 μm while from a colloid chemical point of view, colloids can be of nanometer size. In the fixed angle reflectometry experiments (3.7), the membrane filtered fraction is still supposed to contain colloidals. Sulphide (S^{2-}) was measured according Trüper and Schlegel (1964). Mixed liquor total- and volatile suspended solids were measured according to the Dutch normalized standards (NEN 6621). The optical density (OD600) was measured on a Milton Roy Spectronic 601 spectrophotometer.

3.5 Flocculation tests

These tests were conducted to asses the individual role of the sludge type and viability, the temperature and the presence or absence of oxygen on the flocculation process. Sludge and wastewater were brought together under different experimental conditions and flocculation was measured as the decrease in the turbidity of the paper filtered sludge/water mixture. 100 ml serum bottles were filled with 10 ml wastewater and 5 ml of mesophilic or thermophilic sludge. Sulphide, originally present in the wastewater was removed in advance by stripping with nitrogen gas. The gas phase in the bottles consisted of air or a mixture of N_2/CO_2. Incubation took place for a 24 hour period at 30 or 55 °C in an orbital shaker. Tests were conducted with viable or inactivated sludge samples. Sludge inactivation took place by gamma irradiation (25 kGray, Gammaster, The Netherlands) or $HgCl_2$ treatment (Wolf and Skipper, 1994). The latter inactivation was conducted by incubating the sludge for a 24 h period in a 50 mg l^{-1} $HgCl_2$ solution with sufficient air in the headspace to avoid anaerobic conditions. Blanks with solely 15 ml wastewater were incubated as well. Sampling took place at t=0, t=1, t=6 and t=24 h by opening the serum flasks and measuring the OD600 of the paper filtered sludge/wastewater mixture. The experiments were performed in duplicate.

3.6 Biosorption assays

These assays were conducted to determine the biosorption capacity of mesophilic, thermophilic and municipal activated sludge under anaerobic and aerobic conditions. A fixed volume of 800 ml activated sludge was brought in a cylindrical baffled vessel (12 cm diameter, 21 cm height) in which the temperature was maintained at 30 or 55 °C or at room temperature respectively. A mixture of raw wastewater and treated mesophilic or thermophilic effluent was subsequently added to obtain a final volume of 2 liters. Gentle mixing took place with a Heidolph electronic stirrer (RZR 2051 Control) equipped with a BR11 paddle. Sampling took place at t=0, 5, 10, 20, 40 and 60 min. Biosorption was measured as the decrease in soluble and in colloidal COD in the sludge/water mixture. Control

experiments were performed with sewage and municipal activated sludge to check whether the experimental set-up would yield results according to reported literature data.

Three different types of adsorption assays were performed, two types of assays with mesophilic or thermophilic sludge under anaerobic (1) and aerobic (2) conditions, the third type was the control experiment. In assay 1, raw or diluted wastewater was mixed with sludge under a nitrogen headspace. Sulphide in the wastewater was removed beforehand by stripping the solution with nitrogen gas. Assay 2 was similar to the previously described assay except that pressurized air was sparged through the sludge-wastewater mixture thus biodegradation could prevail. Assay 3 was the control experiment with municipal activated sludge and pre settled sewage conducted at room temperature under a nitrogen headspace.

3.7 Fixed angle reflectometry

The aim of these experiments was to characterize both wastewater and sewage for their hydrophobic or hydrophilic properties and to assess the proposed temperature dependency of the hydrophobic interaction. Reflectometry is an optical technique used to detect the adsorption of molecules from a solution onto a macroscopically flat surface (Cohen Stuart and De Keizer, 2001). The amount of wastewater or sewage colloidals that adsorbs on a flat surface of a known composition was measured in time. The surface characteristics of the colloidals can to some extent be determined by measuring their adsorption on different surfaces: a negatively charged, a positively charged and a hydrophobic surface. Furthermore, the temperature dependency of the hydrophobic interaction was validated by measuring the adsorption of colloidals on a hydrophobic surface as a function of temperature.

In fixed angle reflectometry the reflectivity at the Brewster angle is measured. In this study, it is combined with a stagnation point flow cell indicating that there is actually no liquid flow at the point where adsorption is measured, and thus the flux of molecules towards the adsorbing layer is diffusion controlled. The adsorbed amount is measured on-line and is obtained from changes in reflectivity's of the parallel and perpendicular components of a laser beam (wavelength 632.8 nm). The adsorption is proportional to the reflectometer signal which can be converted into an adsorbed amount according to Abeles' method. A conversion factor is used depending on the properties of the solvent, the adsorbing material and the adsorbing surface itself. As an estimate of the refractive index increment of the adsorbing material a value of 0.163 was used, which is similar to the value for CMC (carboxy methyl cellulose). For more detailed information concerning this technique, the reader is referred to Hecht (1987) or Cohen Stuart and De Keizer (2001).

The adsorbing material consists of a totally flat silicon wafer (Si) with a silica layer (SiO_2) on top on which the adsorbate (for instance sewage colloidals) adsorb. The silica layer itself is negatively charged and can be modified by for instance silane derivates (Giesbers, 2001). Three different surfaces were made by different modifications: 1. a negatively charged surface was obtained by using

a normal silicon wafer with a bare silica layer, 2. a positively charged surface was obtained by adsorbing QNHEC (quaternary ammonium hydroxy ethyl cellulose), a positively charged polymer onto the before mentioned silica wafer (1) and 3. a hydrophobic surface was made by placing the silicon wafer (1) in a 1% DCDMS (dichloro dimethyl silane) solution in water free toluene (Maoz and Sagiv, 1984; Giesbers, 2001). DCDMS reacts with the silica layer on the surface resulting in a methylation of the silica surface (Fig.2).

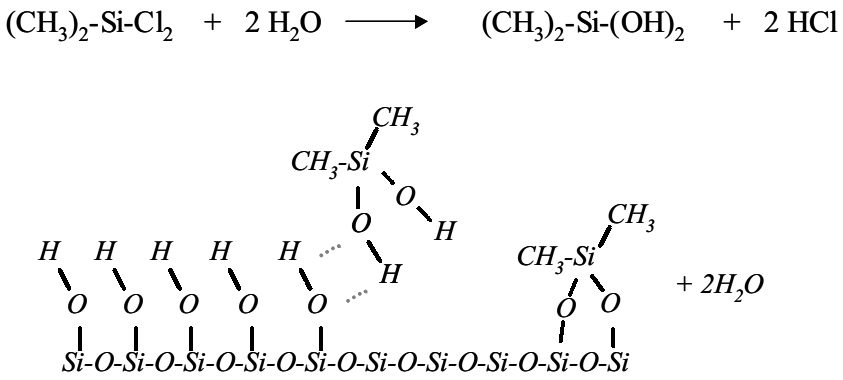

Fig. 2. Methylation of a silica surface with DCDMS (after Giesbers, 2001).

Two series of adsorption experiments were conducted: The first series was conducted at room temperature with three different wastewater and or sewage fractions (raw sample, paper filtered sample and membrane filtered sample) and the three before mentioned adsorption surfaces. The wastewater was diluted 50 times in 50 mM NaCl, pH 7.5. Sewage samples were diluted 5 times in the same solvent.

The second series of adsorption experiments were conducted in the temperature range 20-60 °C with only the membrane filtered fraction of both wastewater and sewage. In these experiments, the positively charged surface and the methylated hydrophobic surface were used. A solution of negatively charged silica particles (Ludox AS-40) was used as a reference material in the experiments with the positively charged surface.

3.8 Sludge characterization

Mesophilic and thermophilic activated sludge were characterized for their surface characteristics (zeta potential and hydrophobicity), the sensitivity towards shear forces and the sludge particle size distribution.

The zeta potential of the non-settleable fraction of the sludge and water samples was measured in triplicate using a Malvern Zetasizer 2000. Bacterial cells were liberated from the sludge matrix by 0, 5, and 10 minutes sonification (Elma Transsonic T700). Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured as an indicator for cell lyses during sonification according to Lessie

and Van der Wijk (1972). The zeta potential was determined for all sludges, reactor influent (wastewater), and both reactor effluents and for pre settled sewage. The ionic strength of the solutions containing the particles was constant during the measurements.

Sludge hydrophobicity was measured by a modified version of the MATH test in which the partitioning of bacteria from the sludge or water sample between n-octane and water is measured. Sludge samples were washed by centrifuging (1000 g, 10 min) and re-suspending the pellets in demineralized water in three repetitive sequences after which the sludge was dispersed by sonification (0, 5 and 10 min). Large particles were removed by sedimentation (90 min, 2 °C) and the suspension was again centrifuged (1000 g, 15 min) and re-suspended in demineralized water. This bacterial suspension was used in the hydrophobicity tests as described by Guellil *et al*. (1998). Application of this method yields a K_e value which is the ratio of biomass in the octane phase over the amount of biomass in the water phase.

Shear sensitivity of the activated sludges was determined in a standardized shear test (Mikkelsen and Keiding, 1999) in which sludge is sheared by intensive mixing in a baffled vessel while the turbidity of the supernatant is measured in time. Supernatant turbidity increases as a result of erosion of cells from the flocs. The experimental set-up was similar to the biosorption assays but stirring speeds were higher. All experiments were conducted at 4 °C to minimize the biological activity, the VSS concentration in the batch vessel was in all cases 4 g l^{-1} and tests were conducted at shear rates (G) of 400, 800, 1200 and 1600 s^{-1}. Supernatant turbidity was measured directly after centrifugation (2 min, 2200 rpm) on a WTW Turb 550. From the course of the turbidity in time, the Kss (shear sensitivity constant), DD (initial degree of dispersion) and DF (dispersible fraction) were calculated according to Mikkelsen and Nielsen (2001). The initial degree of dispersion is defined as the amount of dispersed biomass over the total amount of suspended solids (%) when a sample is taken from the reactor. The dispersible fraction is the sludge fraction that is at maximum dispersible under very high shear conditions (%) whereas the shear sensivity constant is the ratio of dispersed biomass over total solids at G=800 s^{-1} (%).

Sludge particle size distribution was measured using a Coulter LS 230 particle size analyzer (measuring range 0.04-2000 μm). Sludge samples were taken from the reactors, diluted in distilled water and subsequently measured at room temperature.

4. RESULTS

4.1 Flocculation tests

The results of these tests show that flocculation only occurred with the combination of viable, mesophilic sludge, at 30 °C, in the presence of oxygen as measured as a significant reduction in supernatant turbidity (Fig. 3a). The combination of viable thermophilic sludge, in the presence of

oxygen at 55 °C only provided a slight turbidity removal (Fig. 3b). Inactivation of the sludge using gamma irradiation and $HgCl_2$ treatment gave very similar results in the flocculation tests, viz, little or no turbidity removal in all cases. Mixing viable mesophilic sludge with wastewater in the absence of oxygen hardly gave any removal of colloidal COD from the water phase either (Fig. 3c).

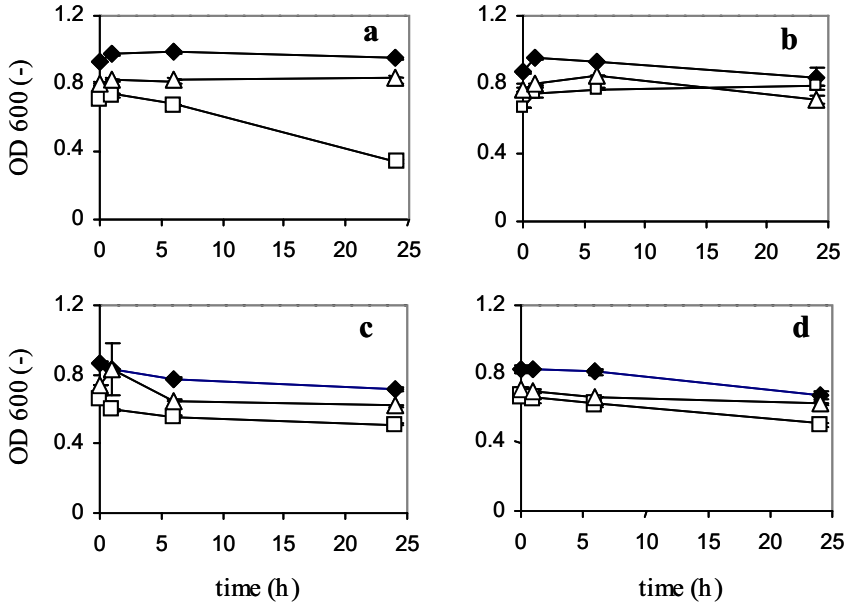

Fig. 3 a-d: Batch flocculation experiment with viable sludge. Error bars in the graph indicate standard deviation, duplicate samples. (♦) wastewater, (□) wastewater + mesophilic sludge, (Δ) wastewater + thermophilic sludge. 3a: T=30 °C, air headspace 3b: T=55 °C, air headspace 3c: T=30 °C, N_2/CO_2 headspace 3d: T=55 °C, N_2/CO_2 headspace

4.2 Biosorption assays

Representative results of the assays 1 and 3 are shown in Fig. 4, depicting the course of soluble and colloidal COD over time during 60 min. In the first assay, in which mesophilic or thermophilic sludge was mixed with wastewater under anaerobic conditions, hardly any biosorption took place. At 30 °C, a slight biosorption of soluble COD occurred while under both temperature conditions the concentrations of colloidal COD in the liquid increased (Table 1). The amount of adsorbed COD per gram MLVSS in the different assays is presented in table 1.

In the control biosorption experiments with sewage and municipal activated sludge (assay 3), on average , 20 ± 6 mg COD g $MLVSS^{-1}$ was removed from the liquid phase under floc loadings varying between 67 and 367 mg COD g $MLVSS^{-1}$ (Table 1). These results show that biosorption was limited in these experiments as well but it constituted a relatively larger part of the initial sewage COD as compared to the experiment with wastewater (Fig. 4 a-c).

Table 1: Biosorption of colloidal, soluble and total COD per gram MLVSS. A negative sign indicates an increase in COD. (assay 1: N_2 anaerobic, no sulphide present; assay 2: aerobic, no sulphide present; assay 3: anaerobic, no sulphide present, municipal activated sludge and sewage).

	Mesophilic			*Thermophilic*			
assay	mg COD-tot g VSS^{-1}	mg COD-col g VSS^{-1}	mg COD-sol g VSS^{-1}	mg COD-tot g VSS^{-1}	mg COD-col g VSS^{-1}	mg COD sol g VSS^{-}	No. of exp.
1	-79 ± 32	-91 ± 36	10 ± 4	-38 ± 20	-28 ± 28	-13 ± 17	3
2	64 ± 1	4 ± 4	61 ± 4	-1 ±41	11 ± 11	-12 ± 30	2
3	20 ± 6	5 ± 5	15 ± 9				8

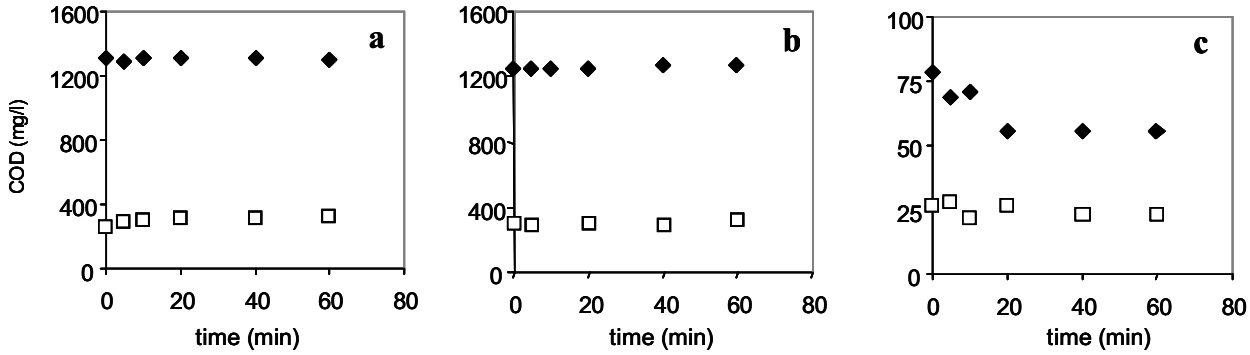

Fig. 4: Biosorption of colloidal (□) and soluble COD (♦) as a function of time. Sulphide is previously removed from the wastewater and nitrogen is bubbled during the experiment, thus anaerobic conditions prevail.
4a: Mesophilic sludge with wastewater (assay 1), 4b: Thermophilic sludge with wastewater (assay 1), 4c: municipal activated sludge with sewage (assay 3).

In assay 2, aeration took place with pressurized air, thus actual biodegradation could occur during the experiment. Table 1 shows that under these conditions, colloidal COD removal is still very limited at both temperatures. At 30 °C, a significantly higher removal of soluble COD (64 ± 1 mg COD g $MLVSS^{-1}$) took place as compared to the first assay. Prolonging the experiment for another 23 hours showed that after the first 2 hours of aeration, when all soluble easily biodegradable COD was removed, levels of colloidal COD dropped at 30 °C while they remained stable at 55 °C (results not shown). At 30 °C, after 24 hours of biodegradation, all colloidal particles were removed from the liquid resulting in a clear supernatant, while at 55 °C colloidal COD levels remained similar throughout the experiment and the supernatant remained turbid.

Assay 2 was repeated in a respirometer under both temperature conditions. Fig. 5a-d shows the oxygen uptake rate (OUR) and COD removal of the different fractions in time. In this experiment, at time zero, 0.75 liters of sludge and 0.75 liters of wastewater without sulphide were added to the respirometer containing 2 liters of mesophilic reactor effluent. The initial OUR was high in both cases and leveled off rapidly. At 30 °C, the OUR remained stable at 14 mg O_2 $l^{-1}h^{-1}$ for 8 hours during

which the levels of colloidal COD in the mixed liquor dropped (Fig. 5a). After 7 hours, the colloidal COD concentration reached zero and the OUR decreased exponentially. From the results of Fig. 5 a and c it follows that colloidal COD removal at 30 °C is related to a simultaneous oxidation of this COD fraction.

At 55 °C, the total amount of oxygen consumed by the sludge was much lower as compared to 30 °C (and COD removal was less as well), although similar amounts of sludge and wastewater were applied. Hardly any removal of soluble and colloidal COD was found while the colloidal COD and turbidity (measured as OD600) increased slightly. In the biosorption assay in the presence of oxygen (assay 2, not performed in the respirometer but in the baffled vessel under low shear conditions), the colloidal COD and turbidity did not increase but remained constant.

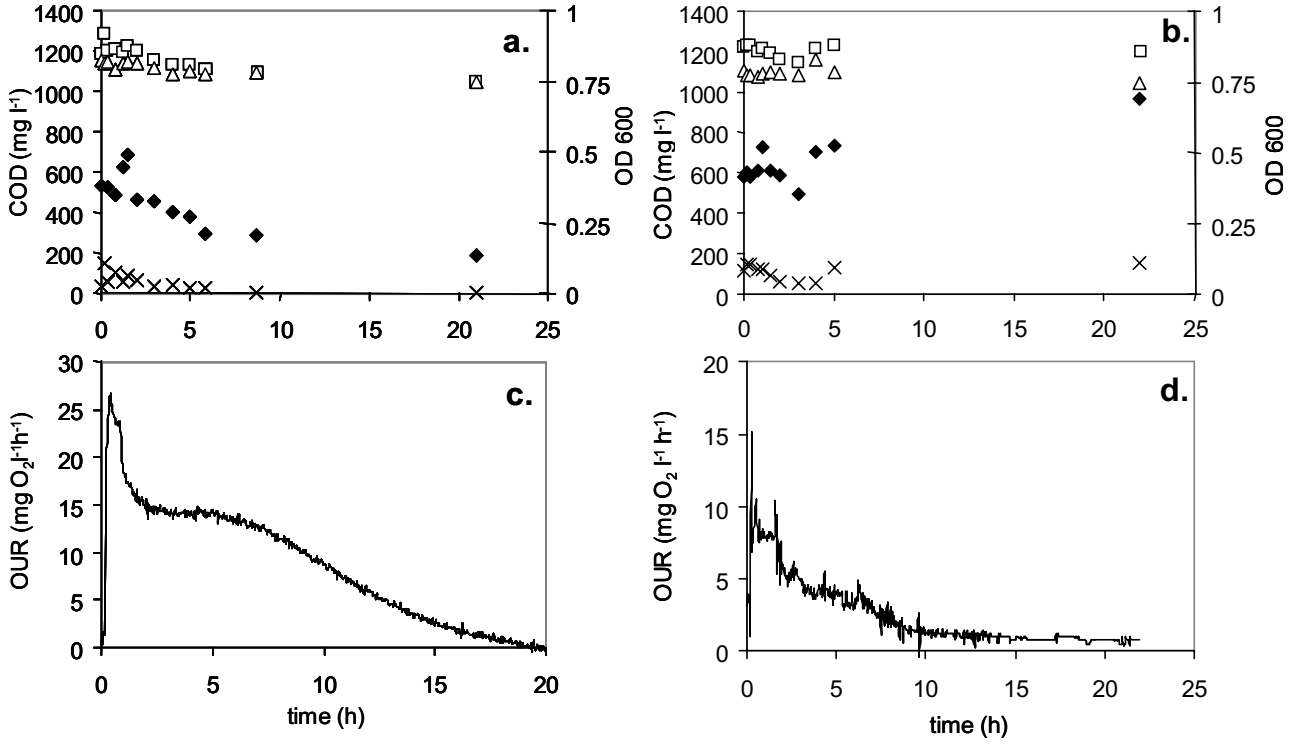

Fig. 5a-d: Course of COD removal during a batch biodegradation experiment during which the OUR was measured simultaneously at 30 (a,c) and 55 °C (b,d). (□) paper filtered COD, (Δ) soluble COD, (×) colloidal COD, (♦) OD 600 of the paper filtered sample.

4.3 Fixed angle reflectometry

Fig. 6 a-d depicts four representative graphs of the adsorption of colloidals from wastewater and sewage onto a flat silica surface in time. Experiments with paper filtered fractions and with the original samples gave similar results but the reflectometer signal contained more noise due to the presence of larger particles in the raw samples. In Fig. 6 a,b, firstly QNHEC is adsorbed on a bare negatively charged silica surface resulting in the first plateau of approximately 1.5 mg m^{-2} of adsorbed

polymer. After coverage of the surface with QNHEC, a membrane filtered fraction of wastewater or sewage was adsorbed on the positively charged surface. Fig. 6a shows that only a minor amount of the membrane filtered wastewater adsorbed on the positively charged surface (0.33 mg m^{-2}) while the membrane filtered fraction of sewage showed a large adsorption (4.3 mg m^{-2}, Fig 6b).

After the second plateau was reached, the reflectometer cell was rinsed with pure solvent (50 mM NaCl). A minor desorption of wastewater material occurred from the silica surface (Fig. 6a) while a larger desorption took place of adsorbed sewage (Fig. 6b).

Fig. 6 c,d show the adsorption of membrane filtered wastewater and sewage on a methylated, hydrophobic surface. Adsorption proceeded rapidly for both samples and a significant adsorption took place in both cases. On average, 0.7 ± 0.14 (mg m^{-2}) of wastewater colloidals adsorbed on the hydrophobic surface (Fig. 6c) which was twice the amount adsorbed on the positively charged surface (Fig. 6a). Rinsing the reflectometer cell with solvent showed a slightly higher desorption from the hydrophobic surface compared to the hydrophilic surface. For the sewage sample, this effect was even more pronounced.

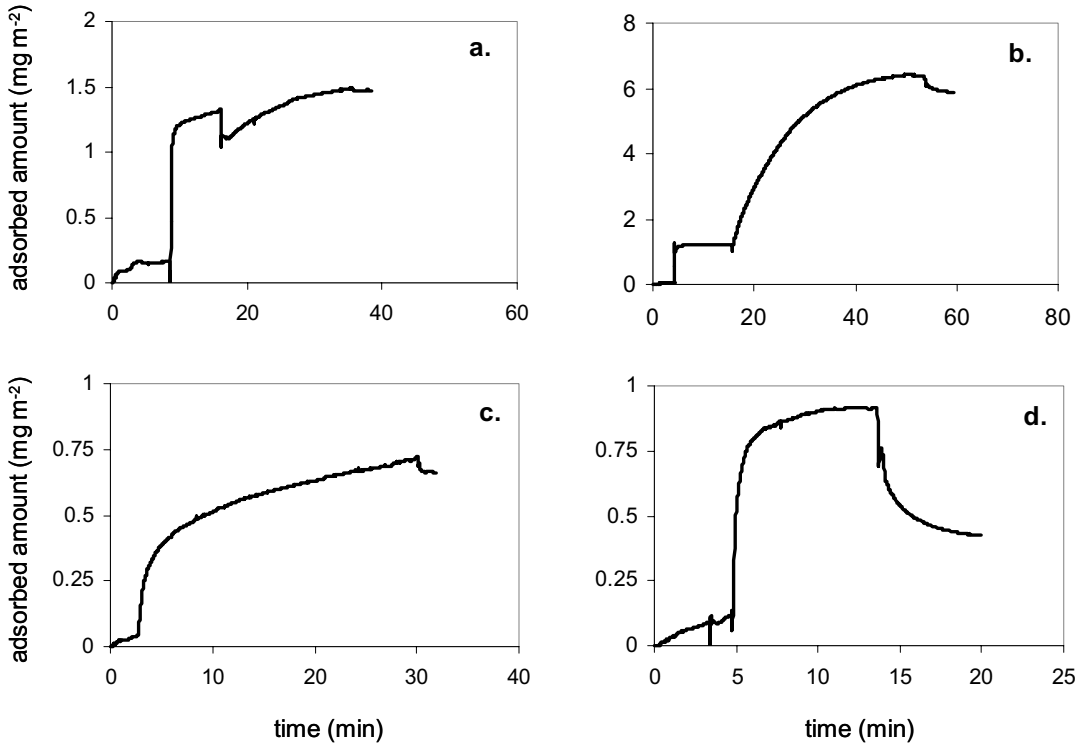

Fig. 6 a-d: Adsorption of a membrane filtered fraction of wastewater or sewage onto a modified silica surface at room temperature.

a: wastewater onto a positively charged surface
b: sewage onto a positively charged surface
c: wastewater onto a hydrophobic surface
d: sewage onto a hydrophobic surface

Experiments were also conducted using a bare negatively charged silica surface. No adsorption of wastewater nor sewage colloidals took place on the negatively charged surface (results not shown).

Table 2 a,b shows the adsorbed amount of different wastewater or sewage fractions on the positively charged and the hydrophobic surface and the initial rates of adsorption. The initial adsorption rates were calculated over the first 60 s of adsorption for the different samples (Table 2a,b). Comparison of the adsorption rates revealed that adsorption proceeded more rapid with sewage colloidals than with wastewater on both surfaces while both the sewage and the wastewater colloidals adsorbed more rapid on a hydrophobic surface compared to a hydrophilic surface.

As mentioned before, significantly more sewage colloidals adsorbed on the positively charged surface when compared to wastewater colloidals. The amount of sewage material that desorbed was also much higher than for wastewater. Furthermore, the amount of sewage colloidals that desorbed from the hydrophobic surface was similar to the amount that desorbed from the positively charged surface.

Table 2a. Sorption of different wastewater and sewage fractions onto a positively charged silica surface at room temperature. tot: total fraction, pf; paper filtered fraction, sol: membrane filtered fraction.

sample	sorption [mg m^{-2}]	desorption [mg m^{-2}]	k-ini [mg $m^{-2}min^{-1}$]	No. of exp.
wastewater -tot	0.5 ± 0	0.06 ± 0	0.1 ± 0.04	2
wastewater-pf	0.54 ± 0.17	0.06 ± 0	0.07 ± 0.02	2
wastewater-sol	0.33 ± 0.03	0.01± 0	0.05 ± 0.02	3
sewage-settled	3.6 ± 0.4	0.55 ± 0	0.47 ± 0.04	2
sewage-sol	4.3 ± 0.5	0.45 ± 0.04	0.51 ± 0.02	3

Table 2b. Sorption of different wastewater and sewage fractions onto a methylated silica surface at room temperature. tot: total fraction, pf; paper filtered fraction, sol: membrane filtered fraction.

sample	sorption [mg m^{-2}]	desorption [mg m^{-2}]	k-ini [mg $m^{-2}min^{-1}$]	No. of exp.
wastewater -tot	0.65 ± 0.03	0.13 ± 0.05	0.27 ± 0.1	2
wastewater-pf	0.72 ± 0.27	0.04 ± 0.02	0.35 ± 0.05	3
wastewater-sol	0.7 ± 0.14	0.02 ± 0.02	0.23 ± 0.08	3
sewage-tot	1.1	0.8	0.9	1
sewage-settled	1.1 ± 0.05	0.65 ± 0.01	1.95 ± 0.25	2
sewage-sol	0.98 ± 0.1	0.44 ± 0.9	1.3 ± 0.4	3

Fig. 7 and 8 depict the adsorbed and desorbed amounts of wastewater and sewage on both surfaces as a function of temperature. Both the amount of adsorbed wastewater material and of the silica particles (Ludox) on the positively charged silica surface remained constant as a function of temperature and hardly any desorption took place (Fig. 7 a,b). Adsorption of components from the sewage increased as a function of temperature just as the desorption increased. On the hydrophobic surface, adsorption of

wastewater and sewage remained relatively constant as a function of temperature while desorption of sewage increased (Fig. 8a,b).

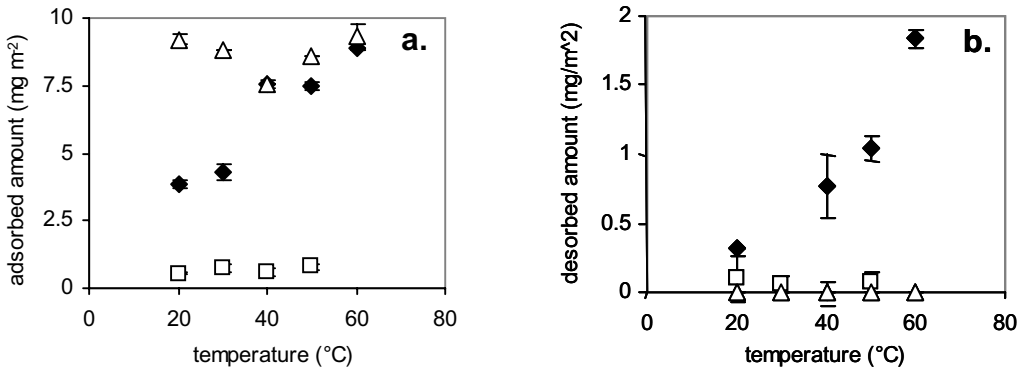

Fig. 7 a,b. Total adsorbed (a) and desorbed (b) amount of wastewater (□), sewage (♦) and Ludox (Δ) on a positively charged silica surface as a function of temperature.

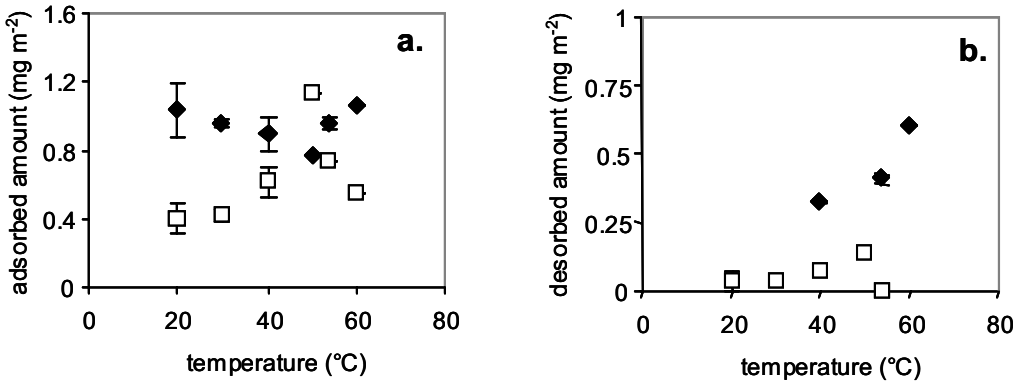

Fig. 8 a,b. Total adsorbed (a) and desorbed (b) amount of wastewater (□) and sewage (♦) on a methylated hydrophobic silica surface as a function of temperature.

4.4 Sludge characterization

The results of sludge hydrophobicity and zeta potential measurements are listed in Table 3. There was a slight but minor difference in de zeta potentials of the mesophilic and the thermophilic sludge (-15.7 ± 0.5 mV and -14.0 ± 0.1 mV, respectively). Sonification of the sludges to release the cells did not affect the measured zeta potentials and no G6PDH activity was measured, thus no cell lyses occurred. Zeta potentials of wastewater and sewage colloidals were respectively -9.9 ± 0.1 mV and –18.9 ± 2.4 mV. The zeta potentials of colloidal material in the mesophilic and the thermophilic effluent were very similar to that of the sludges itself, -16.5 ± 0.4 mV and -15.8 ± 2.1 mV, respectively. Hydrophobic properties of bacteria liberated from mesophilic and thermophilic sludge were found very similar while dispersed biomass from municipal activated sludge was found less hydrophobic than the sludges cultivated on wastewater (Table 3).

The results of the shear sensivity tests are listed in Table 4. Thermophilic activated sludge was clearly shown to be in a more dispersed state as compared to mesophilic sludge; 32% of the sludge total

solids were dispersible under extremely high shear conditions while 11 % was already in a dispersed state when a sample is taken from the reactor. The values were significantly lower for mesophilic sludge (21 % dispersible and 2.9 % initially dispersed) while municipal activated sludge was found to be most stable: 0.6% was initially dispersed while 7% was dispersible under extremely high shear conditions.

Table 3. Zeta potential and hydrophobicity of mesophilic, thermophilic and municipal activated sludge after 5 min sonification.

	Mesophilic sludge	Thermophilic sludge	Municipal activated sludge
zeta potential (mV)	-15.7 ± 0.4	-14.0 ± 0.1	-13.7 ± 0.4
Ke (-)	2.17 ± 0.19	1.93 ± 0.06	0.52 ± 0.1

Table 4. Shear sensivity constants for three sludge types.

	Mesophilic sludge	Thermophilic sludge	Municipal activated sludge
DF (%)	21	32	7
DD (%)	2.9 ± 0.8	11 ± 1.5	0.6 ± 0.07
K_{ss} (%)	12	21	2.3

The relative volume distribution of the sludge particles as a function of their size is depicted in Fig. 9. Thermophilic sludge contained a significantly higher number of small particles compared to both the mesophilic and the municipal activated sludge. The volume percentage of sludge particles with a diameter smaller than 5 µm was 16 % for thermophilic sludge compared to 4% for mesophilic sludge and 1% for municipal activated sludge. The volume percentage of particles larger than 25 µm that can be regarded as flocs were respectively 56%, 84% and 94% for thermophilic, mesophilic and municipal activated sludge.

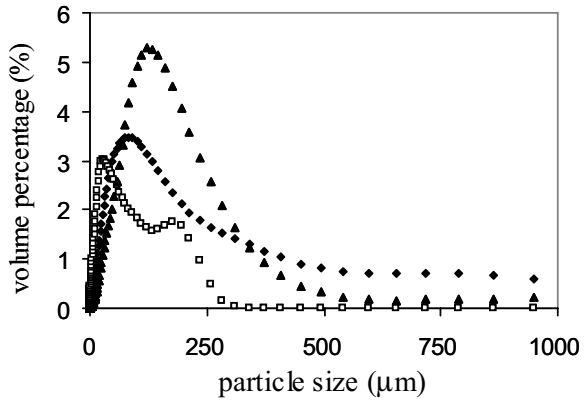

Fig. 9. Relative volume distribution as a function of the particle size. (♦) mesophilic sludge, (□) thermophilic sludge, (▲) municipal activated sludge.

5. DISCUSSION

5.1 Flocculation tests

Flocculation, measured as a decrease in turbidity of the paper filtered sludge/water mixture, only took place when wastewater was incubated with active mesophilic sludge, at 30 °C, in the presence of oxygen. Mesophilic sludge, exposed to 55 °C did not give a significant reduction in turbidity. The removal of colloidal COD is thus not solely linked to the surface characteristics of the mesophilic sludge, but it also needs to be in an active state because when exposed to a temperature of 55 °C, the sludge becomes inactivated. This finding was confirmed by the experiments using radiated and $HgCl_2$ treated sludge. Incubating thermophilic sludge at 30 °C did not give a significant turbidity removal either, indicating that the difference in turbidity removal between 30 and 55 °C is not just a pure temperature effect.

The role of oxygen in the removal of colloidal COD can be linked either to its function as an electron acceptor or to the fact that oxygen affects the sludge characteristics via a change of redox conditions. Changing redox conditions will affect the oxidation state of certain metal ions such as Fe^{2+}/Fe^{3+}. Ferric iron has a higher valence than ferrous iron and is thus able to form stronger bonds with charged EPS components. Nielsen and Keiding (1998) showed that Fe^{3+} in activated sludge can be reduced to Fe^{2+} under anaerobic conditions and that the reduction of Fe^{3+} is associated with a decrease in floc strength. However, results of the present study have shown that inactivated sludge did not flocculate with wastewater when oxygen was present, regardless of the sludge type or temperature. Only when oxygen and viable mesophilic sludge was present, flocculation of sludge and wastewater colloidals took place. Consequently, the necessity of oxygen in bioflocculation is most likely linked to its function as electron acceptor and as a prerequisite for growth.

5.2 Biosorption assays

In the anaerobic assays, under both temperature conditions, an increase in the supernatant colloidal COD was found, confirming the previous findings of the flocculation experiment (5.1). The increase in colloidal COD can be the result of desorption of adsorbed material from the sludge flocs or erosion of cells from the flocs due to shearing. We believe this to be a combination of both phenomena, with floc shearing being of more importance under thermophilic conditions, as based on unpublished data and the shear sensivity experiments (4.4).

The biosorption capacities of both sludges were low under anaerobic conditions (assay 1) and showed little if any difference in behavior. Only when actual biodegradation occurred in assay 2, the differences became evident. The respirogram obtained at 30 °C shows that removal of colloidal COD was linked to the actual biodegradation of this wastewater fraction. This suggests that either in the presence of oxygen bacteria in the mesophilic sludge are able to change their surface characteristics

quite rapidly, i.e. providing more binding sites for colloidals to attach upon or that due to bioconversion, hydrolysis products are removed from the floc matrix and part of the sludge surface area again becomes available for attachment of new colloidals. In the thermophilic assay, the amount of oxygen consumed was smaller compared to the mesophilic assay thus also less colloidal COD was hydrolyzed and eventually converted. This would in turn also affect the biosorption behavior.

The results from assay 3, the biosorption of sewage by municipal activated sludge, were in good accordance with literature data. Rensink and Donker (1991) found values between 2 and 30 (mg COD g $MLVSS^{-1}$) after 30 minutes of biosorption with floc loadings between 20 and 100 (mg COD g $MLVSS^{-1}$). They used sewage of the same municipal wastewater treatment plant as we used in this study. Guellil *et al.* (2001) found values varying between –40 and 100 (mg COD g $MLVSS^{-1}$) after 60 min of biosorption with imposed floc loadings ranging between 40 and 250 (mg COD g $MLVSS^{-1}$). This shows that the experimental set-up that we used to measure biosorption produces representative results.

5.3 Fixed angle reflectometry experiments

5.3.1 Room temperature experiments:

Results of the fixed angle reflectometry experiments at room temperature provide additional information concerning the characteristics of colloids in both sewage and wastewater. However, interpretation of the results is difficult since both samples are heterodisperse systems containing a mixture of particles differing in size, charge and hydrophobicity. In most of the reflectometer experiments, a membrane filtered fraction of the wastewater or sewage was used. This gave the same adsorption curves as for the raw and paper filtered samples but with fewer disturbances. This observation can be attributed to the fact that smaller particles in the membrane filtered fraction have a higher diffusion rate compared to the bigger sized particles in the unfiltered fractions and will adsorb first on the silica surface, as they will reach the surface sooner. The bigger particles were not able to replace the smaller sized particles from the surface and caused some signal noise but did not actually change the adsorbate layer on the silica. This however hampers an easy comparison of these experiments with the biosorption assays since colloidals in the reflectometer experiments are regarded as soluble in the biosorption assays.

Sewage colloidals showed a large and rapid adsorption on both a positively charged and on a hydrophobic surface. Desorption was for both surfaces substantial and almost similar (0.5 mg m^{-2}). Most likely, the desorbing components were surfactants, present in the sewage, that can bind on both a hydrophobic and a hydrophilic surface. In general, adsorption of surfactants is reversible (Torn, 2000).

The total amount of adsorbed components from sewage on the positively charged surface was approximately 9 times higher than the amount of adsorbed wastewater components. It is generally

assumed that saturation in the adsorption only occurs when the charge of the positive surface is fully neutralized by the negative charge of the adsorbing material. A higher amount of adsorbed sewage compared to wastewater thus implies a higher ratio of mass over charge for sewage particles compared to the wastewater particles. For a diffuse electrical double layer in the limit of low potentials, the surface charge density (σ_0) expressed in (C m^{-1}) of the particles is proportional to the surface potential (ψ_0) according to eq. 1 (Kruyt, 1969):

$$\sigma_0 = \varepsilon \kappa \psi_0 \qquad (1)$$

In (1), ε is the dielectric constant of the medium, κ is the reciprocal double layer thickness and ψ_0 is the surface potential (not equal to the zeta potential but a higher zeta potential of a particle does imply a higher surface potential assuming that the distance from the surface to the plane of shear at which the zeta potential is measured is similar in both cases). The amount of mass over charge for a wastewater or sewage particle can be calculated by multiplying the volume of the particle with its density, divided by the surface area multiplied with the surface charge density. For a spherical particle the amount of mass per unit of charge is linearly related to the particle radius and particle density, divided by the surface charge density (eq. 1). The particle density and ε and κ and can be assumed to be identical for sewage and wastewater particles. The absolute value of the sewage zeta potential was markedly higher (-18.9 ± 0.4 mV) as compared to the wastewater zeta potential (-9.9 ± 0.1 mV). This implies that the sewage colloidals need to be a factor 20 larger in diameter to explain the higher amount of adsorbed material. In case a thicker layer of adsorbate develops on the surface, it is also likely that part of the negatively charged adsorbate does not interact with the positively charged surface enhancing the above described effect.

The zeta potential measurements described above and the reflectometry experiments presented in Fig. 6a,c show that the wastewater colloidals were smaller in size, with a lower charge density and were of a more hydrophobic nature than the sewage particles. The latter is evident as the amount of wastewater colloidals that adsorbed on the hydrophobic surface was double the amount that adsorbed on the positively charged surface while for sewage it was the other way around.

For both sewage and wastewater colloidals, adsorption proceeded more rapidly on the hydrophobic surface than on the positively charged surface. This shows that both wastewater and sewage contain more hydrophobic particles compared to charged particles or that charged particles also contain hydrophobic binding sites. Van Loosdrecht *et al.* (1987) have shown that the combination of both a hydrophobic surface and a high surface charge is possible (for instance for bacteria) since the charged groups only require a small percentage of the particle surface area leaving enough space for hydrophobic sites on the surface.

Overall, the reflectometry experiments show that the wastewater contained more hydrophobic, smaller sized colloidals, with a smaller charge density compared to the sewage. In both wastewater and sewage, more particles were of a hydrophobic nature compared to the number of charged particles, or

charged particles contained hydrophobic sites as well. The latter is at least true for surfactants present in the sewage.

5.3.2 Adsorption as a function of temperature

The total amount of wastewater components that were adsorbed on the positively charged surface remained constant as a function of temperature, as for the adsorption of the negatively charged silica particles (Ludox). The latter was expected since both the silica particles and the silica surface are inert and were not supposed to change in their physico-chemical properties with temperature, as for the DLVO interactions themselves. Apparently, the same holds true for the adsorbing wastewater material. However, the amount of adsorbed sewage components increased with temperature, as well as the desorbing amount. Possibly, proteins present in sewage change in their three dimensional structure as a function of temperature, also altering their adsorption properties. The sewage zeta potential was found not to change with the same temperature increase and thus no definite clarification for this effect is available yet. The initial adsorption rates have not been presented as a function of temperature, but over the whole, they increased due to the higher diffusion rates with increasing temperatures.

Adsorption of wastewater and sewage colloidals on the hydrophobic surface was found to be almost independent of temperature (Fig. 8a). This observation shows that the diminished sorption and flocculation properties of the thermophilic sludge cannot be attributed to a lesser adsorption on a hydrophobic surface with an increase in temperature. However, from these results, we could not conclude whether the hydrophobic interaction (the interaction energy) itself became smaller at higher temperatures. For that purpose, more measurements should have been performed with different wastewater concentrations. For the purpose of this research, to examine whether hydrophobic pollutants still bind other hydrophobic sites at elevated temperatures, these measurements suffice.

5.4 Sludge characterization

Comparison of the mesophilic and thermophilic sludge surface characteristics did not yield significant differences in zeta potential and sludge hydrophobicity. Both mesophilic and thermophilic sludge were more hydrophobic than municipal activated sludge (Table 4). From the reflectometry experiments is was evidenced that the wastewater colloidals were of a more hydrophobic nature than the sewage colloidals. The type of substrate thus affects the sludge characteristics. The activated sludge flocs consist of both newly grown biomass as well as wastewater material that is retained in the flocs. Biomass growth was limited due to the small amount of biodegradable COD in the wastewater (Chapter 4) and thus the similarity in sludge and wastewater hydrophobicity is obvious as a large fraction of the floc consists of wastewater components. However, this similarity between sludge and wastewater was not observed regarding the zeta potentials. Zeta potentials of effluent colloidals were

more closely related to the sludges, suggesting that effluent colloidal material arises from the sludge. It should be noted that sludge hydrophobicity and zeta potential were determined from bacterial cells liberated from the sludge matrix by sonification and not from the entire flocs themselves. One may question whether this is representative for the entire sludge floc but better alternatives were not available.

The results of the shear sensitivity measurements, revealing a high dispersed fraction of thermophilic sludge correspond well with these of the particle size distribution measurements and with previous observations showing the presence of dispersed thermophilic biomass in the continuous reactor effluent (Chapter 6). Shear sensitivity is related to the interaction energy between the sludge particles; a higher sensitivity indicates a lower amount of Gibbs energy release upon binding of two particles (Mikkelsen and Keiding, 1999). Aggregation was thus shown to be weaker under thermophilic conditions.

Municipal activated sludge was found more stable as compared to both industrial sludges although the sludge zeta potential was higher and the sludge hydrophobicity was smaller. Based on the DLVO theory, the expectations are just the other way around: a higher zeta potential is expected to result in a higher electrostatic repulsion within the flocs, leading to a higher sludge shear sensivity and consequently smaller floc sizes. Furthermore, both industrial sludges, with seemingly similar surface characteristics (zeta potential, hydrophobicity) had completely different sludge characteristics such as shear sensivity and particle size distribution and were both more sensitive towards shear as compared to municipal activated sludge. These results indicate that other more important factors than solely the zeta potential and sludge hydrophobicity are determining the sludge characteristics.

Mikkelsen and Keiding (2002) observed that activated sludges with high EPS contents were less susceptible towards shear forces and had larger floc sizes than sludges with fewer EPS. They found that a high EPS content also corresponded with a high sludge surface charge and consequently a higher zeta potential. The more stable sludges thus contained more charged exo-polymers. Based on these measurements they conclude that polymer entanglement is a key factor in stabilizing floc structures and is of more importance than electrostatic repulsion within the flocs.

In this research we tried to measure EPS of the different sludges according to a method described by Frølund *et al.* (1996). However, these measurements did not yield reproducible results due to sampling problems from the small lab-scale bioreactors.

5.5 General discussion

Results of both the flocculation and biosorption assays showed that flocculation, measured as colloidal COD removal only took place when actual bioconversion occurred. Colloidal COD removal and flocculation are, from a theoretical perspective, affected by surface characteristics such as sludge

surface charge and sludge hydrophobicity. However, no significant differences in these parameters were found for mesophilic and thermophilic activated sludge.

Results of the fixed angle reflectometry experiments showed that the amount of wastewater components adsorbing on a hydrophobic surface did not decrease at higher temperatures, i.e. the proposed hypothesis concerning the temperature dependency of the hydrophobic interaction could not explain the observed differences in flocculation behavior between mesophilic and thermophilic sludge.

Using the DLVO theory, the total interaction energy between two particles (for instance bacteria) can be calculated as a function of their separation distance, provided that their zeta potentials, diameter and ionic strength of the medium are known (Kruyt, 1969). This calculation was made for a bacterium in an activated sludge floc when encountering a bacterium from the reactor influent (=influent colloidal material). We approximated the interaction between a floc and a bacterium by the interaction between two bacteria since activated sludge flocs are very porous in nature (Li and Ganczarczyk, 1990). As an estimation of the Stern potential, we used the measured zeta potentials of the mesophilic and thermophilic sludge and wastewater colloidals (Table 3), assuming a bacteria diameter of 1 μm, ionic strength of the medium was 0.04 M (estimated by analyzing the ionic composition of the reactor effluent), Hamaker constants were varied from 0.2-2 $*10^{-21}$ J which are estimates for bacterial cells and blood platelets. Calculation of the total interaction energy between two bacteria as a function of their separation distance shows that in theory flocculation is very unlikely. The secondary energy minimum is not deep enough to cause reversible flocculation and flocculation in the primary minimum is hampered by the electrostatic repulsion when particles approach each other more closely (5-9 kT at 2 nm separation distance).

However, flocculation and biosorption experiments showed that flocculation actually did occur (in case of viable mesophilic sludge in the presence of oxygen) while on the basis of theoretical calculations, flocculation is unlikely due to long range electrostatic repulsion. The only long-range interaction not included in the DLVO theory are polymer interactions. Due to polymer adsorption on for instance a bacterium, both stabilization and flocculation of a suspension can occur in other ways than predicted by DLVO interactions alone. Polymers can adsorb in a compact or in a more loosely oriented structure. Polymers that adsorb in a compact structure either improve or worsen colloidal stability directly by changing the original surface characteristics. The original surface is coated with a new polymer surface affecting both the surface charge and the Hamaker constant. This can favor flocculation (a lower surface charge and a higher Hamaker constant or high hydrophobicity) or hamper flocculation (via a higher surface charge).

When the adsorbed polymer is more loosely structured and extends into the solution, two phenomena can occur: 1. bridging flocculation or 2. steric repulsion. The loosely structured polymers are able to penetrate the electrical double layer surrounding the colloidals as they are highly solvated and almost

freely penetrable for counter ions. If both surfaces are not more than partly covered with a polymer layer with a thickness exceeding the electrical double layer thickness, then one and the same polymer may attach to both surfaces and bridge between them. Provided sufficient affinity between the polymer and the particle surface, it causes the particles to aggregate due to bridging flocculation. In case of an excess of polymer on each particle, interpenetration of the polymers results in steric repulsion and subsequent stabilization of the particles in dispersed state in the solution. Steric repulsion arises mainly from the entropy loss encountered by the interacting polymers and is thus temperature dependent. The mechanism of polymer bridging is to some extent similar to the mechanism of the divalent cation bridging theory (dcb theory) as proposed by Sobeck and Higgins (2002). Polymer bridging can take place between the polymer and a cation (dcb theory) but also directly between a polymer and some other surface.

Interactions between polymer coated surfaces are affected by changes in the quality of the solvent (i.e. solvent polymer interaction) which are temperature dependent. A higher solvent quality results in an extension of the polymer into solution while a worsening solvent quality results in a contraction. The net result of an increase in temperature on the state of the exopolymers, resulting in flocculation or stabilization is hard to predict, especially since little is known concerning the chemical composition of exopolymers of mesophilic and thermophilic bacteria. Differences in amount and composition of exopolymers of mesophilic and thermophilic bacteria can obviously also lead to a different flocculation behavior. Besides exo-polymers, also differences in the behavior and location of exo-enzymes of both sludges can affect the biosorption of colloidal COD as the removal of this fraction was found to be directly linked to its actual biodegradation.

Summarizing the above, we are still unable to clarify the fundamental phenomena underlying the differences in mesophilic and thermophilic flocculation behavior. However, theoretical calculations using the DLVO theory and the invalidation of the temperature dependency of the hydrophobic binding point to the importance of exopolymers (EPS) and exo-enzymes in bioflocculation and further study in this direction is required.

6. CONCLUSIONS

Biosorption capacities of mesophilic and thermophilic activated sludge as used in the present study are low under anaerobic conditions. Significant removal of colloidals from the water phase only takes place with mesophilic biomass provided that actual biodegradation occurs. Thermophilic activated sludge was found to be more dispersed, containing a higher volume fraction of small particles and is more sensitive towards shear forces than mesophilic activated sludge. Binding of hydrophobic pollutants on an artificial hydrophobic surface was not significantly affected by variations in temperature and can thus not account for the large observed differences in flocculation behavior.

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8

COMBINED CALCIUM AND BOD REMOVAL IN A NOVEL THERMOPHILIC UP-FLOW FLUIDIZED BED REACTOR

ABSTRACT

A novel reactor concept has been developed that combines calcium removal and BOD conversion under mesophilic as well as under thermophilic conditions. Soluble calcium, present in many industrial wastewaters, precipitates as a result of forced aeration in the aerobic bioreactor. The calcium carbonate precipitates in turn act as a carrier material for biofilm growth. This results in dense rapidly settling sludge granules covered with a biofilm surface. These sludge characteristics facilitate the application of short hydraulic retention times while maintaining a high biological activity in the reactor.

Vogelaar,J.C.T., F. van der Wal and G. Lettinga, submitted.

1. INTRODUCTION

Process water from the paper and board industry that uses recycled wastepaper as raw material contains high concentrations of both calcium and biodegradable COD. These process water characteristics, combined with a high water temperature and the absence of toxic compounds make it very suitable for anaerobic treatment (Habets and Knelissen, 1985; 1997). However, aerobic post treatment is still required for the removal of rest BOD and sulphide. Furthermore, scaling problems caused by calcium are of great concern to most paper mills and calcium needs to be removed as well from the process water. Previous research showed that activated sludge post treatment functioned satisfactory when polishing the anaerobic effluent of a board mill under both mesophilic as well as under thermophilic conditions (Chapter 5, 6). Sulphide and COD were biologically oxidized while calcium was removed by calcium carbonate precipitation in the aeration tank. Extensive calcium carbonate precipitation occurs when dissolved carbon dioxide (partly present as bicarbonate in the anaerobic pretreatment) is stripped from the solution via the forced aeration in the aerobic bioreactor (see eq. 1).

$$2\ HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

Removal of carbon dioxide results in a shift of the equilibrium to the right hand side, resulting in more calcium carbonate precipitation.

The only disadvantage of activated sludge post treatment is the low loading rate that can be applied and consequently a relatively long hydraulic retention time is needed for a simple polishing step. A strategy to overcome this problem is by increasing the biomass concentration in the aerobic bioreactor. Generally, higher biomass densities can be obtained in a bioreactor by using granular sludge as compared to flocculent types of sludge. Granular sludge may develop by auto-granulation or by biofilm formation on an inert carrier. In case the wastewater contains a high amount of calcium, calcium carbonate precipitates may act as a carrier material for biofilm growth. This hypothesis is used in the development of the aerobic, granular sludge bed bioreactor that we investigated in the present experiment. This system uses calcium carbonate precipitates as carrier material.

Consequently, the aim of this study was to develop an aerobic bioreactor designed for efficient post treatment of anaerobic effluents with a high calcium content. Two identical EGSB type reactors were operated in parallel, one at 30 °C and the other at 55 °C. Reactor performance was monitored in terms of volatile fatty acids (VFA) and calcium removal as a function of the hydraulic retention time. Successful operation of the bioreactors beyond the critical dilution rate for dispersed biomass growth proves that actual biomass immobilization has taken place on the granules. Batch experiments were performed with mesophilic and thermophilic sludge to assess the maximum VFA degradation rates.

2. MATERIALS AND METHODS

2.1 Bioreactors

Two identical temperature controlled EGSB (Expanded Granular Sludge Bed) reactors were operated at 30 and 55 °C (Fig. 1). The inner reactor diameter was 10 cm, total reactor height was 2 meters giving a total reactor volume of 5.5 liters. Concentrated synthetic medium was fed from the bottom of the reactors via two different stock solutions and was diluted at the inlet point with tap water. The hydraulic retention time (HRT) was varied from 4 hours to 1 hour. A recycle flow was imposed in order to obtain a constant liquid upflow velocity of 6 (m h^{-1}). Aeration took place with a mixture of pure nitrogen gas and oxygen. This set-up enables a variable nitrogen/oxygen ratio in order to uncouple mixing from oxygen supply. Table 1 lists the oxygen supply rate (OSR) and imposed organic loading rates (OLR) of both reactors. The pH was controlled and kept constant at 7.2. Dissolved Oxygen (DO) was measured using WTW EO90 oxygen electrodes.

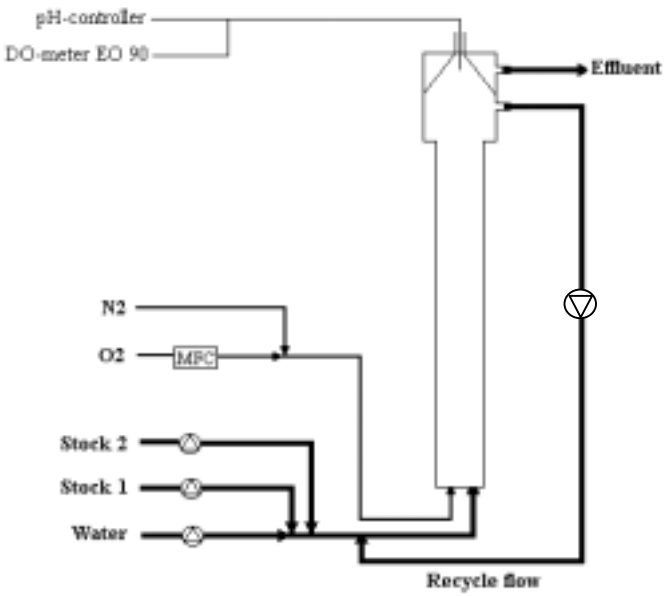

Fig. 1. Experimental set-up of the aerobic upflow reactors (MFC: mass flow controller).

Table 1. Organic loading rate (OLR) and oxygen supply rate (OSR) of both reactors. The organic loading rate is calculated for VFA alone and for both VFA and citric acid (2-nd row).

	HRT 4 h	HRT 1 h
OLR (g VFA-COD l $reactor^{-1}$ day^{-1})	1.2	4.8
total OLR (g COD l $reactor^{-1}$ day^{-1})	2.1	8.3
OSR (g O_2 l $reactor^{-1}$ day^{-1})	34	103
total gas flow (vvm)*	0.12	0.15
percentage oxygen in gas mixture	17%	56%

* vvm: volume of gas sparged per reactor volume per minute.

2.2 Batch experiments

The maximum VFA conversion rates of the developing mesophilic and thermophilic sludges were assessed in batch experiments in a temperature controlled baffled vessel. The biomass was kept in suspension by intensive stirring with a Heidolph RZR 2051 electronic stirrer. Aeration took place with pressurized air.

2.3 Seed sludge

500 ml of settled anaerobic granular sludge from a UASB reactor located at a paper mill was used as inoculant in both reactors. The sludge was solely mend as an initial carrier material for calcium carbonate precipitation and for biofilm growth and was thus in this sense sacrificed. No inoculation with aerobic biomass took place.

2.4 Reactor Influent

Synthetic medium, consisting of VFA, calcium, bicarbonate and nutrients was used as reactor influent. The exact medium composition is listed in Table 2. This synthetic wastewater was diluted 20 times before entering the bioreactors leading to actual concentrations of the main components in the reactor influent of 200 (mg l^{-1}) VFA-COD, 500 (mg l^{-1}) Ca^{2+}, and 2000 (mg l^{-1}) HCO_3^-. After 80 days of operation, citric acid was added as a biodegradable complexing agent for calcium in order to avoid premature calcium carbonate precipitation in the stock solutions. Actual process waters from the paper and board industry contain humic acids that act as natural complexing agents, keeping part of the calcium in solution under conditions of over saturation (Stevenson, 1982). The amount of citric acid added was capable of keeping 21% of the influent calcium in solution as calculated from the stoiciometry of the complexation reaction.

Table 2. Composition of reactor influent, all concentrations expressed per litre stock solution.
* The trace element solution is described by Zehnder *et al*., (1980)

Component	period 1 (day 0-77)	period 2 (day 77-182)
Stock solution 1		
Acetate	2 g COD	2 g COD
Propionate	1 g COD	1 g COD
Butyrate	1 g COD	1 g COD
$CaCl_2 \cdot 2\ H_2O$	33.75 g	37.17 g
$Ca(OH)_2$	1.725 g	-
Citric acid	-	4.86 g
NH_4Cl	0.7 g	0.7 g
$FeSO_4 \cdot 7\ H_2O$	10 mg	10 mg
$MgSO_4 \cdot 7\ H_2O$	100 mg	100 mg
Trace element solution*	0.125 ml	0.125 ml
Stock solution 2		
$NaHCO_3$	55 g	55 g
K_2HPO_4	160 mg	160 mg

2.5 Analyses

The Chemical oxygen demand (COD) was determined according to APHA (1995). Mixed liquor total- and volatile suspended solids, (MLTSS/MLVSS) were determined according to the Dutch normalized standards (NEN 6621). Sludge samples were taken from the reactor after the sludge was allowed to settle for one minute in the reactor. Volatile fatty acids (VFA) of pre-centrifuged samples were measured on a Hewlett Packard GC, model 5890 A. The GC was equipped with a 2 m × 2 mm glass column, packed with Supleco port (100-120 mesh) coated with 10% fluorad FC431. Carrier gas was nitrogen saturated with formic acid. Calcium (both in particulate and soluble form) was determined by an atomic absorption spectrometer (Model AA975, Varian, Springvale, Australia) according to NEN 6446 using a 422.7 nm wavelength lamp. Particulate calcium in the samples was solubilized using a destruction step with a mixture of nitric and hydrochloric acid. The fraction dissolved calcium was obtained by membrane filtration (Whatman GF/F) of the original sample. Lanthaan chloride solution was used to dilute all samples. Microscopic observations and photographs of sludge granules were made by light microscopy (Olympus BH-2/C-35AD-4).

3. RESULTS AND DISCUSSION

3.1 VFA removal

Both reactors were started directly at their set-points temperatures. Anaerobic granular biomass rapidly fell apart under the prevailing aerobic conditions but the fragments were retained in the system acting as a nuclei for calcium carbonate precipitation. In this way, calcium carbonate precipitation on the reactor wall was avoided. At 55 °C, a stable reactor operation in terms of VFA removal was established within two weeks (Fig. 2). At both 4 and 1 hour hydraulic retention time the removal efficiencies were almost 100%. The temporary reactor crash at day 170 was due to an empty gas bottle. The smaller disturbances observed at day 80 and 120 were mainly due to agglomeration of sludge particles which resulted in mass transfer limitations. These effects were distinctly more severe in the 30 °C reactor. After discharge of these cemented sludge granules in the mesophilic reactor, the removal efficiencies rapidly recovered and remained stable at 98-100% (Fig. 2, day 125 and onwards).

Measured steady state DO values were 4.2 (mg O_2 l^{-1}) at 30 °C and 1.5 (mg O_2 l^{-1}) at 55 °C at 4 hours HRT. At a HRT of 1 hour, these values were respectively 0.9 and 0.2 (mg O_2 l^{-1}). Calculated oxygen saturation concentrations in the reactors for their specific temperatures and oxygen partial pressure (Chapter 2) are 6 (mg O_2 l^{-1}) at 30 °C and 4.2 (mg O_2 l^{-1}) at 55 °C at 4 hours HRT. At the 1 hour HRT, these values were significantly higher due to the increased oxygen partial pressure (Table 1) in the oxygen/nitrogen mixture: 19.8 (mg O_2 l^{-1}) at 30 °C and 13.7 (mg O_2 l^{-1}) at 55 °C, respectively. Based

on these figures and the applied conversion rates and by assuming a yield factor, a rough estimation can be made of the overall oxygen transfer coefficient (K_la) for both temperatures and hydraulic retention times. The main conclusion from these calculations is that the K_la decreased in time as the hydraulic retention time was lowered from 4 hours to 1 hour. The worsened oxygen transfer efficiency could be attributed to the clearly visible calcium scaling on the aeration stones. This resulted in larger gas bubbles which means a smaller specific surface area (a) and hence a lower K_la .

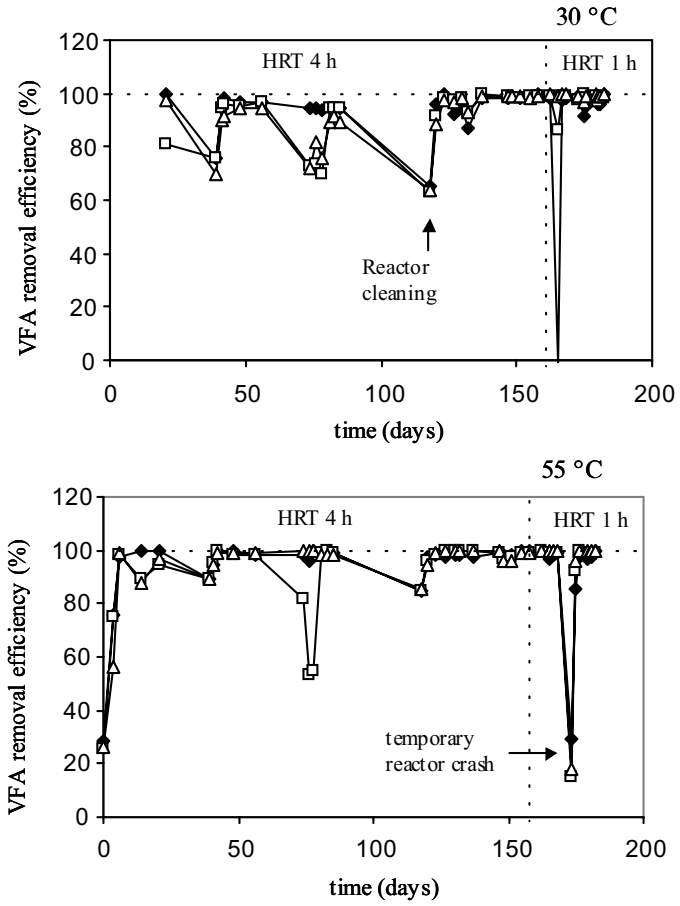

Fig. 2. VFA removal efficiencies at 30 °C and 55 °C over the entire operational period. (♦) acetate, (□) propionate, (Δ) butyrate.

Aerobic seed sludge was found not to be necessary for the start-up of both the mesophilic and the thermophilic bioreactor treating these simple substrates. Bacterial contaminations present in the pressurized gases or water developed into stable biofilms under the conditions prevailing in the bioreactors resulting in an efficient VFA removal.

Operation of the bioreactors at 4 hours HRT did not necessarily require immobilization of biomass as the maximum growth rate of free swimming bacteria oxidizing VFA at 30 and 55 °C still exceeds the reactor dilution rate. Based on maximum growth rates obtained for biomass grown on acetate, the critical dilution rates are estimated to be 2 (h) at 30 °C and 1.4 (h) at 55 °C (Chapter 3). The

observation that a 100% VFA removal efficiency was maintained at a hydraulic retention time of 1 hour thus proves that actual immobilization of biomass did occur on the granules. This was also confirmed by results of batch experiments with granular biomass because these showed a high VFA removal rate, especially when compared to the rates prevailing in the continuous reactors (Table 3). The average VFA removal rates in the continuous reactors were calculated on the basis of the removal efficiencies and the assessed biomass concentrations in the reactors. The results of the batch experiments showed that significantly higher sludge loading rates could be imposed on the system. However, under continuous reactor operation, oxygen transfer limitations will eventually determine the minimum hydraulic retention times that can be reached. Determination of these boundary conditions is the next area of study.

Noteworthy is also the observation of the substantially higher maximum VFA degradation rate found in the batch experiment for mesophilic sludge as compared to thermophilic sludge. Generally, higher specific conversion rates are expected under thermophilic conditions (Chapter 3). These data should however be taken with some precaution since they represent a single experiment in which the initial substrate concentrations were not identical.

The biodegradability of citric acid was evaluated in batch experiments with granular sludge and a neutralized citric acid solution. Citric acid removal was estimated from the COD reduction in time. We found degradation rates of 123 (mg citric acid-COD g VSS^{-1} h^{-1}) at 30 °C and 154 (mg citric acid-COD g VSS^{-1} h^{-1}) at 55 °C. Obviously, citric acid degradation did occur but it was hard to verify this under continuous reactor operation. The fact that still 10% of the soluble calcium (Table 5) was found back in the effluent suggests that approximately 50% of the citric acid was converted under continuous reactor operation. This estimation was also confirmed by the effluent soluble COD concentration after subtracting the VFA in the effluent.

Table 3. Maximum VFA degradation rates obtained in batch experiments and rates calculated from continuous reactor operation at 1 hour HRT, all expressed in (mg VFA-COD g VSS^{-1} h^{-1}).
Sludge samples for batch experiments were taken from the reactors when operated at HRT 1 (h).

	VFA degradation rates (mg VFA-COD g VSS^{-1} h^{-1})	
	maximum rates batch experiment	measured rate under continuous operation
30 °C	351	5.2
55 °C	131	4.7

3.2 Sludge characteristics

In both reactors a sludge with a very high density developed as a result of the extensive calcium carbonate precipitation. Total solids concentrations of settled sludge samples were in the order of 400 (g TSS l^{-1}) and consisted for 96-98 % of inorganic material (Table 4). Despite the small fraction of

organic material in the sludge, still a high concentration of volatile solids (12-20 g VSS l^{-1}) could be maintained in the reactor. This enables the system to achieve an efficient removal of organic pollutants at likely even lower detention times as applied in this study.

Table 4. TSS and VSS concentrations of settled mesophilic and thermophilic granular sludges ± standard deviations. The figures in between brackets represent the number of measurements.

	TSS (g l^{-1})		VSS (g l^{-1})	
Reactor	30 °C	55 °C	30 °C	55 °C
HRT 4 h	374 ± 183 (13)	493 ± 285 (13)	16 ± 6 (13)	12 ± 5 (13)
HRT 1 h	390 ± 197 (4)	572 ± 174 (4)	15 ± 3 (4)	21 ± 5 (4)

Table 5. Calcium removal efficiency in both reactors ± standard deviations. The figures in between brackets represent the number of measurements.

Reactor temperature	Total calcium removal (%)		Soluble calcium removal (%)	
	30 °C	55 °C	30 °C	55 °C
HRT 4 h	79 ± 7 (10)	81 ± 9 (10)	87 ± 9 (11)	93 ± 7 (11)
HRT 1 h	79 ± 5 (5)	81 ± 18 (5)	82 ± 7 (5)	91 ± 3 (5)

Figure 3 shows two light microscopic photographs of mesophilic and thermophilic granular sludge obtained from the reactors operated at 4 (h) HRT. In these pictures, still some free swimming dispersed bacteria are visible. These dispersed organisms were washed out when the HRT was further reduced to 1 (h). At 30 °C, some protozoa were detected while these were absent at 55 °C. Most of the calcium carbonate precipitates were found to be covered with a thin biofilm.

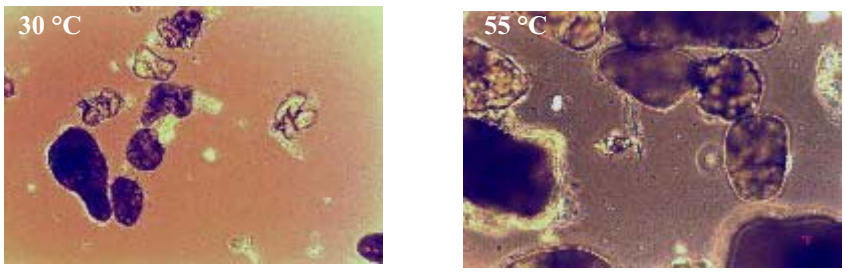

Fig. 3. Light microscopic pictures of mesophilic (30 °C) and thermophilic (55 °C) granules. Magnification 100*.

Calcium removal proceeded efficiently in both reactors, 79 % total calcium was removed in the mesophilic reactor at both 1 and 4 hours HRT while 82 to 87 % was removed in the thermophilic reactor (Table 5). The higher removal efficiency under thermophilic conditions can be explained by the decreasing calcium carbonate solubility with increasing temperatures. The removal efficiency for soluble calcium was higher than for total calcium as part of the smaller calcium carbonate precipitates were washed out of the reactors.

4. CONCLUSIONS

The results of this preliminary study show the feasibility of applying a system in which calcium and BOD are removed in a single bioreactor. As such, the up-flow reactors function as crystallization reactors were sufficient biological activity for an efficient BOD conversion is immobilized on the grains. Mesophilic and thermophilic biofilms were able to develop on the calcium carbonate precipitates, even under these typical conditions of a high shear stress and with the risk of encapsulation of biomass by precipitation. This holds a great potential for minimizing the hydraulic retention time in an aerobic biological post treatment system as compared to activated sludge treatment. Many questions however still need to be resolved regarding conversion rates, oxygen transfer efficiency and whether granulation will still take place with actual wastewaters from various industries.

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9

GENERAL DISCUSSION

1. INTRODUCTION

Thermophilic aerobic (post) treatment is an evolving new technology that will gain more interest in the years to come. Reasons for this are in general not the proposed benefits over mesophilic treatment but in fact the actual needs to purify hot wastewater streams. As water and energy prizes rise, industries, and in particular the pulp and paper industry have a tendency for more process water reuse and thus also for hot process water treatment. This general discussion will focus on: (1) the physico-chemical effects of temperature on process water treatment, (2) the characteristic features of thermophilic aerobic wastewater treatment and (3) application of thermophilic aerobic wastewater treatment in the paper and board industry.

2. TEMPERATURE EFFECTS ON PHYSICO-CHEMICAL PARAMETERS

Since most physico-chemical parameters are influenced by temperature, they will affect the aerobic conversion process under high temperature conditions. The most important parameters and their impact on the treatment process are listed in Table 1.

Table 1: Physico-chemical changes of water properties under thermophilic conditions (modified after LaPara and Alleman, 1999)

Parameter	**Expected change**	**Impact on thermophilic aerobic wastewater treatment**
Water vapor pressure	increase	Increased evaporation, heat losses, decreasing solubility of gases
Solubility of gases	decrease	Reduced transfer efficiency of under saturated gases (e.g., O_2) Increased stripping of super saturated off-gases (e.g., CO_2)
Diffusion rate	increase	Improved gas transfer efficiency Improved mixing efficiency
Solubility of salts	increase/decrease	Higher permissible concentrations of most organics and in organics Lower solubility of carbonate salts
Viscosity	increase	Improved gas transfer efficiency Improved mixing efficiency
Surface tension	decrease	Improved gas transfer efficiency Increased tendency for foaming

2.1 Reactor temperature

The water vapor pressure increases in a non-linear fashion with temperature. As a result, more process water evaporates from the aeration tank under the high temperature conditions and significant amounts of heat are lost due to the high enthalpy of evaporation of water. Figure 1 depicts the estimated temperature of the mixed liquor in a thermophilic aerobic bioreactor as a function of the

oxygen transfer efficiency. The oxygen transfer efficiency is defined as the percentage of oxygen transferred from the gas phase to the water phase when compared to the total amount of oxygen blown in the reactor via the aeration system. A low oxygen transfer efficiency thus implies that a high aeration rate is required for conversion of a certain amount of COD. The temperature of the reactor influent is 55 °C. The temperature of the reactor contents itself is estimated from a steady state heat balance including heat production due to microbial conversion and heat losses with the exhaust air, evaporated water and convection through the reactor wall. Roels and Heijnen (1980) have shown that on aeration with compressed air in a bubble column, the power dissipation due to gas expansion does not need to be taken into account in the heat balance. Expansion of the aeration gas in the mixed liquor causes a motion of the liquid with the expansion work dissipated as heat. However, this effect is fully compensated as on the expansion of an ideal gas at constant temperature, heat is taken up by the gas and thus the net effect is zero.

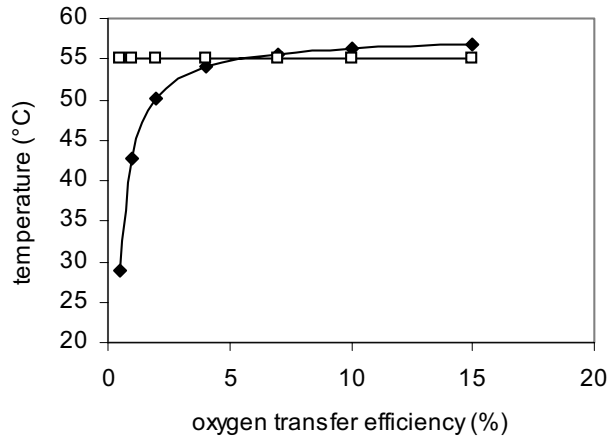

Fig. 1: Estimated reactor temperature as a function of the oxygen transfer efficiency. (□) reactor influent, (♦) reactor effluent. The organic loading rate is 4.9 (kg COD $m^{-3}day^{-1}$).
Assumptions: cylindrical reactor, height: 20 m, diameter: 5 m, 1 cm stainless steel and 5 cm glass wool insulation with heat conductivity coefficients of respectively 50 and 0.04 (W $m^{-1}K^{-1}$). Influent biodegradable COD: 1000 (mg COD l^{-1}), average free energy release upon oxidation of process water COD of 3.5 (kcal g COD^{-1}), observed yield: 0.1; ambient air temperature: 15 °C. The required air flow rate is calculated directly from the oxygen requirements for a 100% COD conversion (including the biomass yield of 0.1) and the oxygen transfer efficiency. Off-gas is assumed to be at reactor temperature and 100% relative humidity.

From Figure 1 follows that an approximate oxygen transfer efficiency of 5% needs to be attained to maintain the reactor temperature in the thermophilic range. In aerobic bioreactors such as the CIRCOX reactor (Heijnen *et al*., 1993) and in activated sludge systems with fine bubble aeration and a rather deep aeration tank, these efficiency values are feasible. In Figure 2, a heat balance is made over the biological reactor as described in Fig. 1 at a hydraulic retention time of 4.9 hours, an organic

loading rate of 4.9 (kg COD $m^{-3}day^{-1}$) and 5% oxygen transfer efficiency. The amount of heat generated by biological conversion is almost equal to the heat losses by the exhaust air, resulting in only a minor change in the reactor temperature (0.5 °C). The heat losses due to convection via the reactor wall are almost negligible when compared to the heat losses due to evaporation of water and the hot exhaust air.

Expected water losses due to evaporation are rather small, only 2.6% of the influent flow is lost in case the exhaust air is fully saturated with water at 55 °C, at an oxygen transfer efficiency of 1 %. In our lab-scale reactors, evaporation was distinctly higher (up to 20% at 55 °C) due to the much smaller reactor scale.

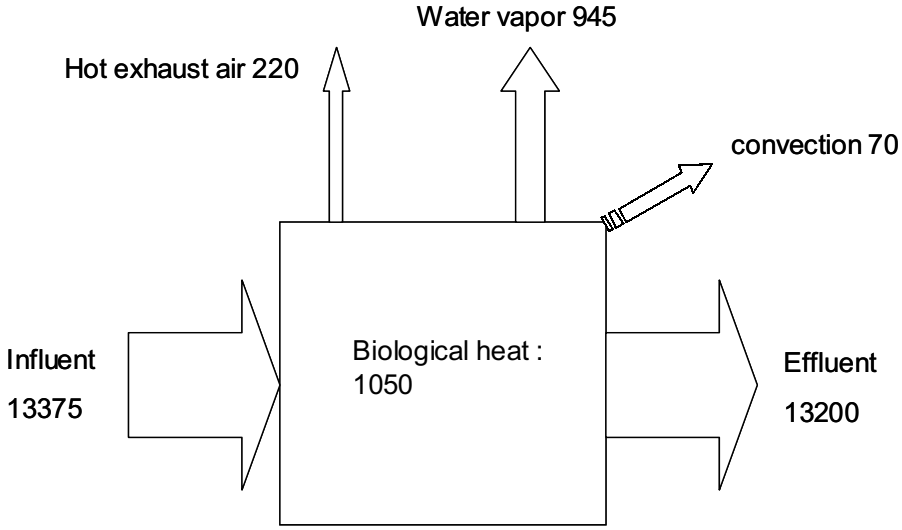

Fig. 2: Heat balance over the biological reactor as described in Fig. 1 at a hydraulic retention time of 4.9 (h), OLR 4.9 (kg COD $m^{-3}day^{-1}$) and 5% oxygen transfer efficiency. Figures are in (MJ h^{-1}).

2.2 Oxygen transfer

In Chapter 2 it was shown that the increased oxygen diffusion rate almost completely offsets the lower oxygen saturation concentration under thermophilic conditions. This results in a constant oxygen transfer rate in the temperature range of 20-55 °C (Fig. 2A-chapter 2). However, we calculated the oxygen transfer rate assuming a zero oxygen concentration in the liquid phase. When assuming a certain residual oxygen concentration in the mixed liquor, the OTR does decrease as a function of temperature (Fig. 2B-chapter 2). This effect becomes increasingly important with temperatures exceeding 60 °C. Beyond 60 °C, the increasing water vapor pressure decreases the oxygen saturation concentration to such an extent that it cannot be compensated fully anymore by the increasing overall oxygen transfer coefficient (K_la), resulting in a severe decrease in the oxygen transfer rate (Boogerd *et al*., 1990; Chapter 2).

At temperatures exceeding 60 °C, aeration with pressurized air may not be sufficient as it would require high air flow rates, imposing a high shear stress on the sludge. In chapter 7, it was evidenced that thermophilic activated sludge is more susceptible for shear forces than mesophilic sludge, leading to floc disintegration and a loss of activity in case a secondary settler is used for biomass retention. Under these circumstances, the use of pure oxygen instead of pressurized air may be an attractive alternative.

Besides oxygen, also the solubility of other gases such as methane, H_2S, NH_3 and CO_2 decreases. A lower concentration of dissolved CO_2 results in a slightly higher reactor pH and a more extensive precipitation of calcium carbonate. Both phenomena were observed in this research.

2.3 Foaming

As temperature rises, the surface tension of water decreases, possibly leading to more severe foaming problems. In this study, foaming problems occurred in both mesophilic and thermophilic reactors i.e. activated sludge and continuous flow without cell recycle as described in chapter 3,5,6. Excessive foaming in these experiments could always be attributed to short term reactor upsets such as pH, and temperature shocks. Probably these disturbances cause partial cell lyses and concomitant liberation of cell components (phospholipids, proteins) which act as foam stabilizers. Based on the experience gained in our study, we do not expect significant differences in foaming behavior between mesophilic and thermophilic conditions, at least not governed by a pure temperature effect.

3. CHARACTERISTICS OF THERMOPHILIC AEROBIC WASTEWATER TREATMENT

When comparing thermophilic aerobic wastewater treatment with conventional mesophilic treatment three main differences will manifest:

1. a lower removal of complex soluble COD (Chapter 4,5,6; LaPara *et al.,* 2001)
2. less cohesion between thermophilic activated sludge flocs leading more easily to floc disintegration and sludge settling problems, and a diminished adsorption of colloidal material to the sludge flocs (Chapter 5,6,7)
3. a non-simultaneous degradation of polymeric substances under thermophilic conditions (Bomio *et al*., 1989; LaPara *et al*., 2000a; Kosseva *et al*., 2001; this study, unpublished data)
4. kinetics of conversions. The maximum growth rate of aerobic biomass on acetic acid is a factor 1.5 higher at 55 °C than at 30 °C. Biomass decay rates double with the same temperature increase (Chapter 3).

1). The differences in removal of complex soluble COD between both temperature conditions is most likely due to the inability of the thermophilic biomass to degrade the same variety of components as the mesophilic biomass (Chapter 4). Häner *et al*. (1994a,b) added ^{14}C-acetate batch wise to thermophilic aerobic biomass and found that part of the acetate was converted into slowly biodegradable (or inert) soluble microbial products (SMP). Part of these SMP were subsequently biodegraded after their production but a significant fraction was left over after 110 (h) of incubation (Häner *et al.,* 1994a). The experiments were performed under oxygen limiting conditions which might affect the results. Nevertheless, these findings show that SMP production is a process that should be taken into account under thermophilic conditions.

In chapter 3, we found that the effluent soluble COD concentration of a thermophilic reactor fed with acetate was lower as compared to that of a 30 °C reactor (excluding acetate in the effluent). In Chapter 4, batch biodegradation experiments at 30 and 55 °C are described with glucose as the only organic substrate. After 35 days of biodegradation similar residual COD concentrations were obtained at 30 and 55 °C. This residual COD could only arise as a by product from microbial metabolism or from decay and lyses of cells since glucose and acetate are completely biodegradable. These results show that possibly under thermophilic conditions, more SMP are formed as metabolic by-products but given sufficient time, these can still be biodegraded. The difference in effluent soluble COD between a mesophilic and a thermophilic reactor treating a complex wastewater thus represents a fraction from the influent that can be biodegraded by the mesophilic biomass but not by the thermophilic biomass.

The causes for the inability of the thermophilic biomass to degrade these components are still unknown. Possibly, a smaller thermophilic microbial diversity as reported by Konopka *et al.* (1999) and LaPara *et al.* (2000a) could cause the lower COD removal although in this research, no significant reduction in species richness was observed between mesophilic and thermophilic sludge (Chapter 6).

2). Intercellular cohesion within thermophilic activated sludge flocs appeared to be smaller as compared to mesophilic flocs. This was evidenced by a higher shear sensitivity of the thermophilic sludge flocs and a higher dispersible fraction (Chapter 7). As a result, thermophilic floc sizes are smaller as compared to mesophilic flocs when cultivated on the same wastewater and the same shear conditions (Chapter 7) and thus active biomass is more easily washed out with the effluent (Chapter 6). In addition to a smaller internal cohesion within the flocs, also the adhesion of influent wastewater colloidals onto the flocs was smaller, resulting in a lower removal efficiency of colloidals from the influent (Chapter 5,6). The underlying cause for this phenomenon is still unclear. Protozoa are known to use free living bacteria in the mixed liquor as a food source but their absence under thermophilic conditions was not found to be the cause for the higher effluent turbidity at 55 °C (Chapter 6). The hydrophobic interaction did not decrease significantly within the temperature range of 30-55 °C and could not explain the difference in flocculation behavior (Chapter 7). Theoretical calculations using

the DLVO theory revealed that sludge flocculation and adsorption of colloidals is unlikely to occur under both temperature conditions unless bacterial polymers are present on the cell surface (Chapter 7). These polymers are able to penetrate the electrical double layer surrounding the bacteria/colloidals resulting in bridging flocculation or steric repulsion. Steric repulsion results in a stabilization of the colloidals in the liquid phase whereas polymer bridging governs sludge flocculation and sorption of colloidals. Whether bridging flocculation or steric repulsion occurs depends on the polymer concentration on the cell surface and on the solvent polymer interactions. The latter interactions are highly temperature dependent but of course also depend on the type of polymers involved. Mesophilic and thermophilic aerobic bacteria may have different exo-polymers but little is known of the actual composition and structure of these polymers.

So far, it seems most likely that the different flocculation behavior under both temperature conditions is the result of changes in polymer interactions but till this moment it is unclear whether this is a pure temperature effect or that differences in polymer composition are the main cause.

Hydrolysis of colloidal material by activated sludge was found to be related to the biosorption of this fraction by the sludge (Chapter 7). Frølund *et al.* (1995) and Goel *et al*. (1998) have shown that the hydrolytic activity of activated sludge is mainly located in the sludge floc matrix, whereas for pure bacterial cultures, the hydrolytic activity is mainly located in the bulk liquid (Goel *et al*., 1998). As thermophilic activated sludge grows more dispersed, relatively more enzymes may be excreted to the bulk liquid. These are obviously lost with the effluent in case of continuous reactor operation. Of particular interest is also the measured lower hydrolysis rate of suspended and colloidal COD in the BOD5 test (Fig 3d-Chapter 4) at 55 °C compared to 30 °C. Under thermophilic conditions, in general, higher hydrolysis rates are expected but these were not found in this experiment. Presumably, the hydrolysis rate is also affected by the sludge flocculation behavior. Flocculation of colloidals onto the sludge flocs brings the polymeric substrate in close contact with the exo-enzymes. In the absence of flocculation, both substrate and enzyme may not remain attached to each other, hindering the biodegradation process.

3). In unpublished experiments we observed that gelatin was rapidly and completely biodegraded by thermophilic activated sludge from the lab-scale reactors described in Chapter 5,6. However, soluble starch was hardly converted under thermophilic conditions. Literature reports also mention a non-simultaneous degradation of polymeric substances under thermophilic conditions (LaPara *et al*., 2000a; Kosseva *et al*., 2001) with proteins being degraded preferentially over carbohydrates. Bomio *et al.* (1989) could not detect any lipase, amylase, pectinase or cellulase activity in thermophilic aerobic sludge cultivated on wasted sewage sludge. They concluded that thermophilic bacterial populations preferentially utilize proteinaceous material as a carbon source. These observations are of

significant interest when studying the applicability of thermophilic aerobic bioconversions but obviously require further study.

4). Kinetics

In general, a higher conversion rate and a lower sludge production is expected under thermophilic conditions (LaPara and Alleman 1999). In Chapter 3, we indeed found a lower observed biomass yield when comparing mesophilic and thermophilic bioreactors fed with acetate. Due to the higher maintenance requirements under thermophilic conditions, the observed yield decreased more rapidly as a function of the sludge retention time at 55 °C when compared to 30 °C. This results in a lower sludge production (and a higher oxygen demand) when oxidizing a fully biodegradable substrate. However, sludge production is not only governed by biomass growth and decay but also by entrapment of influent suspended and colloidal particles. The anaerobic effluent of the cardboard producing paper mill at Zülpich, Germany, contains significant amounts of such slowly biodegradable fractions. These are retained in the sludge, resulting in an almost equal sludge production at both temperatures (Chapter 5).

In Chapter 3 it was shown that maximum growth rates for biomass grown on acetate at 55 °C are 50% higher than for mesophilic biomass. As theoretical yields are similar at both temperatures, then at maximum 50% higher conversion rates are to be expected per gram of active biomass. However, maintenance requirements approximately double with the same temperature increase. This leads to a lower biomass concentration in a thermophilic reactor compared to a mesophilic analogue provided that both reactors are operated at the same organic loading rate. As the increase in the decay rate is not fully compensated by the increase in the maximum growth rate, the overall maximum volumetric conversion rate of a thermophilic bioreactor will be lower as compared to a mesophilic reactor, especially at longer sludge retention times. This was confirmed in batch experiments with sludge grown on acetate at a 13 and 43 (h) SRT (Table 6, Chapter 3), in the short term BOD experiments (Fig. 2a,b, Chapter 4) and in the biosorption experiments (Fig 3c,d Chapter 7). In the batch experiments of Chapter 4 and 7, the maximum oxygen uptake rate (OUR) at 55 °C was similar (Chapter 4) or even lower (Chapter 7) as compared to 30 °C. The reason for this is obviously the lower amount of active biomass in the 55 °C sludge. In contrast to the results of the batch experiments as presented in Chapter 3, this was not only the result of a higher decay rate. In Chapter 4 and 7, activated sludge, cultivated on anaerobic effluent from Zülpich was used in the batch experiments. In the thermophilic activated sludge reactor, less COD was converted resulting in a lower biomass growth (Chapter 4,5,6) and more active biomass was lost with the effluent due to erosion of flocs and dispersed growth (Chapter 6).

In practice however, thermophilic activated sludge reactors treating a soluble substrate will not be operated at the same organic loading as a mesophilic bioreactor. As the biomass concentration in a

thermophilic bioreactor tends to be lower, a higher organic loading rate will be imposed in order to increase the biomass content. In this respect, thermophilic treatment has a kinetic advantage over mesophilic treatment. In theory, when treating the same wastewater, a thermophilic reactor can be smaller compared to a mesophilic system, provided an efficient sludge retention and sufficient oxygenation capacity is achieved. These kinetic advantages are however of little use when treating the effluent of an anaerobic reactor. The limiting step in the treatment of such effluents will be the retention and partial hydrolysis/oxidation of solids. Solids retention is not as efficient under thermophilic conditions as compared to mesophilic conditions and the few results so far show that the hydrolysis rate is not necessarily faster at 55 °C than at 30 °C.

4. THERMOPHILIC AEROBIC TREATMENT FOR WATER SYSTEM CLOSURE IN THE PAPER AND BOARD INDUSTRY

Process water of the paper and board industry using recycled wastepaper as raw material is characterized by a relatively high concentration of easily biodegradable COD (depending on the wastepaper quality) with little toxic compounds (Webb, 1985; Habets and Knelissen, 1985; 1997). Under these circumstances, a thermophilic anaerobic pretreatment system combined with aerobic post treatment will be the most cost effective treatment scenario. The thermophilic anaerobic effluent is likely to contain H_2S, calcium, bicarbonate alkalinity, a small amount of easily biodegradable substrates and suspended and colloidal material that is either washed through the anaerobic pretreatment or that is eroded from the anaerobic granules.

The aim of the thermophilic aerobic bioreactor is to remove calcium, rest BOD, sulphide, suspended and colloidal material and possibly anionic trash. Calcium must be removed in order to prevent scaling problems and sulphide because it causes bad smells in the mill and in the product. A lower process water COD, containing little VFA, will result in a lower dissolution of calcium carbonate in the process water during the pulping process and limits biofilm growth. Furthermore, micro-organisms present in the process water should be retained in the aerobic bioreactor to prevent biofilm formation in the paper mill. Minimizing the amount of anionic trash in the process water reduces the costs of cationic polymer dosage on the paper machine.

A thermophilic aerobic bioreactor can not fulfill all these requirements. Calcium was found to be removed easily by precipitation (Chapter 5). Sulphide and other malodorous compounds are rapidly oxidized (Chapter 4) or stripped with the off-gas. The amount of anionic trash, measured as cationic demand was hardly affected by biological treatment (unpublished data) and no significant differences were observed between mesophilic and thermophilic treatment. The obtained soluble COD removal efficiencies were almost similar under mesophilic and thermophilic conditions (Chapter 4,5,6). Most

likely, the slight differences in removal efficiency will have no effect on the paper production process and a slightly higher process water COD can be tolerated.

The main difference between mesophilic and thermophilic treatment is the retention of colloidal material in the sludge (Chapter 5). Colloidal material in the process water mainly consists of micro-organisms (Chapter 6) which are washed through the thermophilic reactor. Furthermore, also some thermophilic biomass is lost with the effluent (Chapter 6). In general, a relatively high concentration of suspended solids can be tolerated in the aerobic effluent in case the water is re-used directly in the pulping process. However, when these solids are mainly composed of bacteria, especially anaerobic acidifiers, this could cause bacterial re-growth in the mill causing problems related to biofilm formation. In view of that, the supply of nutrients in the biological treatment plant is of crucial importance. Malmqvist *et al*. (1999) studied the effect of internal process water treatment on the biofilm formation potential of the process water of a paper mill. They found that an optimum dosage of nutrients for biological treatment was of more importance than a 100% removal of biodegradable COD. Excess dosage of nitrogen and phosphorous in the biological treatment led to a rapid biofilm formation in the process water, also when little biodegradable COD was present in the water. Furthermore, in their study they observed that dispersed growing biomass in the moving bed biofilm reactor was washed out with the effluent, enhancing the biofilm formation later on in the process. Consequently, the effluent colloidals can cause problems related to biofilm growth in the paper mill. However, this is still a hypothetical threat and should be studied under practical conditions in a paper mill with a completely closed water system.

For application in the board industry, a modified version of an activated sludge system is likely to be the most cost effective option for removing both calcium and rest BOD. In case a higher water quality without any suspended solids is required (for instance to be used on the sprayers of the paper machine or when biofilm growth is to extensive), then part of the treated water can be upgraded using sand filters or be treated by flocculation with organic polymers. In the latter option, it would be worthwhile to return the flocculated colloidals back into the aeration tank as they also comprise thermophilic biomass.

In Chapter 5, it was also shown that when a different retention agent is used on the paper machine (in this case bentonite), almost all colloidal material could be removed from the process water by flocculation on the paper machine. The colloidal material thus ends-up in the produced board. In this scenario, no additional flocculants need to be used after the biological treatment as most of the effluent colloidal material after treatment arises from the influent (Chapter 5). In practice, these different scenarios need to be evaluated: whether to apply an additional coagulation/flocculation step or not, and if so, to apply this after the biological treatment system or directly on the paper machine itself.

In Chapter 8 of this thesis, we described preliminary experiments with a novel up-flow reactor in which calcium carbonate granules developed with a biofilm growing on the surface. Influent soluble calcium precipitated with carbonate due to stripping of carbon dioxide. Most likely, these granules will also develop in a full scale reactor treating anaerobically pretreated effluents. As a prerequisite, a sufficiently high concentration of calcium and bicarbonate alkalinity is necessary combined with a short hydraulic retention time and high shear conditions in the aeration tower. In this scenario, the aerobic bioreactor functions more like a precipitation reactor, capable of removing soluble COD and H_2S. However, the removal of suspended and colloidal material remains very poor.

For thermophilic treatment of other types of wastewater, containing little if any calcium, probably biofilm reactors such as the CIRCOX (Heijnen *et al*., 1993), moving bed biofilm reactor (Broch-Due *et al*., 1994) and the rotating biological contactor (Strom and Chung, 1985) are the best feasible reactor configurations. Other studies reveal that stable thermophilic biofilms can be formed under thermophilic conditions while biomass retention does not depend on sludge settleability (Perrtula *et al.,* 1991;Visvanathan and Nhien, 1995; Jahren *et al*., 2002). However, it is not clear from their results to what extent colloidal material can be removed in these reactors since most research so far was focused on total COD removal or dealt with removal of soluble substrates (Perrtula *et al*., 1991). Jahren *et al*. 2002 note that an additional treatment step is required to remove biomass from the effluent that was sloughed off from the carrier material. Their study also reported a higher effluent VSS concentration as compared to the influent. This is probably due to dispersed bacterial growth when operating such systems at relatively long hydraulic retention times. An additional sludge separation step is thus required or the system should be operated at very short HRTs.

An alternative method for retention of suspended and colloidal material is to use membrane bioreactors. Some thermophilic membrane bioreactors (MBR) have already been implemented in different paper mills (Ramaekers *et al.*, 2001) and were shown to function satisfactory. However, it is not clear so far whether a thermophilic MBR is also the most cost effective option for this type of applications. Based on the current insights described in this thesis, it is recommended to further research the application of more simple and robust biofilm processes under thermophilic conditions.

SAMENVATTING

Produktie van papier en karton gaat in het algemeen gepaard met een hoog water en energieverbruik. In Europa wordt jaarlijks ongeveer 70 miljoen ton papier en karton geproduceerd. De benodigde hoeveelheid schoon water voor de produktie van 1 ton papier is ongeveer 20 kubieke meter. Dit resulteert in een totaal waterverbruik in Europa van ongeveer 1.4 miljard kubieke meter water per jaar (Kappen *et al.*, 1999). In Nederland alleen wordt ongeveer 35 miljoen kubieke meter grondwater gebruikt bij de produktie van papier en karton.

Karton wordt vrijwel uitsluitend gemaakt van gerecycled oud papier. In het produktie proces wordt het gerecyclede oud papier intensief gemengd met heet water waardoor de papier vezels in oplossing gaan. Het water/vezel mengsel wordt vervolgens naar de papiermachine verpompt alwaar het op een vilt gespoten wordt. Het water zakt door het vilt heen en de vezels blijven achter. Na het drogen van deze achtergebleven vezels is eenvoudig gesteld een strook karton ontstaan.

Om de papiermachine optimaal te laten functioneren vindt het produktieproces plaats bij een hoge temperatuur van ongeveer 55 °C. Het koude grondwater wordt daartoe opgewarmd door stoominjecties en door de warmteafgifte van de vele pompen in de fabriek zelf. De combinatie van èn een hoog waterverbruik èn een hoge procestemperatuur maken de produktie van papier en karton tot een erg energie-intensief proces. Een besparing op het waterverbruik leidt dan ook direct tot een vermindering van het energieverbruik. Om die reden worden in veel papier en kartonfabrieken de waterkringlopen in meer of mindere mate gesloten, d.w.z. er wordt meer warm water hergebruikt waardoor er minder schoon, koud water opgewarmd behoeft te worden.

Echter, naarmate het water vaker hergebruikt wordt, raakt het in sterkere mate vervuild met allerlei ongewenste componenten uit het oud-papier. Deze vervuilende stoffen kunnen het produktie proces verstoren en ook de produktkwaliteit wordt minder. Om dit tegen te gaan moet er òf meer schoon water gebruikt worden, òf de vervuilende stoffen moeten m.b.v. een extra zuiveringsstap uit het water verwijderd worden. Deze zuiveringsstap behoort dan wel bij 55 °C plaats te vinden.

Proceswater van de kartonindustrie bevat een hoge concentratie gemakkelijk afbreekbaar organisch materiaal en is derhalve goed te behandelen in een anaerobe bioreactor. Hierin zetten bacteriën onder zuurstofloze (anaerobe) condities organisch materiaal om in biogas. Daarna is nog een aerobe zuiveringsstap noodzakelijk waarbij de restfractie van het organische materiaal en eventuele stankstoffen geoxideerd worden door bacteriën die daarvoor zuurstof gebruiken. Bovendien vindt in deze reactor precipitatie van calciumcarbonaat plaats waardoor het kalkafzettend vermogen van het proceswater afneemt. Het onderzoek zoals dat in dit proefschrift is beschreven heeft betrekking op deze laatste aerobe zuiveringsstap onder thermofiele condities, d.w.z. bij 55 °C.

De hoge temperatuur van het zuiveringsproces heeft zowel zijn weerslag op de bacteriën die bij deze temperatuur leven alswel op een aantal fysisch-chemische processen. De temperatuurseffecten op de

microbiologie staan in de volgende paragraaf beschreven. Wat betreft de fysisch-chemische processen is met name de overdracht van zuurstof uit de lucht naar de waterfase van belang. De beschikbaarheid van opgelost zuurstof voor de bacteriën kan namelijk een beperkende stap worden in de biologische omzetting.

Naarmate de temperatuur stijgt neemt de oplosbaarheid van zuurstof in het water af waardoor de drijvende kracht voor de zuurstofoverdracht minder wordt. Dit effect wordt echter vrijwel geheel gecompenseerd door een toename in de zuurstofdifusiesnelheid waardoor de totale zuurstofoverdrachtssnelheid in het temperatuursbereik van 20-55 °C vrijwel constant blijft (hoofdstuk 2). Zuurstofoverdrachtsproblemen zijn dus niet te verwachten onder thermofiele condities mits er volstaan kan worden met een lage concentratie opgelost zuurstof in de vloeistoffase.

TEMPERATUURS EFFECTEN OP DE MICROBIOLOGIE

Bij een vergelijking tussen een thermofiel aeroob zuiveringssysteem en een mesofiele (30°C) referentiereactor komen een aantal punten van verschil naar voren. Dit zijn:

1. Een verminderde verwijdering van opgelost complex organisch materiaal onder thermofiele condities. Dit is beschreven in de hoofdstukken 4,5,6 en in LaPara *et al*., 2001b.
2. Een verminderde cohesie binnen thermofiele actiefslibvlokken waardoor deze gemakkelijker uit elkaar vallen (H 7), kleiner in afmeting zijn (H 7) en moeilijker bezinken (o.a. Tardiff and Hall, 1997; Tripathi and Allen, 1999). Bovendien vindt onder thermofiele condities minder adsorptie van colloidaal materiaal uit het afvalwater aan actief slib vlokken plaats (H7) waardoor een troebel effluent vaak het gevolg is (H 5,6).
3. Een sequentiële afbraak van verschillende soorten polymeren onder thermofiele condities in tegenstelling tot een gelijktijdige afbraak onder mesofiele condities (Bomio *et al*., 1989; Lapara *et al.,* 2000a; Koseva *et al*., 2001, ongepubliceerd materiaal van deze studie)
4. Kinetiek van de omzettingen. De maximale groeisnelheid op azijnzuur (en dus ook de maximale omzettingssnelheid) is een factor 1.5 hoger bij 55 °C in vergelijking tot 30 °C. De biomassa afstervingssnelheid neemt in hetzelfde temperatuursbereik met ongeveer een factor 2 toe.

1. Bij de behandeling van een complex afvalwater kan een verschil in verwijderingsrendement voor opgelost CZV twee oorzaken hebben: mogelijk kan de thermofiele biomassa niet dezelfde complexe moleculen oxideren die wel door de mesofiele biomassa omgezet kunnen worden of er wordt onder thermofiele condities meer inert materiaal geproduceerd. Produktie van inerte opgeloste stoffen (SMP) kan plaats vinden door afsterving van biomassa: een deel van de bacteriecel blijkt dan niet

meer afbreekbaar te zijn, of deze stoffen worden geproduceerd als bijprodukt van het substraatmetabolisme.
Bij de thermofiele behandeling van een volledig afbreekbaar substraat (azijnzuur) werd hetzelfde CZV verwijderingsrendement verkregen in vergelijking tot de mesofiele behandeling (H 3). De restfractie die in deze experimenten overblijft kan alleen onstaan zijn uit afsterving van biomassa of als gevolg van substraat metabolisme. Dit toont dus aan dat er onder thermofiele condities niet significant meer opgelost inert materiaal geproduceerd wordt dan onder mesofiele condities. De verschillen in verwijderingsrendementen bij de behandeling van een complex, niet volledig afbreekbaar substraat moeten dus het gevolg zijn van het onvermogen van de thermofiele biomassa om dezelfde varieteit aan opgelost complex organisch materiaal af te breken als waar de mesofiele biomassa wel toe in staat is. Mogelijk is dit te wijten aan een kleinere microbiële diversiteit in het thermofiele slib in vergelijking tot mesofiel slib zoals gerapporteerd door Konopka *et al*. (1999) en LaPara *et al*. (2000a). In dit onderzoek (H6) werden echter geen grote verschillen in diversiteit tussen de beide slibsoorten aangetroffen.

2. De minder goede flocculatie van thermofiel actief slib heeft tot gevolg dat deze gevoeliger is voor afschuifkrachten en dat de vlokken sneller uit elkaar vallen. Hierdoor zijn ze kleiner in afmeting in vergelijking tot de mesofiele actief slib vlokken (H 7) en bezinken ze moeilijker. Bovendien worden colloidale deeltjes uit het influent minder goed vastgehouden in de vlokken waardoor het effluent van de thermofiele zuivering vaak troebel is in tegenstelling tot het heldere mesofiele effluent. Indien het influent echter geen colloidale deeltjes bevat kunnen deze ook niet uitspoelen en kan wel een helder thermofiel effluent verkregen worden (H 5). Dit geeft dus aan dat met name de concentratie influent colloidaal materiaal van invloed is op de thermofiele effluent kwaliteit (H 5,6). Een deel van de effluent deeltjes bestaat echter ook uit thermofiele bacteriën (H 6).
De reden voor de slechtere flocculatie van de thermofiele biomassa is nog niet volledig achterhaald. De afwezigheid van protozoa onder thermofiele condities kon de troebelheid van het thermofiele effluent niet verklaren aangezien het mesofiele effluent nog steeds helder bleef, ook als de daar aanwezige protozoa afgedood werden m.b.v. specifieke remmers (H 6). De temperatuurs afhankelijkheid van de hydrofobe interactie bleek minimaal te zijn en kon ook het verschil in flocculatiegedrag niet verklaren (H 7). Op basis van berekeningen met de DLVO theorie bleek dat ook onder mesofiele condities flocculatie zeer onwaarschijnlijk was en dat dit alleen door brugvorming met bacteriële exo-polymeren op kon treden. Polymeer interacties zijn sterk temperatuursafhankelijk en in plaats van flocculatie door brugvorming kan ook sterische repulsie optreden. Hierdoor zullen de bacteriën niet flocculeren maar stabiel in de oplossing aanwezig blijven. Ook kan de chemische samenstelling van de exo-polymeren op thermofiele biomassa verschillen van die van de mesofiele biomassa waardoor er in plaats van flocculatie juist repulsie optreedt. Hoe dit mechanisme precies

functioneert is nog onduidelijk. Wel is uit onderzoek gebleken dat het flocculatie gedrag van de beide slibsoorten niet alleen op basis van pure kolloid-chemie te verklaren is maar dat de biomassa ook biologisch actief moet zijn. De biosorptie van colloidaal materiaal bleek direct gekoppeld te zijn aan de biologische afbraak van die fractie (H 7).

3. Uit ongepubliceerd materiaal van deze studie en uit literatuur gegevens bleek dat macromoleculen van verschillende aard, b.v. eiwitten en koolhydraten onder thermofiele condities sequentieel werden afgebroken in tegenstelling tot een gelijktijdige afbraak onder mesofiele condities. Eiwitten werden sneller en volledigere afgebroken dan koolhydraten. In hoofdstuk 4 bleek dat de hydrolyse en oxidatie van de gesuspendeerde afvalwater fractie langzamer plaatsvond onder thermofiele condities dan onder mesofiele condities. Over het algemeen wordt aangenomen dat de hydrolysesnelheid juist toeneemt met een stijging van de temperatuur. Een verklaring voor dit fenomeen is mogelijk dat de thermofiele biomassa niet over dezelfde diversiteit aan exo-enzymen als de mesofiele biomassa beschikt of de enzymen komen niet goed in contact met het substraat door de slechtere flocculatie bij 55 °C.

4. Uit de resultaten van hoofdstuk 3 blijkt dat de maximale groeisnelheid van thermofiele biomassa gegroeid op azijnzuur 50% hoger ligt dan die van mesofiele biomassa gegroeid op hetzelfde substraat. Dit houdt in dat de maximale specifieke substraatomzettingssnelheid voor thermofiele biomassa dus ook 50% hoger ligt dan voor mesofiele biomassa. De biomassa-afstervingssnelheid neemt echter met een factor 2 toe bij een temperatuursstijging van 30 naar 55 °C. Dit betekent, met een identieke bruto yield voor de beide slibsoorten, dat de netto biomassa aanwas in een thermofiele reactor lager is dan in een mesofiele reactor. Dit effect wordt slechts ten dele gecompenseerd door de hogere specifieke substraatomzettingssnelheid van het thermofiele slib. Het gevolg hiervan is dat de maximale substraat omzettingssnelheid van een mesofiele reactor hoger is dan die van een thermofiele bioreactor mits beide systemen bij dezelfde belasting bedreven worden (H 3). De actieve concentratie biomassa in de thermofiele reactor is namelijk significant lager in vergelijking tot de mesofiele reactor. De organische belasting in een thermofiel systeem kan echter verhoogd worden om tot eenzelfde biomassa concentratie in dit systeem te komen als in de mesofiele reactor. Onder die omstandigheden kan dus in de thermofiele reactor met een kortere hydraulische verblijftijd (dus een hogere belasting) worden volstaan in vergelijking met een mesofiel systeem en ligt ook de maximale substraatomzettingssnelheid 50% hoger in de thermofiele reactor in vergelijking tot de mesofiele reactor.

TOEPASSING VAN THERMOFIELE AEROBE NAZUIVERING IN DE PAPIER EN KARTON INDUSTRIE

Het doel van een thermofiele aerobe nazuivering toegepast in de kartonindustrie is met name het verwijderen van rest CZV, stankstoffen en calcium. Calcium verwijdering bleek zeer effectief te verlopen onder thermofiele condities aangezien de oplosbaarheid van calcium afneemt met een stijging van de temperatuur (H5). Ook stankstoffen werden snel biotisch dan wel abiotisch geoxideerd (H 4). Wat betreft de verwijdering van rest CZV werd niet hetzelfde rendement behaald in vergelijking tot een mesofiel systeem. Dit levert voor toepassing in de kartonindustrie waarschijnlijk geen probleem op gezien de hoge CZV concentratie die in het proceswater getolereerd kan worden. Van meer belang is lagere verwijdering van colloidaal materiaal in de thermofiele nazuivering. Colloidale deeltjes in het thermofiele effluent bestaan vrijwel allemaal uit bacteriën die afkomstig zijn uit het influent of uit het thermofiele slib zelf (H6). Doorgaans kunnen hoge concentraties gesuspendeerd materiaal in het effluent getolereerd worden als het water hergebruikt wordt in het pulpingsproces. Het is echter wel de vraag of deze bacteriën geen problemen met betrekking tot slijmvorming in het proceswater zullen geven. Als het water hergebruikt wordt voor hoogwaardiger doeleinden, bijvoorbeeld op de sproeiers van de papiermachine moet het verder behandeld worden met bijvoorbeeld een coagulatie stap en snelle zandfiltratie om deeltjes te verwijderen.

Voor toepassing in de kartonindustrie is met name een gesuspendeerd slib systeem zoals actief slib geschikt omdat daar zowel de biologische omzetting alswel als calciumcarbonaat precipitatie plaats kunnen vinden. Een nieuwe ontwikkeling is hier de combinatie van een precipitatiereactor met de biologische omzetting in één behuizing zoals beschreven in hoofdstuk 8. Het voordeel van dit systeem boven een actief slib systeem zijn de te verwachte kortere hydraulische verblijftijden en de geringere behoefte aan gronddoppervlakte. In een dergelijk systeem zullen echter geen deeltjes vastgehouden worden.

Indien een proceswater slechts weinig of geen calcium bevat is een thermofiele nabehandelingstap met een biofilmsysteem het meest voor de hand liggend. Op die wijze kunnen slibbezinkbaarheidsproblemen namelijk voorkomen worden. Wat betreft deze reactortypen is echter nog weinig bekend met betrekking tot de invang van colloidaal materiaal in de biofilm en meer onderzoek op dit gebied is vereist.

Toepassingen voor thermofiele aerobe nazuivering buiten de kartonindustrie zijn legio. Kringloopsluiting is een trend die plaats vindt in de witpapierindustrie en op termijn waarschijnlijk ook in de levensmiddelenindustrie. Voor dergelijke toepassingen zal de effluent kwaliteit van een thermofiel gesuspendeerd slib of biofilm systeem niet voldoende zijn en komen mogelijk membraan processen in aanmerking.

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LIST OF ABBREVIATIONS

θ	theta factor, typical value approximately 1.024 (-)
τ_p	response time of the oxygen probe (s)
a	interfacial surface area (m^2m^{-3})
AS	activated sludge
ASM 1	Activated Sludge Model No. 1
BKME	bleach kraft mill effluent
BOD	biochemical oxygen demand (mg O_2 l^{-1})
BOD-st	short term BOD (mg O_2 l^{-1})
c	dissolved oxygen concentration in the bulk liquid (mg O_2 l^{-1})
c_e	apparent oxygen saturation concentration (mg O_2 l^{-1}).
COD	Chemical Oxygen Demand (mg O_2 l^{-1})
c_s	saturation concentration of dissolved oxygen at the specified temperature, pressure and salinity (mg O_2 l^{-1}).
CSTR	continuously stirred tank reactor
D	dilution rate (h^{-1})
D_{cr}	critical dilution rate (h^{-1})
DAPI	4,6-diamidino-2-phenylindole
d_b	bubble diameter (m)
DD	initial degree of dispersion (%)
DF	dispersible fraction (%)
DGGE	denaturing gel gradient electrophoresis
DLVO	Derjaguin, Landau, Verweij and Overbeek (theory)
DO	dissolved oxygen (mg O_2 l^{-1})
DOC	dissolved organic carbon (mg l^{-1})
D_{ol}	oxygen diffusion coefficient in the liquid phase (m^2 s^{-1})
EGSB	expanded granular sludge bed (reactor)
EPS	exo-polymeric substances
FISH	fluorescent *in-situ* hybridization
f_p	fraction of inert COD in heterotrophic biomass (-)
G	turbulent shear rate (s^{-1})
HRT	hydraulic retention time (h)
k_d	first order decay rate (h^{-1})
K_e	ratio of bacteria in octane phase over water phase (-)
K_l	oxygen transfer coefficient (m h^{-1})
$K_la(T)$	overall oxygen transfer coefficient at temperature T (h^{-1})
K_s	monod constant (mg COD l^{-1})
K_{ss}	shear sensitivity constant (%)
MBBR	moving bed biofilm reactor
MBR	membrane bioreactor

MLSS	mixed liquor suspended solids (g SS l^{-1})
MLVSS	mixed liquor volatile suspended solids (g VSS l^{-1})
NSSC	semi chemical neutral ammonium sulphite pulping process
OC	oxygenation capacity (mg O_2 l^{-1} h^{-1}).
OD600	optical density measured at 600 nm
OLR	organic loading rate (g COD $l^{-1}day^{-1}$)
OSR	oxygen supply rate (g O_2 $l^{-1}day^{-1}$)
OTR	oxygen transfer rate (mg O_2 l^{-1} h^{-1})
OUR	oxygen uptake rate (=respiration rate) (mg O_2 l^{-1} h^{-1})
PCR	polymerase chain reaction
R	gas constant (=8.314) (J $mole^{-1}$ K^{-1})
S	readily biodegradable substrate (mg COD l^{-1})
SBR	sequencing batch reactor
S_I	initially present inert soluble COD (mg COD l^{-1})
SMP	soluble microbial products
S_p	soluble inerts generated from biomass decay (mg COD l^{-1})
S_s	readily biodegradable substrate (mg COD l^{-1})
t	time (s)
T	temperature (^{0}C in eq 2., K in eq. 5.)
TMP	thermo mechanical pulping process
UASB	upflow anaerobic sludge blanket reactor
UF	ultrafiltration
v_{bs}	bubble rise velocity relative to the liquid (m s^{-1})
VFA	volatile fatty acids (mg COD l^{-1})
X_h	heterotrophic biomass concentration (mg biomass COD l^{-1})
X_I	initially present inert particulate COD (mg COD l^{-1})
X_p	particulate inerts generated from biomass decay (mg COD l^{-1})
X_s	slowly biodegradable substrate of particulate nature (mg COD l^{-1})
Y	theoretical yield (g biomass COD g substrate COD^{-1})
Y_{obs}	observed yield (g biomass COD g substrate COD^{-1})
ε	dielectrical constant (C V^{-1} m^{-1})
θ = SRT	Sludge Retention Time (h) or (day)
κ	reciprocal thickness of the electrical double layer (m^{-1})
μ	growth rate (h^{-1})
μ_{max}	maximum growth rate (h^{-1})
σ_0	surface charge density (C m^{-2})
ψ_0	surface potential (mV)
ψ_D	stern potential (mV)
ψ_{zeta}	zeta potential (mV)

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CURRICULUM VITAE

Jacob Cornelis Theodorus (Jaap) Vogelaar werd op 19 mei 1973 geboren te Hekelingen, gemeente Spijkenisse. Na een viertal jaren HAVO aan de Angelus Merula te Spijkenisse werd het VWO diploma in 1991 behaald aan de naburige christelijke scholengemeenschap Blaise Pascal. In datzelfde jaar begon hij aan de studie Bioprocestechnologie aan de Landbouwuniversiteit te Wageningen (LUW). Hier volgde hij een vrije orientatie milieubiotechnologie welke werd ingevuld met afstudeervakken microbiologie en milieutechnologie. De praktijktijd werd doorgebracht in Amman, Jordanië met de opstart van een tweetraps pilot UASB reactor voor de zuivering van huishoudelijkafvalwater.

In 1997 studeerde hij af en werd vervolgens AIO bij de sectie Milieutechnologie van de LUW. Het onderzoek dat hij uitvoerde in de periode 1997 - 2002 staat beschreven in dit proefschrift.

Per 1 september 2002 werkt hij als procestechnoloog voor Paques Natural Solutions b.v. te Balk.

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