INVESTIGATIONS ON AEROBIC THERMOPHILIC TREATMENT OF PULP MILL EFFLUENT

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MASTER OF TECHNOLOGY

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DECLARATION

I hereby declare that the work entitled "INVESTIGATIONS ON AEROBIC THERMOPHILIC TREATMENT OF PULP MILL EFFLUENT" has been carried out in the Department of Biotechnology, Durban Institute of Technology, Durban, South Africa, under the supervision of Prof. S. Singh, Ph.D. The work embodied in this thesis is original and has not been submitted in part or in full for any degree or diploma of this or any other institution.

Date: 10/03/04

Durban

(P. Reddy)
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Thermophilic treatment of wastewaters increases in importance as industries shift from end-of-pipe treatment towards integrated process water treatment. The need for treatment of process water 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 pulp industry, high process water temperatures prevail and thus wastewater treatment needs to take place under thermophilic conditions. This thesis describes research in which the aerobic treatment of paper and pulp mill effluent was investigated under thermophilic conditions.

The objectives of this study were, firstly, to identify a suitable inoculum for thermophilic degradation of pulp mill effluent and this was achieved by screening from various sources i.e., activated sludge, heating water, soil and compost. The second objective was to determine the feasibility of aerobic thermophilic degradation of pulp mill effluent using temperatures of 40°C, 50°C and 60°C. Batch, fed-batch and continuous experiments would enable the feasibility of degradation of pulp mill effluent. Prior to fed-batch systems, batch systems were optimised by determining the following parameters: 1) Effect of temperature on degradation of pulp mill effluent, 2) Effect of biomass concentration on degradation of pulp mill effluent, 3) Effect of aeration on degradation of pulp mill effluent, and 4) the effect of nutrient supplementation.
Once batch systems were optimised, the fed-batch systems came into play, and this system demonstrated much potential for thermophilic degradation of pulp mill effluent, which finally led to developing a continuous system, where degradation was optimal.
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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

South Africa is a major international producer of pulp and paper products. With 1860 million tones of pulp produced during 1991, it ranked twelfth amongst the world’s top producers. In 1992, this country was the tenth largest producer of pulp and 22\textsuperscript{nd} biggest supplier of paper and board in the world. The industry contributes at least 3\% of the gross national product and is a major employer. The pulp and paper industry is also a major polluter. Chlorination is generally one of the stages of pulp bleaching. It involves addition of chlorine or chlorine dioxide and oxidation to remove residual lignin in the pulp. Chlorine and chlorine dioxide reacts with lignin in the pulp to form chlorinated organic compounds. The effluents containing the chlorolignins are highly coloured and can cause serious environmental problems because some of these compounds are in part toxic and mutagenic (Christov and Prior, 1998).

There has been considerable concern about the effect of chlorinated organic matter in pulp mill effluents on the environment. Some members of this family are known to be toxic, mutagenic, persistent, and bioaccumulating, and are thought to cause numerous harmful disturbances in biological systems. The manufacturer of paper generates significant quantities of wastewater, as high as 60m\textsuperscript{3}/tonne of paper produced. The raw wastewaters from paper and board mills can be potentially very polluting. Indeed, a recent survey within the UK industry has found that their chemical oxygen demands can be as high as 11000 mg/l (Thompson et al., 2000).
The paper and board industry is actively investigating closed 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 (Vogelaar et al., 2000). A sequenced anaerobic-aerobic treatment system as the most cost-effective option for in-line treatment of process water from a papermill using recycled wastepaper as a 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 (LaPara and Alleman, 1999).

Clearly the limiting factor preventing the wide spread use of thermophilic biotechnology in environmental engineering is the cost of raising reactor temperatures. Two situations exist in which this cost can be avoided:

i) when wastewater are produced hot, and

ii) when the wastewater are highly concentrated such that heat released during contaminant biodegrading is sufficient for the autothermal operation. The former condition exists at pulp and paper industries.

The temperature of combined wastewater from mills using a large proportion of mechanical pulp is high as most of the input pulping energy (approximately 5GJ/ton pulp) is dissipated as heat. Even with the much lower thermal inputs to the water system that are typical of papermaking (0.2-0.8 GJ/ton), process or wastewater temperatures well above 40°C are obtainable. Biological treatment of such wastewater is not normally attempted due to fears
about process instability, which in the case of aerobic plants, is caused by the adverse effect of high temperatures on biomass diversity. As a result, cooling systems are required to bring temperatures down to within the range for mesophilic operation. These can be either of the normal cooling tower design or heat exchangers using open or closed cooling circuits (Webb, 2001).

Effluent compositions among paper mills are not identical, since they may adopt combinations of the number of technologies available in each unit process involved in manufacturing pulp and paper. As a result, no single specific technology can be applied to the treatment of effluents from all mills, since the process diversities may preclude its acceptability. So it should be understood that each paper mill is a large complex, highly interactive operation and that instability in one area may have a greater impact than expected in another area. Consequently, the treatment of wastewaters from the pulp and paper mills tends to become mill-specific, and it is for this reason that the knowledge of possible contaminants present in the wastewater, their origins and degree of toxicity and available treatment technologies are essential (Ali and Sreekrishnan, 2001).

The main treatment process used at pulp and paper-mill plants is primary clarification, and in some cases, it is then succeeded by secondary treatment, generally of a biological nature. Secondary effluent treatment of pulp-mill effluents has been traditionally based on domestic wastewater treatment methods. Aerated lagoons and activated sludge systems are the principle methods employed for biodegradation of organic material. The effectiveness of these systems is generally measured by the removal of biological oxygen demand (BOD) and total suspended solids. However, the increased emphasis on the removal of priority
pollutants such as chlorinated compounds and resin acids has led to efforts to better understand their removal mechanisms and the factors affecting their degradation (Thoms, 2000).

Tertiary processes for further treatment, or colour removal, are rare at present, but may become more common in the future if legislation becomes more stringent (Thompson et al., 2000). The pulp mill effluent streams released from wood pulping and papermaking processes are at high temperatures (45°C-60°C) and must be cooled prior to biological treatment to ensure the activities and stability of the microbial communities in conventional biological treatment systems. Cooling such hot streams of millions of litres per day is extremely expensive. Therefore, biological treatment of pulp mill effluents at elevated temperatures would be advantageous.

Rudolfs and Amberg (1953) investigated the biological treatment for white water discharged from the board mills at temperatures up to 50°C. BOD removal in excess of 90% was possible only with the addition of chemical coagulant (alum). Gehm (1956) successfully treated kraft mill influent for 3 months at temperatures from 50°C to 53°C at a pH of 9.5 to 9.8. Compared with treatment at low temperatures (30°C to 38°C), similar BOD removals were observed. Dissolved oxygen concentration was non-detectable, but apparently had no adverse effect on process performance. Settling characteristics were “excellent” with sustainable mixed liquor solids concentration up to 3000 mg/L.

Carpenter et al. (1968) compared the aerobic treatment of pulp and paper wastewater at temperatures of 26°C, 37°C, 42°C, 47°C and 52°C. Effluent BOD increased at higher temperatures from a maximum removal percentage of 80.5% at
37°C to a lower value of 37% at 52°C. A low sludge volume index (SVI) was measured at thermophilic temperatures (47°C and 52°C) indicative of dispersed microbial growth. Pertulla et al. (1991) treated sulphite mill condensates containing acetate and ammonia at 65°C in packed-bed bioreactors, attempting to save energy by decreasing cooling requirements before treatment and reheating prior recycling the treated effluent. Extensive growth was observed, even to the point of clogging an activated carbon packing media. Overall, acetate removal was similar to that of mesophilic processes.

Rintala and Lepisto (1993) treated bleached kraft mill effluent by both anaerobic-aerobic and aerobic bench-scale processes at 55°C. Both systems studied provided levels of COD and adsorbable organic halogen (AOX) removal similar to analogous mesophilic systems. Barr et al. (1996) investigated the effects hydraulic residence time (HRT), solids residence time (SRT), and temperature on the aerobic treatment of kraft pulping effluent. Similar BOD removal was observed at 41°C -50°C as mesophilic temperatures, and a higher COD removal was reported. Settling characteristics improved with higher temperatures. The authors attributed these unique results to the use of an external clarification system, which extended the SRT to approximately 15 days.

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 m³ per tonne of paper caused by a low pulp quality, based on straw, bagasses or jute and by the use of outdated paper machines (Gupta, 1994). Table 1.1 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 metres of groundwater were used by the Dutch paper and board industry.
Table 1.1. Specific water usage in the Netherlands (Joore, 1999)

<table>
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<th>Product group</th>
<th>Specific water usage in time (m³ tonne⁻¹)</th>
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<tr>
<td>Massive board</td>
<td>ND</td>
</tr>
<tr>
<td>Corrugated board</td>
<td>ND</td>
</tr>
<tr>
<td>Graphic paper</td>
<td>ND</td>
</tr>
<tr>
<td>Tissue</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
</tr>
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</table>

ND: No data

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 m³ tonne⁻¹ of paper, assuming a groundwater temperature of 10°C and a process water temperature of 55°C results in energy savings of approximately 10⁴ MJ tonne⁻¹ of paper. Previously water savings were accomplished by good onsite clarification processes and wastewater treatment strategies. 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 (Vogelaar et al., 2000).

According to Habets and Knelissen (1997) different groups of compounds together with microorganisms accumulate in the process water as water cycles are closed. These compounds include, 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 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 fibre mat. Dosage of cationic polymers is thus a crucial step in the paper production process and increasing polymer demands lead to additional production costs (Habets and Knelissen, 1997).

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. An aerobic post treatment mainly removes residual 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 satisfactorily 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 off-site. Board production takes place at 55°C providing optimum runability 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 per tonne of paper can be achieved in this scenario.

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.

1.2 LITERATURE REVIEW

1.2.1 What are thermophiles?

Microorganisms and their enzymes have numerous industrial, medical and environmental applications. These processes frequently involve exposure to extremes of temperature, pressure, ionic strength, pH and organic solvents and hence there is a continuing need to isolate microorganisms/enzymes, which are able to function and catalyse specific reactions under these imposed conditions. Such organisms are found naturally in diverse range of environments, which whilst considered extreme by man, are optimal for their growth and development. Recently the term ‘extremophile’ has been used to describe them and within this grouping are included thermophiles, psychrophiles, acidophiles, alkalophiles,
halophiles, osmophiles, barophiles, radiation resistant and heavy metal tolerant organisms. Although originally considered to be no more than ‘scientific curiosities’, it is now widely accepted that many extremophiles have unique properties which have considerable biotechnological and, therefore, commercial significance (Herbert, 1992).

Microbiologists have long appreciated that temperature profoundly affects the activities and distribution of microorganisms in natural environments. The temperature range of growth has often been used to classify groups of organisms. The common divisions are psychrophiles (5°C - 20°C), mesophiles (15°C - 40°C) and thermophiles (45°C - 100°C). It is with this latter group that the most exciting developments have occurred in recent years. Brock (1986) defined thermophilic organisms as those, which proliferate at temperatures greater than 55°C - 60°C. This classification, used by many microbiologists, has some practical significance because these temperatures are almost exclusively related to geothermal activity, and no known eukaryotic organism can grow above 60°C. From a biological waste treatment point-of-view however, common terminology generally includes any process operating at temperature of 45°C or higher as thermophilic. This definition distinguishes these high temperature processes from transitional systems such as conventional anaerobic sludge digestion operated at 35°C - 40°C.

Thermophilic organisms can be found among bacteria, archaea, fungi and algae (Sonnleitner and Fiechter, 1983b). The group of moderate thermophiles that predominates in thermophilic aerobic wastewater treatment systems operating at 55°C belongs to the bacterial domain while most hyperthermophilic bacteria are archaea, (the exceptions being Aquifex and Thermotoga species). 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, 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 thermostability 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 tetra-ether 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
α-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 archaeb (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, archaeb 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.

Running biotechnological processes at elevated temperatures has many advantages. The increase of temperature has a significant influence on the bioavailability and solubility of organic compounds. The elevation of temperature is accompanied by a decrease in viscosity and increase in the diffusion coefficient of organic compounds. Consequently, higher reaction rates due to smaller boundary layers are expected. Of special interest are reactions involving less soluble hydrophobic substrates such as polyaromatic aliphatic hydrocarbons and
fats and polymeric compounds such as starch, cellulose, hemicellulose and proteins. The bioavailability of hardly biodegradable and insoluble environmental pollutants can also be improved dramatically at elevated temperatures allowing efficient bioremediation. Furthermore by performing biological processes at temperatures above 60°C, the risk of contamination is reduced and controlled processes under strict conditions can be carried out. The number of genes from thermophiles that have been cloned and expressed in mesophiles is also increasing sharply (Niehaus et al., 1999) and will impact on the ability to genetically manipulate these organisms to improve their biotechnological capabilities.

1.2.2 Habitats and growth of thermophiles

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. In 1996, bacteria were isolated from boiling hot springs at Yellowstone National Park. Bacteria were not just surviving there; they were growing and flourishing with active enzymes. Subsequently, prokaryotes have been detected growing around black smokers and hydrothermal vents in the deep sea at temperatures at least as high as 115°C. Microorganisms have been found growing at very low temperatures as well. In super-cooled solutions of H₂O as low as 20°C, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near 0°C.

Thermophilic fungi have not been found in hyperthermal environments. However, other niches such as soil and self heating masses of organic debris such as compost heaps, hay, piles of wood chips and manure are excellent sources for the isolation of thermophilic fungi. Composting is an aerobic process that degrades
moist solid organic waste in a process that starts off at mesophilic temperatures, but due to self-heating that results from microbial activity, may reach thermophilic conditions as temperatures rise up to 80°C. This is reflected in the microbial composition of compost that is mediated by both mesophilic and thermophilic organisms that are chiefly Gram-positive and include *Bacillus*, *Streptomyces*, *Thermonospora*, *Saccharomonospora* and *Thermoclinomyces*. It is apparent that thermophilic *Bacilli* dominate and more selective methods are required to isolate the actinomycetes. Also different media allow the proliferation of some species in preference to others (Redman *et al.*, 1999).

Because the primary goal of thermophilic wastewater treatment processes is to reduce the level of organic compounds in the waste stream, the dominant microorganisms are almost certainly aerobic heterotrophs. Of the previously described thermophilic organisms, therefore, only species of *Bacillus*, *Thermus* and *Actinomycetes* can grow in these reactors. Thermophilic *Bacillus* spp. are a heterogenous group which have been isolated from thermal areas such as hot springs and deep sea vents as well as non-thermal locations such as soils, compost, surface waters, sewage and spoiled food. The distribution of *Thermus* spp. is considerably less ubiquitous. These bacteria are almost exclusively isolated from hot springs or deep-sea vents, although they had been found in domestic hot water heaters and compost (LaPara and Alleman, 1999).

In 1996, it was reported that compost in many different types (garden and kitchen wastes) sewage sludge, industrial composting systems contain high numbers of bacteria of the genus *Thermus* which grow on organic substrates at temperatures from 40°C-80°C with optimum growth between 65°C and 75°C. The
numbers were as high as $10^7$ to $10^{10}$ g$^{-1}$ dry weight of compost therefore this is a rich source of thermophiles (LaPara and Alleman, 1999).

Natural habitats of thermophilic microorganisms are in any case exotic locations such as thermal springs and geysers, thermal basins and deep sea geothermal vents (Brock, 1986). However, most moderate thermophiles can be found everywhere on earth (Sonnleitner and Fiechter, 1983b) 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 1.2).

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). Surucu (1999) studied the growth requirements of a mixed culture of thermophilic bacteria degrading a highly concentrated substrate and found that 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 essential. 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, it has been reported that, yeast extract or peptone was added when a synthetic wastewater was 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.

1.2.3 Isolation and cultivation of thermophiles

Growth of thermophiles poses a number of problems that require modifications of traditional methods. At higher incubation temperatures there is more evaporation of both solid and liquid media. This is increased if growth in liquid culture is accompanied by sparging with gases. Condensation can lead to confluent growth on solid media, which may lead to problems in isolating pure cultures from thermophilic environments or during genetic studies (Edwards, 1990). At temperatures in excess of 65°C-70°C most plastic agar dishes will melt and agar itself becomes unstable. Media constituents become more thermolabile, for example, there will be a much more rapid caramelization of sugars with increasing temperature.

At temperatures above 55°C it is preferable to use agar at 2-4% to decrease evaporation. At temperatures in excess of 70°C, it is necessary to employ other
solid media such as silica gel. A recent development is a clarified gum from *Pseudomonas* species sold under the trade name of 'gelrite' which relies on divalent cations such as magnesium or calcium to solidify it and which is suitable as a solid medium at high temperatures. It has been used to culture extremely thermophilic microorganisms from submarine hydrothermal vents and remained solid at 120°C and at vapour pressures and hydrostatic pressures to 265 atm (Edwards, 1990). Gas solubility generally decreases with rising temperature. For example, at 30°C, oxygen solubility of water is 237 nmoles ml, at 50°C it is 171 and at 70°C, only 120. Therefore cultivation of cultures that require gassing (aerobes or anaerobes) must insure that there is a large liquid surface to gas ratio which can be achieved using baffled flasks, high rates of agitation and reduction of liquids to flask volume ratio.

Since most true thermophiles do not grow at ambient or room temperatures, storage of purified or enriched thermophilic microorganisms at temperatures of 20°C is sufficient to prevent growth and deterioration. To avoid complications caused by the growth of mesophiles in freshly collected samples causing unfavourable pH shifts of formation of bacteriocides by mesophilic fungi, it is good practice to keep the samples around 10°C during extended transport times (Brock, 1986)

As with mesophilic bacteria, there are many ways of enriching and isolating thermophiles. Besides the temperature, selective conditions to be used in enrichment will primarily depend on the physiological and nutritional type of organism to be isolated. Most of the presently known thermophiles have been isolated using the enrichment culture. For aerobic organisms, at incubation
temperatures above 50°C, the use of cotton plugs should be avoided. Above this temperature closed flasks are needed to prevent the evaporation of liquid. To replenish the oxygen supply during growth the flasks are flushed through sterile cotton filters with or O2-N2, the interval of flushing depending on the growth and incubation temperature.

Another method is to use a large pressurized gas reservoir connected to the flasks via small tubes and hypodermic needles. When aerobic cultures or gas-utilizing cultures are continuously flushed with gas at temperatures above 50°C the incoming gas should be humidified by first passing it through a sterile cotton filter and then through a sterile water reservoir. The water reservoir should preferably be at the same temperature as the culture to obtain the proper humidification. When anaerobic thermophiles are enriched by mesophilic sludge and mud samples only a low number of thermophiles are present, but the inoculum contains a considerable amount of fermentable substrates. The sample can be incubated in the enrichment medium without addition of a carbon and energy source until gas production has considerably decreased, usually occurring after 10 to 40 hours. Then, after an adjustment of the pH, the selective substrate is added (Brock, 1986).

Thermophiles are widespread and many of them, especially aerobic and anaerobic spore-formers, can be easily isolated from nearly any soil. Spore-forming thermophiles have even been isolated from Arctic ice. However, with decreasing environmental temperature, the numbers of thermophiles usually decrease. Thus for the investigation of a mesophilic environment, larger samples have to be used. For example, in Georgia soil, the number of organisms growing
on 0.5% glucose-yeast extract medium under anaerobic conditions at 60° and 70°C was around $10^3$ to $10^4$ g$^{-1}$ soil sample, whereas in soil from around hot springs of Yellowstone National Park the numbers were $10^2$ to $10^3$ fold higher. If water samples are used, a ratio of samples to buffered medium of 5:1 or at least 1:1 is desirable (Redman et al., 1999).

For organisms sensitive to changes in mineral content, etc, enrichment can also be carried out in undiluted samples supplemented with a nitrogen source and the selective carbon and energy sources. However, it seems that samples at least buffered with medium give better results. Enrichment cultures allow for the organisms to grow faster under the culture conditions employed. The isolation method of chance is direct isolation without enrichment, by filtration as a concentration step.

Thermophiles can be grown in sufficient quantities in fermenters. High stirring rates are required for the transfer of gases into the liquid but they do not cause overheating problems as often encountered when growing mesophiles. Special attention has been given to the gas transfer rate, since it is often the rate-limiting step in culturing gas-utilizing thermophiles. The mass culture of thermophiles requires sterile conditions very similar to mesophiles. Although the contamination risks are lower than with mesophiles, spore-forming aerobic and anaerobic thermophiles are ubiquitous and can cause airborne infections. In addition, the time of heat sterilization has to be increased, as the spores of thermophiles are very heat resistant (Brock, 1986).
Table 1.2. Overview of suspended growth systems at elevated temperature ranges and their treatment efficiencies

<table>
<thead>
<tr>
<th>Temp. range °C</th>
<th>Influent</th>
<th>System</th>
<th>SRT-loading rate g COD g MLVS⁻¹ day⁻¹</th>
<th>Temp. adaptation period</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-50</td>
<td>Pre settled board mill white water</td>
<td>B</td>
<td>Short, not mentioned SRT loading: 0.08-0.29</td>
<td>2 weeks</td>
<td>Good solids separation but higher effluent turbidity at 50°C</td>
<td>Rudolfs and Amberg (1953)</td>
</tr>
<tr>
<td>30-53</td>
<td>Kraft mill waste water</td>
<td>AS</td>
<td>SRT unknown loading: 0.5</td>
<td>Not mentioned, probably 3 months</td>
<td>Excellent sludge settling characteristics, nothing mentioned about effluent turbidity</td>
<td>Gehm (1956)</td>
</tr>
<tr>
<td>21-46</td>
<td>Citrus wastes</td>
<td>AS</td>
<td>SRT unknown loading: 1.3</td>
<td>10 days</td>
<td>Decreasing treatment efficiency at temperatures beyond 37°C</td>
<td>Dougherty and McNary (1958)</td>
</tr>
<tr>
<td>26-52</td>
<td>Simulated craft effluent</td>
<td>AS</td>
<td>SRT and loading unknown</td>
<td>2-3 weeks</td>
<td>Lower SVI at 55°C but more suspended solids in the effluent</td>
<td>Carpenter et al. (1968)</td>
</tr>
<tr>
<td>4-55</td>
<td>Synthetic sewage</td>
<td>B-AS</td>
<td>SRT 5 days 2 days loading: 0.58</td>
<td>Not mentioned</td>
<td>96% soluble BOD removal but biological solids did not settle properly compared to 35°C</td>
<td>Carter and Barry (1975)</td>
</tr>
<tr>
<td>35-50</td>
<td>Powdered milk</td>
<td>SBR</td>
<td>SRT unknown loading: 1.4</td>
<td>Not mentioned</td>
<td>Effluent TSS increased with increasing temperatures</td>
<td>Duke et al. (1981)</td>
</tr>
<tr>
<td>20-37</td>
<td>Industrial waste water</td>
<td>Pilot AS</td>
<td>SRT 1-10 days loading: 1.3</td>
<td>Not mentioned</td>
<td>Colloidal material was present in the influent and the effluent</td>
<td>Jackson (1983)</td>
</tr>
<tr>
<td>53</td>
<td>Cardboard waste water</td>
<td>CSTR</td>
<td>SRT: 1-10 days loading: 1-3</td>
<td>Not mentioned</td>
<td>Effluent turbidity due to colloids could only be removed by addition of a polymer</td>
<td>Jackson (1983)</td>
</tr>
<tr>
<td>53</td>
<td>Cardboard waste water</td>
<td>Pilot AS</td>
<td>SRT: 1-10 days loading: 1-3</td>
<td>Not mentioned</td>
<td>Increased effluent turbidity at elevated temperatures</td>
<td>Flippin and Eckenerfelder (1994)</td>
</tr>
<tr>
<td>28-52</td>
<td>Bleach kraft and chemical wastes</td>
<td>Full-scale AS</td>
<td>SRT and HRT unknown loading: 0.7-1.8</td>
<td>Total 4 months for a 15°C temperature increase</td>
<td>BOD removal, reactor MLVSS and effluent solids thermophilic and mesophilic were comparable</td>
<td>Barr et al. (1996)</td>
</tr>
<tr>
<td>35-50</td>
<td>Pre settled BKME</td>
<td>AS</td>
<td>SRT: 5-15 days loading: ≥0.8 at 50°C</td>
<td>Acclimatization period not mentioned</td>
<td>Poor sludge settling and low biomass growth resulted in low contaminant removals at 50°C</td>
<td>Tardiff and Hall (1997)</td>
</tr>
<tr>
<td>20-50</td>
<td>Simulated mechanical newprint white water</td>
<td>SBR</td>
<td>SRT: 20 days loading: ≥0.5 at 50°C</td>
<td>Total 4 months for a 15°C temperature increase</td>
<td>Poor sludge settling and low biomass growth resulted in low contaminant removals at 50°C</td>
<td>Tardiff and Hall (1997)</td>
</tr>
<tr>
<td>35-60</td>
<td>BKME</td>
<td>SBR</td>
<td>SRT: 10-15 days loading: ≥0.6 at 60°C</td>
<td>Acclimatization period not mentioned</td>
<td>Decreased COD removal and higher suspended solids concentration in the thermophilic reactors</td>
<td>Tripathi and Allen (1999)</td>
</tr>
<tr>
<td>24-44</td>
<td>Pharmaceutical wastes</td>
<td>Pilot AS</td>
<td>SRT: 3 days loading: 1.6</td>
<td>Decreased COD removal and higher suspended solids concentration in the thermophilic reactors</td>
<td>Bulking sludge observed at temperatures beyond 39°C turbid supernatant</td>
<td>Bastin et al. (1999)</td>
</tr>
<tr>
<td>25-56</td>
<td>Recycle mill waste water</td>
<td>Lab SBR</td>
<td>SRT: 9 days loading: ?</td>
<td>Severe filamentous bulking at temperatures beyond 40°C and a lesser COD removal beyond 45°C</td>
<td>Decreased COD removal and higher suspended solids concentration in the thermophilic reactors</td>
<td>Norris et al. (2000)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Influent</th>
<th>Reactor System</th>
<th>Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>NH₄Ac and evaporator condensate NSSC mill</td>
<td>Biofilm (diatomite carriers)</td>
<td>Acetate removal rates: 0.7 and 0.5 g l⁻¹ h⁻¹</td>
<td>Perrtula et al. (1991)</td>
</tr>
<tr>
<td>55</td>
<td>BKME</td>
<td>Biofilm on PUR</td>
<td>Thermophilic anaerobic/aerobic treatment gave stable COD removal efficiencies</td>
<td>Rintala and Lepisto (1993)</td>
</tr>
<tr>
<td>30, 38, 45 and 55</td>
<td>Modified domestic waste water</td>
<td>Aerated filter</td>
<td>Suspended solids removal decreased with increasing temperatures</td>
<td>Viswanathan and Nhien (1995)</td>
</tr>
<tr>
<td>55</td>
<td>Simulated white water</td>
<td>MBR and UF</td>
<td>MBR gave better results compared to UF alone</td>
<td>Ragona and Hall (1998)</td>
</tr>
<tr>
<td>60</td>
<td>Methanol and evaporator condensate</td>
<td>MBR</td>
<td>Methanol removal is more efficient using the synthetic waste water</td>
<td>Berube and Hall (1999)</td>
</tr>
<tr>
<td>37-52</td>
<td>White water</td>
<td>Suspended carrier biofilm</td>
<td>70-85% soluble COD removal, cloudy effluent</td>
<td>Malmqvist et al. (1999)</td>
</tr>
<tr>
<td>55</td>
<td>TMP white water</td>
<td>MBBR</td>
<td>60-65% soluble COD removal, yield similar to mesophilic treatment</td>
<td>Jahren et al. (2002)</td>
</tr>
</tbody>
</table>

1.2.4 Microorganisms involved in thermophilic aerobic conversions

In the past, many microbiological studies have been made on pure cultures of thermophilic microorganisms (Kuhn et al., 1980; Sonnleitner et al., 1982; Cometta et al., 1982; Becker et al., 1997). Bacterial diversity in Autothermal Thermophilic Aerobic Digestion (ATAD) and thermophilic composting processes were mainly studied, using classical microbiological methods (Sonnleitner and Fiechter, 1983c, d; Strom and Chung, 1985; Hensel et al., 1989; Beaudet 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 and Chung, 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 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.

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 hybridization (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
phylum 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
(oblige 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°C - 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 α-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.

1.3 THERMOPHILIC AEROBIC WASTEWATER TREATMENT

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 (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 was observed over a 24 h period at 50°C-65°C (Sonnleitner, 1983a). 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 (Vogelaar *et al.*, 2000).

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 1.2) and other reactor configurations such as biofilm processes or membrane bioreactors (Table 1.3).
1.4 ACTIVATED SLUDGE

Activated sludge can be defined as “a mixture of microorganisms which can contact and digest biodegradable materials (food) from wastewater” (Department of Natural sources, 2001a). It is a suspended growth process that began in England (Blitton, 1999; Horan, 1990). This process has since been adopted worldwide as a secondary biological treatment for domestic and industrial wastewaters (Blitton, 1999; Ramalho, 1983). The process consists of an aerobic treatment that oxidises organic matter to CO$_2$, H$_2$O and new cell biomass. Air is introduced by diffused or mechanical aeration (Blitton, 1999). At the same time flocculant sludge is formed. Microscopic examination of this sludge shows that is formed by a heterogenous population of microorganisms. This population changes continually in nature in response to variation in the composition of the wastewater and environmental conditions (Ramalho, 1983).

1.4.1 Formation of the floc

The bacterium that is in the sludge develops into small chains or clumps as they grow. They are still very active and motile, which makes it difficult for them to settle. The reason for this is that the slime layer, which helps them to stick together, has not yet developed. As the sludge is allowed to age, the bacteria lose their motility and accumulate more slime. This helps the chains and clumps to stick together which then grows bigger until a floc is formed. If the organisms develop properly, under the right conditions, the flocs get larger and more compact and begin to settle (Department of Natural Resources, 2001a).
The reason why flocs are formed is still partly unexplainable. However it is known that there are various factors that play a part in the formation of an activated sludge floc, *viz.*, 

i. Many bacteria form slime capsules, which is built up mainly of polymeric compounds that glues the cells together

ii. Bacteria have a negative charge and the positively charged ions around them contribute to joining of cells

iii. Networks of extremely thin fibrils are formed by some bacteria around the cell. These are mostly composed of cellulose or another polysaccharide. It is this network that contributes to further joining of cells and trapping of bacteria (Eikelboom and Bujisen, 1983).

**1.4.2 Composition of the floc**

A sludge floc consists of a whole range of microorganisms, as detailed below. It is this great diversity, which makes an activated sludge system so flexible and enables a large number of different compounds to be simultaneously metabolised.

**1.4.2.1 Bacteria**

Bacteria make up the largest component of the microbial community in all biological wastewater treatment process. The numbers are in excess of $10^6$ bacteria mL$^{-1}$ (Horan, 1990). It is mainly the gram-negative bacteria that make up this major component. The bacteria are responsible for oxidation of organic matter and nutrient transformations and produce polysaccharides and other polymeric materials, which
aid in the flocculation of microbial biomass. Filamentous microorganisms are also found. Table 1.4 shows the bacterial genera found in standard activated sludge, using culture-based techniques (Blitton, 1999).

<table>
<thead>
<tr>
<th>Genus group</th>
<th>Total isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comamonas pseudomonas</td>
<td>50.0</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>5.8</td>
</tr>
<tr>
<td>Pseudomonas (fluorescent group)</td>
<td>1.9</td>
</tr>
<tr>
<td>Paracoccus</td>
<td>11.5</td>
</tr>
<tr>
<td>Unidentified (gram negative rods)</td>
<td>1.9</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>1.9</td>
</tr>
<tr>
<td>Flavobacterium-Cytophaga</td>
<td>13.5</td>
</tr>
<tr>
<td>Bacillus</td>
<td>1.9</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>1.9</td>
</tr>
<tr>
<td>Coryneform</td>
<td>5.8</td>
</tr>
<tr>
<td>Arthrobacter</td>
<td>1.9</td>
</tr>
<tr>
<td>Aurebacterium-Microbacterium</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Table 1.4. Description of aerobic heterotrophic bacteria in standard activated sludge (Blitton, 1999)**

1.4.2.2 **Fungi**

Even though fungal filaments are occasionally observed in activated sludge, the growth of fungi is not usually favoured. They may grow abundantly but only
under specific conditions of low pH, toxicity and nitrogen-deficient wastes. The predominant genera found are *Geotrichum*, *Penicillium*, *Cephalosporium*, *Cladosporium* and *Alternaria* (Bitton, 1999).

1.4.2.3 Protozoa

Protozoa being eukaryotic organisms demonstrates a wide diversity in form and mode of life. They are generally unicellular, motile and classified on the basis of their morphology, more particularly their mode of locomotion (Horan, 1990). Protozoa are also significant predators of bacteria in activated sludge (Bitton, 1999). The presence of particular types of protozoans is related to effluent quality and plant performance. They play secondary but important role in purification of aerobic wastewater. The protozoans in activated sludge fall in three classes, viz., *Sarcodina*: amoeba; *Mastigophora*: the flagellates and *Ciliophora*: the ciliates free swimming, crawling and stalked (Department of Natural Resources, 2001a).

1.4.2.4 Rotifers

These are large, somewhat stretched multicellular organisms (Eikelboom and Buijsen, 1983). They are rarely found in large numbers. Their principal role is removal of bacteria and aiding in floc formation by secretion of a mucous. Rotifers require a longer time to become established and their presence indicates increasing stabilization of organic wastes (Department of Natural Resources, 2001a).
1.4.3 Effect of filamentous bacteria on floc formation

Three different types of activated sludge flocs can be distinguished based on the amount of filamentous organisms, viz.,

i. **Ideal floc**: In this floc the filamentous organisms and floc forming organisms grow in “balance”. Here the filamentous organisms grow inside the floc providing it with structure and strength. There are a few filaments that protrude out from the floc surface, but these are in sufficient quantities and would not cause settling problems in the activated sludge, creating a clear supernatant.

ii. **Pinpoint floc**: In this type of floc there are either no filaments present or a very few. The floc is very small and if the glycolax is not properly developed, the flocs are weak and easily sheared and broken. The large flocs settle and compact quickly while these smaller aggregates do not settle well creating a turbid supernatant.

iii. **Filamentous bulking sludge**: Here the filamentous organisms are predominant and they grow both inside and outside the flocs, penetrating the bulk solution. With this type of sludge, poor settling rates are known to occur. However when they do settle a clear supernatant is produced (Jenkins *et al.*, 1993).

1.4.4 Effect of filamentous organisms on activated sludge floc structure

The overall filamentous organism level in activated sludge is extremely important in determining its settling and compacting characteristics (Jenkins *et al.*, 1993). In operation of a treatment plant the principal aim is always the production of a clear effluent, this cannot always be achieved. Table 1.5 provides information on the problems
regarding solids separation in activated sludge. Two of the major problems affecting biological waste treatment today are filamentous bulking and foam production caused by filamentous microorganisms (Dueholm et al., 2001).

**Table 1.5. Causes and effects of activated sludge separation problems (Blitton, 1999)**

<table>
<thead>
<tr>
<th>Type of problem</th>
<th>Cause of problem</th>
<th>Effect of problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersed growth</td>
<td>Microorganisms do not grow flocs but are dispersed, forming only small clumps or single cells</td>
<td>Turbid effluent</td>
</tr>
<tr>
<td>Slime (jelly); Viscous bulking (also referred to as nonfilamentous resulting in bulking)</td>
<td>Microorganisms are present in large amounts of extracellular slime</td>
<td>Reduced settling and compaction rates. Virtually no solid separation.</td>
</tr>
<tr>
<td>Pin-floc (or pinpoint floc)</td>
<td>Small, compact, weak, roughly spherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly.</td>
<td>Low sludge volume index (SVI) and a cloudy, turbid effluent</td>
</tr>
<tr>
<td>Bulking</td>
<td>Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling</td>
<td>High SVI-very clear supernatant</td>
</tr>
<tr>
<td>Rising sludge</td>
<td>Denitrification causes release of N$_2$ gas which attaches to flocs and causes them to float to surface of clarifier</td>
<td>A scum of activated sludge forms on the surface of secondary clarifier</td>
</tr>
<tr>
<td>Foaming/scum formation</td>
<td>Caused by nondegradable surfactants or the presence of filamentous bacteria, such as <em>Nocardia</em> sp. or <em>Microthrix parvicella</em></td>
<td>Foam causes the activated sludge solids to float to surface of treatment unit. Foam accumulates and putrefies. Solids can also overflow into secondary effluent.</td>
</tr>
</tbody>
</table>
1.4.5 Bulking sludge

Bulking sludge may be defined as sludge which only settles slowly and compacts poorly because of an excessive growth of filamentous and/or zoogloeal organisms (Eikelboom and Buijsen, 1983). Bulking problems have been well documented (Wanner, 1997). A widely used sludge settleability parameter is the sludge volume index (SVI) (Casey et al., 1995). The following formula shows the way in which this parameter is determined.

\[ \text{SVI} = \frac{V + V \times 10^4}{\text{MLSS}} \]

where:
- \( V \) is the volume of settled sludge after 30 minutes (mL/L), and
- \( \text{MLSS} \) is the mixed liquor suspended solids (mg/L).

The SVI is expressed in mL per gram and is thus the volume occupied by one gram of sludge. A high SVI (>150mL/g) indicates bulking conditions, whereas an SVI below 70mL/g indicates the predominance of pin (small) flocs (Blitton, 1999).

1.4.6 Parameters affecting activated sludge performance

When designing and operating activated sludge systems a number of parameters must be taken into consideration to ensure that the biological treatment and organic removal rates operate at maximum efficiency. These parameters that were of importance include: sludge loading or \( S_0/X_0 \) ratio; sludge age, mixed liquor suspended solids and the wastewater characteristics.
1.4.6.1 Sludge loading or $S_0/X_0$

The $S_0/X_0$ ratio is the initial substrate concentration to the initial biomass concentration ($S_0/X_0$ as COD/biomass). At low ratios, a high amount of biomass is supplied with a low quantity of substrate and no or negligible cell multiplication takes place during exogenous substrate removal. Atkinson (1999) stated under these conditions complete organics oxidation takes place which results in an effluent of high quality and sludge flocculating and settling well. At higher ratios, a low amount of biomass is supplied with a higher quantity of substrate. The number of microorganisms increases during the exogenous substrate removal, which is indicated by a large reduction of COD and biomass growth (Chudoba et al., 1992). Also at this ratio the microorganisms do not form flocs and are generally dispersed which causes settleability problems (Atkinson, 1999).

1.4.6.2 Sludge age

The performance of activated sludge systems depends on the mean cell retention time (MCRT). MCRT is also referred to as solids retention time (SRT) or sludge age. When the well-formed flocs settle and separate from the effluent, a portion of this settled sludge would be wasted from the system. The fraction of solids which are wasted will determine the average amount of time which the biomass will occupy the reactor. The sludge age can therefore be defined as the mass of sludge in the reactor divided by the mass of sludge wasted per day (Atkinson, 1999).
1.4.6.3 Mixed liquor suspended solids (MLSS)

The MLSS is the concentration of suspended solids, which constitutes to the resident biomass. The value of the MLSS offers the systems operator a crude measure of the biomass contained within the process (Atkinson, 1999).

1.4.7 Wastewater characterization

Wastewater provides a source of substrate and nutrients for the microbial consortium of activated sludge and thus its composition must be monitored to obtain maximum process efficiency. Also in the design of the activated sludge process, the chemical characteristics of the wastewater to be treated are significant factors to be considered.

1.4.8 Thermophilic activated sludge treatment

In the Nordic countries, activated sludge is the most frequently applied treatment system in the pulp and paper industry (Saunamaki, 1997). Thus far, results concerning the application of activated sludge above temperatures of 40°C have been unsuccessful and sometimes contradictory. There appears to be two major characteristics of thermophilic activated sludge hindering its application, viz., i) an increased effluent turbidity (Rudolf and Amberg, 1953; Carpenter et al., 1968; Flippin and Eckenfelder, 1994; Visvanathan and Nhien, 1995) and ii) 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 earlier 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, Bastin et al. 1999) and high loading rates (Carpenter et al., 1968; Jackson, 1983; Duke et al., 1981 and 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°C-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°C-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.

1.4.9 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. An overview of these studies is depicted in Table 1.3. 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 difficulty. 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 Lepisto (1993)
and Jahren et al. (2002) mainly focused on COD removal. They showed that biofilm processes could 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; Berube and Hall, 1999; Ramaeckers et al., 2001). Membrane bioreactors have the advantage of 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 Berube 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 (Berube and Hall, 2000).

1.5 PROBLEM STATEMENT

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 treatment system for application in a pulp and paper mill. The purpose of this treatment system is to remove residual COD from the effluent using batch, fed-batch and continuous systems.

1.6 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 describes screening of thermophilic microorganisms for the degradation of effluent.

Chapter 3 evaluates a feasibility study of a thermophilic activated sludge process for the treatment of pulp mill effluent and optimization of physico-chemical parameters for enhanced degradation of effluent using batch experiments with pulp mill sludge and sewage sludge.
Chapter 4 describes a degradation of pulp mill effluent with pulp mill sludge and sewage sludge at thermophilic temperatures using fed-batch and continuous experiments.

Chapter 5 presents a general discussion of the obtained results.
CHAPTER 2: SCREENING OF THERMOPHILIC ORGANISMS FOR THE DEGRADATION OF PULP MILL EFFLUENT

2.1 ABSTRACT

The nutritional, temperature and pH requirements of four sources of thermophilic microorganisms (activated sludge, heating water, soil and compost) were studied. The screening procedure was performed on agar plates and involved three incubation temperatures (40°C, 50°C and 60°C), a pH range of 5-8, on five different media, viz., potato dextrose agar (PDA), tryptone soy agar (TSA), chemically defined medium (CDM), effluent, and effluent supplemented with the components of CDM. Activated sludge exhibited the best growth across the pH and temperature range investigated, and was also capable of growing well on minimal media, implying, that the thermophilic microorganisms from this source did not require nutrient supplementation to the same extent as the other sources. The thermophiles isolated from each source demonstrated that a neutral to basic medium was preferred to grow optimally. Across the temperature range, activated sludge had the most stable thermophilic microbial population. From the screening trials, activated sludge was the most suitable source of thermophilic microorganisms capable of degrading the bleach pulp mill effluent.
2.1 MATERIALS AND METHODS

Activated sludge (SAPPI plant), heating water (SAPPI-pilot plant), soil (samples from different locations of the SAPPI plant) and compost piles (Cato Ridge) were screened for thermophilic microorganisms. Samples from SAPPI were selected on the basis of obtaining thermophilic microbes with inherent capabilities for effluent degradation. Compost piles operate at a maximum of 80°C, which therefore serves as a rich source of thermophilic microbes.

The samples were screened for thermophilic microorganisms on agar plates, using Potato dextrose agar (PDA-Oxoid), Tryptone soy agar (TSA-Biolab) and a chemically defined medium (CDM) (Redman et al., 1999), raw bleach effluent supplemented with CDM components (Appendix 1) over a pH range from 5.0-8.0. Each sample of 100 μl volume was transferred in duplicate onto plates (pH 5.0-8.0) and then incubated for 24 h at 40°C, 50°C and 60°C respectively. Plates were then examined for microbial growth.
2.2 RESULTS AND DISCUSSION

The activated sludge sample from SAPPI-Enstra contained microorganisms that demonstrated good growth on PDA, TSA, CDM and CDM+Effluent at pH 5.0 across all three temperatures, however at 50°C and 60°C respectively, the effluent agar plates were unable to support any microbial growth. Microorganisms present in the heating water sample grew sparsely on PDA, TSA and CDM+Effluent (pH 5.0) across the temperature range, while the temperature range was unable to support any microbial growth on the CDM and Effluent medium. The soil samples contained microorganisms that demonstrated dense growth across the temperature range on TSA plates, and at 40°C on PDA plates only. Sparse growth was observed for the soil sample at: 50°C and 60°C on the PDA medium, 40°C on CDM plates and across all three temperatures for the CDM+Effluent agar plates. The microorganisms present in the compost sample exhibited dense growth at: 40°C, 50°C and 60°C on TSA plates, 40°C and 50°C on the PDA medium and at 40°C on the CDM+Effluent medium. No growth was observed at any of the temperatures on the effluent agar plates. The three temperatures supported sparse growth on the CDM plates (Table 2.1.)
### Table 2.1. Screening of thermophilic microorganisms from four sources on different media at pH 5.0

<table>
<thead>
<tr>
<th>Sources</th>
<th>Temperature (°C)</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>TSA</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>40</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>++</td>
</tr>
<tr>
<td>Heating water</td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td></td>
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</tr>
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<td></td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>+</td>
</tr>
</tbody>
</table>

- : no growth
+ : sparse growth (under 10 colonies)
++ : dense growth (entire plate covered)
Table 2.2. Screening of thermophilic microorganisms from four sources on different media at pH 6.0

<table>
<thead>
<tr>
<th>Sources</th>
<th>Temperature (°C)</th>
<th>Media</th>
<th>PDA</th>
<th>TSA</th>
<th>CDM</th>
<th>Effluent</th>
<th>CDM + Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge</td>
<td>40</td>
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<td>+</td>
<td>++</td>
<td>+</td>
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</tr>
</tbody>
</table>

- : no growth  
+ : sparse growth (under 10 colonies)  
++ : dense growth (entire plate covered)
Table 2.3. Screening of thermophilic microorganisms from four sources on different media at pH 7.0

<table>
<thead>
<tr>
<th>Sources</th>
<th>Temperature (°C)</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
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<td>Activated sludge</td>
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- : no growth
+ : sparse growth (under 10 colonies)
++ : dense growth (entire plate covered)
Table 2.4. Screening of thermophilic microorganisms from four sources on different media at pH 8.0.

<table>
<thead>
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<th>Sources</th>
<th>Temperature (°C)</th>
<th>Media</th>
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<td>CDM</td>
<td>Effluent</td>
<td>CDM + Effluent</td>
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<td>++</td>
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</tr>
</tbody>
</table>

- : no growth
+ : sparse growth (under 10 colonies)
++ : dense growth (entire plate covered)
Fig. 2.1. Thermophilic microorganisms (expressed as a percentage) isolated from the four sources at 40°C, 50°C and 60°C, respectively
At pH 6.0, the activated sludge sample exhibited good growth at all three temperatures on PDA, TSA, CDM and CDM+Effluent. Growth was sparse at 40°C and 50°C, and completely absent at 60°C on the effluent agar plates. Good growth was only observed at 40°C and 50°C on the PDA plates for the heating water sample. Yet again, the three temperatures were unable to support any microbial growth from the heating water sample on the effluent agar plates. Good growth (Soil sample) was prominent on: PDA and TSA plates at all three temperatures, CDM at 40°C and 50°C, and on the CDM+Effluent plates at 40°C only. The soil sample revealed no microbial growth at any of the three temperatures on the effluent agar plates. Microbial growth patterns on PDA, TSA and CDM at pH 6.0 for the compost sample was similar to the growth patterns at pH 5.0, with the exceptions being: i) sparse growth was observed at 40°C on the effluent agar plates and ii) better growth was observed at 50°C on the CDM+Effluent plates (Table 2.2.).

The three temperatures supported dense growth at pH 7.0 for the activated sludge sample on PDA, TSA, CDM and CDM+Effluent. At this pH (7.0) growth (activated sludge) was evident at all three temperatures on the Effluent agar plates. The heating water sample demonstrated good microbial growth: at all three temperatures on PDA and TSA plates, and at 50°C and 60°C on the CDM plates. Growth was absent (heating water) was observed on the effluent agar plates. Microbial growth from the soil sample was only absent at 50°C and 60°C on the effluent agar plates only. For the compost sample, microbial growth was evident
throughout the media and temperature range, with the exception of the effluent agar plates (Table 2.3.).

At pH 8.0, the activated sludge sample demonstrated identical growth patterns on the media across the temperature range as at pH 7.0. The heating water sample demonstrated good microbial growth: at 40°C on PDA, and at 40°C, 50°C and 60°C on TSA plates. Growth (heating water) was only absent at 50°C and 60°C on the Effluent agar plates. The soil sample also demonstrated similar good growth patterns (PDA, TSA and CDM) as at pH 7.0, with the only exception being that at 60°C on the PDA plate, growth was sparse. Growth was absent at all three temperatures on the effluent agar plates and at 60°C on the CDM+Effluent plate. Microorganisms contained within the compost sample were unable to grow at the three temperatures on the effluent agar plates and at 60°C on the CDM+Effluent plates (Table 2.4.).

Experimental research on industrial wastewater treatment with thermophilic aerobic bacteria was first reported about 50 years ago. Since then, the problem has attracted the attention of many researchers (Middlebrooks and Coogan, 1969; Loll, 1976; Ginnivan et al., 1981; Couillard and Zhu, 1993; Zvauya et al., 1994; Tripathi and Allen, 1998), but in spite of this, there is only limited knowledge of the microbiological aspects of thermophilic aerobic treatment. One problem is that each type of wastewater to be treated by this method requires a separate determination of the optimal process parameters and also the choice of the optimal composition of the mixed populations generally used (Cibis et al., 2002).
The biological treatment of fats and oils under thermophilic conditions is expected to be advantageous due to favourable changes in most physical properties of these hydrophobic compounds with increasing temperature. The melting point of fats and long-chain fatty acids is often well above ambient temperatures. Above their melting temperature, i.e. in the liquid state, these substances become more accessible to microorganisms and their lipolytic enzymes (Becker et. al., 1999). Becker et al. also showed that a newly isolated thermophilic aerobic bacterium *Bacillus thermoleovorans* IHI-91 had the potential of rapid and complete lipid degradation at 65°C and that the strain was also able and suitable for the treatment of lipid-rich wastewater exemplified by the high-strength wool scouring wastewater.

Limited information is available regarding the microbial diversity specifically supported by thermophilic aerobic wastewater treatment reactors. Of the attempts that have made to isolate cultures from thermophilic aerobic wastewater treatment reactors, only *Bacillus* and *Bacillus*-like organisms have been found. A bioreactor treating liquid manure was augmented with the thermophilic actinomycete *Thermomonospora fusca*, but process performance was essentially unaffected, indicating that the growth of the organism was not selectively favoured. The biology of thermophilic aerobic wastewater treatment reactors differs from conventional activated sludge microflora in that nitrifying bacteria, floc-forming organisms, or protozoa and other life forms are not present. Because the primary goal of thermophilic wastewater treatment process is to reduce the level of organic compounds in the waste stream the dominant microorganisms are almost always
aerobic heterotrophs. Of the previously described thermophilic organisms, therefore, only certain species of Bacillus, Thermus, and Actinomycetes can proliferate in these reactors. Thermophilic Bacillus spp. are a heterogeneous group which have been isolated from thermal areas such as hot springs and deep sea vents, as well as from non-thermal locations such as soils, composts, surface waters, sewage, and spoilt food. The distribution of Thermus spp. is considerably less ubiquitous. These bacteria are almost exclusively isolated from hot springs or deep sea vents although they have been found in domestic hot water heaters and composts. The thermophilic actinomycetes are not generally considered to grow well in liquid, and thus are not likely to be relevant to high wastewater treatment (Lapara and Alleman, 1999).

The result indicated that activated sludge was a good source of thermophilic microorganisms (Tables 2.1-2.4). TSA was the medium that supported the best growth at all three temperatures, probably due to the amount of nutrients, as it is a complex medium. Potato dextrose agar fairly supported thermophilic growth while the CDM was unable to support growth at 60°C. CDM is a minimal medium implying that its composition is known and that it does not have as many nutrients as complex media.

For the heating water sample, once again TSA supported the best thermophilic growth at all three temperatures (Tables 2.1-2.4). At 60°C, PDA was unable to support any microbial growth. The CDM revealed growth at all three temperatures.

In general, all inoculum sources exhibited growth over the pH and temperature range investigated. The activated sludge inoculum gave the best growth across the
temperatures and pH investigated (Tables 2.1-2.4). Heating water and compost tended to give less growth with CDM (a minimal medium) than with PDA and TSA. This would tend to indicate that these sources require more nutrients for optimal growth. Activated sludge gave similar growth with the minimal medium (CDM) as with the complex supplements (PDA and TSA). This indicates that activated sludge would probably not require nutrient supplementation to the same extent as the other inoculum sources. In general, good growth was observed across the pH range 6.0-8.0, whereas previous experiments gave no growth at pH 4.0 (unpublished data). This implied that these thermophiles preferred a neutral to basic medium to grow optimally.

At pH 6.0, effluent + CDM supported growth of thermophiles from all four inoculum sources at the three temperatures, however the effluent alone supported growth of the compost inoculum at 40°C and the activated sludge inoculum at 40°C and 50°C, respectively (Table 2.2). At pH 7.0, results were similar to pH 6.0, the exception being (i) that the effluent did not support growth of thermophiles from compost at all three temperatures, and (ii) growth was observed at 60 °C for the activated sludge sample (Table 2.3). At pH 8.0, effluent + CDM supported growth of thermophiles from all four sources, except for the soil sample at 60°C (Table 2.4). The effluent alone only supported thermophilic growth for the activated sludge sample at all three temperatures and at 40°C for the heating water sample only. In general, activated sludge once again proved to be the best inoculum. With the effluent alone, some growth was observed across the temperature and pH range investigated,
while with the effluent + CDM, good growth was observed across the pH and temperature range. Therefore, the activated sludge from SAPPI-Enstra plant was used as inoculum for further experiments.

The activated sludge sample contained the highest and most stable thermophilic microbial population (82.5 – 87.5%) across all three temperatures (Fig 2.1.). The thermophilic populations present in the soil and compost samples favoured growth at 40°C as compared to 50°C and 60°C, while the thermophiles present in the heating water sample grew at a constant rate across the three temperatures (47.5 – 52.5%). This confirms that activated sludge was a good source of thermophilic microorganisms.

Microbial diversity is a fundamental aspect of nature. The very fact that thermophiles can only manifest themselves in certain environments is clearly evident from this screening procedure.
CHAPTER 3: DEGRADATION OF PULP MILL EFFLUENT BY THERMOPHILIC ORGANISMS USING BATCH SYSTEMS

3.1 ABSTRACT

Batch studies were conducted with two types of activated sludge (pulp mill sludge and sewage sludge) at 40°C, 50°C and 60°C to determine the feasibility of thermophilic degradation of bleach pulp mill effluent. The effects of increasing aeration, biomass concentration and nutrient addition were the parameters investigated, to determine the maximum degradation. Preliminary batch studies confirmed the feasibility of thermophilic degradation, as COD removal achieved with the pulp mill sludge was 55.2%, 37.6% and 31.4% at 40°C, 50°C and 60°C after 5 days, respectively while the COD removal with sewage sludge was 50.2%, 37.3% and 26.98% under the same conditions. Degradation was further improved, using the same inocula in subsequent experiments and this confirmed that an acclimatization period is required, prior to degrading the bleach pulp mill effluent. A biomass concentration of 3.5 g/L with both sludge types demonstrated better COD removal as compared to a biomass concentration of 2.15 g/L. Increasing aeration (doubling the aeration) rates had a negligible effect on the final COD values. Additional nutrient supplementation had slight increase in COD removal as compared the standard nutrient supplementation. Maximum degradation occurred at 40°C for both sludge types (pulp mill sludge reduced the COD’s between 45% - 70.1%, while sewage sludge reduced the COD’s between 37.18% - 52.9%)
3.2 MATERIALS AND METHODS

3.2.1 Bleach pulp mill effluent

Bleach pulp mill effluent was obtained from SAPPI-Enstra (Springs, Gauteng), from the inlet to the primary clarifier on a weekly basis, and transported to the laboratory within two days in 25 L polypropylene containers. The effluent was stored at 4°C prior to use. Prior to experiments, urea and potassium di-hydrogen phosphate were added to give a COD: N: P ratio of 100:5:1 to simulate conditions at SAPPI-Enstra. The pH of the effluent was adjusted to 7.0 with either concentrated orthophosphoric (H₃PO₄) acid AR grade-Unilab or 10 M sodium hydroxide (NaOH).

3.2.2 Inocula

Pulp mill sludge inoculum was obtained from the return activated sludge plant at SAPPI-Enstra, Springs, Gauteng, South Africa while the sewage sludge inoculum was obtained from eThekwini Northern Waste Treatment Water Works, Kwa-Zulu Natal, South Africa.

Previously the inocula (pulp mill sludge) received from SAPPI-Enstra and eThekwini Northern Waste Treatment Works was stored at 4°C until required. There were strong indications, however, that the inoculum was turning non-viable/anaerobic. The storage techniques for the both sludges of biomass has been corrected and the inocula are now stored in a large aerated containers, fed daily with SAPPI-Enstra effluent. On receiving the pulp mill and sewage sludge from the respective plants, the sludges were immediately transferred to two 20 L glass fish
tanks. Fish tank (Petpro air pumps) (2 pumps having 4 ports for aeration) and a Rena pump connected to silicone tubing were used to aerate the fish tanks containing the pulp mill and sewage sludge. Viability of the sludge was maintained by removing 2 L of the contents from the fish tanks daily and adding 2 L of fresh pH adjusted effluent. The pH of the sludges were monitored daily. The viability of the sludges was maintained on a continuous basis in order to use the sludges as inocula for experiments. Biomass concentrations were maintained from 2 to 4 g/L. This system only served as a biomass reservoir and no scientific readings were recorded. The set-up for maintenance of inocula is shown in Fig 3.1.

Fig. 3.1. Schematic diagram of a fish tank used for the maintenance of inocula showing 1 and 2: air pump; 3: cover; 4: glass tank; 5: recirculating pump
3.2.3 Shake flask tests

Two potential problems were identified here. Firstly, the initial inocula for the shake flask tests were probably too small (below 0.5 g/L). Whilst this would not affect the ultimate degradation, a lengthy retention time would be required to reach maximum degradation. This was corrected by increasing the biomass concentration in the inoculum reservoir, and subsequently ensuring (by calculation) that the inoculum concentration in the flasks was at least 2 g/L to 4 g/L.

The second problem concerned the availability of oxygen in the shake flasks. It was strongly suspected that the rate of oxygen transfer in the previous shake flask trials was insufficient to maintain aerobic growth. Whilst the shake flask trials were carried out strictly according to the "standard" protocol for shake flask trials (150 rpm on a shaking incubator), the biomass concentrations in the current trials were significantly higher than that in "standard" shake flask trials. Accordingly, while agitation may be an adequate means to aerate "standard" shake flask experiments, it is most probably inadequate for the higher biomass concentrations used here.

To overcome the above, flasks were aerated, after being heated to the appropriate temperatures in water baths. Initially compressed oxygen was used for aeration, and thereafter "fish tank" aerators replaced this, which facilitated good mixing for heat transfer. De-ionised water was added to the flasks to maintain the liquid volume, due to evaporation losses.
3.2.4 Batch cultivation trial at thermophilic temperatures

Experiments A and B (as described below) were performed to assess the feasibility of degradation of pulp mill effluent degradation.

3.2.4.1 Experiment A

SAPPI – Enstra untreated bleach pulp mill effluent (pH 7.0) was transferred into 2 L Erlenmeyer flasks (working volume, 1 L) and inoculated with pulp mill sludge and sewage activated sludge (2.5 g/L). The inoculated flasks were incubated at 40°C, 50°C and 60°C in water baths. The volumes of the flasks were also controlled daily, to overcome evaporative losses, by the addition of deionised water. COD analyses were performed daily after inoculation for a period of 5 days.

3.2.4.2 Experiment B

Flasks containing untreated bleach pulp mill effluent (pH 7.0) were inoculated with the pulp mill sludge and sewage sludge developed from experiment A, and this was accomplished by centrifuging the entire contents of the flasks in experiment A and reconstituting the pellets (biomass) only, in effluent as experiment B. The flasks were incubated at 40 °C, 50°C and 60°C. Samples were removed on a daily basis and COD analyses were performed over a 5 day period.
3.2.5 Optimisation studies for enhanced degradation of pulp mill effluent using batch experiments

3.2.5.1 Effect of biomass concentration on degradation of pulp mill effluent

To determine the effect of increasing biomass concentration on degradation of pulp mill effluent, flasks were inoculated with two biomass concentrations of 2.15 g/L and 3.5 g/L from both activated sludges for the degradation of pulp mill effluent. The pH of the effluent was maintained at 7.0 throughout the experiments. Flasks were incubated at 40°C, 50°C and 60°C in separate water baths. COD analyses were performed daily after inoculation over 5 days. The optimum biomass concentration of inocula was fixed in subsequent experiments.

3.2.5.2 Effect of aeration on degradation of pulp mill effluent

Flasks containing effluent, pulp mill sludge and sewage sludge, respectively were incubated at 40°C, 50°C and 60°C in water baths. Aeration was varied as follows: i) single pump aeration (referred to as normal aeration-NA), and ii) two pump aeration (referred to as double aeration-DA). The biomass concentrations of both sludges were maintained at 3.5 g/L. Samples were removed daily over a 5 day period and COD values were determined.

3.2.5.3 Effect of nutrient addition on degradation of pulp mill effluent

Prior to biological treatment of the effluent at the SAPPI-Enstra plant, nutrients containing nitrogen (Urea) and phosphate (Phosphoric acid) are added to give a COD:N:P ratio of 100:5:1. This was done so that the food to microorganisms
ratio creates an environment favourable to the floc forming organisms versus filamentous organisms, which in turn provides good settling characteristics for the sludge.

Flasks containing both sludge types respectively were incubated at 40°C, 50°C and 60°C. Nutrient additions were varied as follows: i) COD:N:P at a ratio of 100:5:1, referred to as standard nutrients (SN) which is applied at SAPPI-Enstra, and ii) COD:N:P at a ratio of 100:10:2, referred to as double nutrients (DN). The biomass concentrations of both sludges maintained was 3.5 g/L. Samples were removed daily over a 5 day period and COD values were determined.

3.2.6 Analytical methods

3.2.6.1 Mixed liquor suspended solids (MLSS)

MLSS is defined as the total amount of organic and mineral suspended solids contained in the mixed liquor of the activated sludge reactor. This value offers the system operator a crude measure of the biomass contained within the process. Refer to Appendix 2.

3.2.6.2 COD analyses

COD is the measure of the oxygen equivalent (expressed as mg/L) of the oxidisable material content of a sample that is susceptible to oxidation by a strong chemical oxidant. This method employs a standard potassium dichromate solution as the oxidant in the presence of sulphuric acid and silver sulphate catalyst. The chloride
interferants are removed with mercuric sulphate and the residual dichromate is back titrated with ferrous ammonium sulphate. Refer to Appendix 2.

3.3 RESULTS AND DISCUSSION

3.3.1 Experiment A vs Experiment B

Experiment A was conducted to determine whether aerobic thermophilic treatment of bleach pulp mill effluent was feasible. The flasks inoculated with pulp mill sludge at 40°C demonstrated a decrease in COD after 24 hours (708 mg/L), and a final COD value of 420 mg/L was obtained after 5 days (Fig. 3.2). Flasks inoculated with pulp mill sludge at 50°C and 60 °C also show decreased COD values, at a gradual rate finally reduced to 572 mg/L at 50°C and 650 mg/L at 60°C by day 5. This confirmed that degradation does occur at thermophilic temperatures, however not as efficient as at 40°C. The SAPPI activated sludge plant operates between 25°C-37°C and therefore degradation is more pronounced at 40°C, as compared to 50°C and 60°C.

The overall % COD removal for pulp mill sludge varied from 24.5-55.2%, 17.03-37.6%, and 6.12-31.4% for the batch systems operating at 40°C, 50°C and 60°C respectively. The % COD removal of the batch system inoculated with pulp mill sludge at 50°C and 60°C were significantly lower than the batch system at 40°C. These results are coincide with previous findings of Tripathi and Allen (2000), for bleach pulp mill effluent studied at 45°C, 55°C and 60°C in a sequencing batch reactor (SBR). From the results it indicates that 40°C seems to be the ideal
temperature for degradation of bleach pulp mill effluent. Tripathi and Allen (2000) achieved % COD removal at the following temperatures: 45°C (63±8%), 55°C (52±8%) and 60°C (50±6%), while operating a SBR over 40 weeks.

The flasks inoculated with sewage sludge were able to degrade effluent to 468 mg/L at 40°C, 580 mg/L at 50°C and 690 mg/L at 60°C by day 5 (Fig 3.3.). The overall % COD removal for the batch system inoculated with sewage sludge varied from: 21.5-50.2%,
Fig. 3.2. COD removal and degradation profiles of batch experiment A using pulp mill effluent inoculated with pulp mill sludge at thermophilic temperatures
Fig. 3.3. COD removal and degradation profiles of batch experiment A using pulp mill effluent inoculated with sewage sludge at thermophilic temperatures.
15.5-37.3% and 3.4-26.98%, for 40°C, 50°C and 60°C, respectively. The same trends were observed with both sludge types, i.e., higher temperature results in decreased COD removal. From the results, pulp mill sludge demonstrated higher COD removal than sewage sludge at 40°C, 50°C and 60°C. Due to acclimatisation factors it was expected that sewage sludge being sourced from a domestic wastewater treatment facility would not degrade the effluent as efficiently as pulp mill sludge. A subsequent concern on the viability of pulp mill sludge in this study was questioned as the sludge was normally in transit for 48 hours prior to experimentation. Studies conducted by Tripathi and Allen (2000) indicated that organisms present in activated sludge required an acclimatisation period prior to degrading the bleach pulp mill effluent efficiently at thermophilic temperatures.

The two sludge types (pulp mill sludge and sewage sludge) used in experiment A served as the inocula for experiment B. The flasks with pulp mill sludge were able to degrade the pulp mill effluent much better as compared to experiment A. After 5 days the COD’s were reduced to 374 mg/L, 465 mg/L and 578 mg/L at 40°C, 50°C and 60°C, respectively, while after 5 days sewage sludge reduced the COD’s to 445 mg/L, 539 mg/L and 625 mg/L at 40°C, 50°C and 60°C respectively (Figs. 3.4. & 3.5.). The overall % COD removal for pulp mill sludge was: 21.1-59.8%, 11.1-48.4% and 6.5-37.2% at 40°C, 50°C and 60°C respectively, while the overall % COD removal for sewage sludge was: 17.6-52.4%, 8.2-41.1% and 2.7-32.4% at the same temperatures. In comparison to the results of experiment A with pulp mill sludge, experiment B with pulp mill sludge demonstrated an increase in COD removal (±
4.6% at 40°C, ± 10.8% at 50°C and ± 5.8% at 60°C). The sewage sludge in experiment B compared to the sewage sludge in experiment A also demonstrated an increase in COD reduction at all of the temperatures (2.2% at 40°C, 3.8% at 50°C and 5.42% at 60°C). The increase in degradation at all three temperatures for both sludge types in experiment B as compared to experiment A confirms that better or improved degradation is attributed to an acclimatisation period. Pulp mill sludge was still the better inoculum of the two demonstrating an increase of COD removal at each of the temperatures investigated (7.4% at 40°C, 7.3% at 50°C and 4.8% at 60°C).
Fig. 3.4. COD removal and degradation profiles of batch experiment B using pulp mill effluent inoculated with pulp mill sludge at thermophilic temperatures
Fig. 3.5. COD removal and degradation profiles of batch experiment B using pulp mill effluent inoculated with sewage sludge at thermophilic temperatures
higher COD removal at each temperature. It is clearly evident that an acclimatization process is required for efficient COD removal as the inocula demonstrated better degradation as compared to experiment A.

3.3.2 Effect of biomass concentration on degradation of pulp mill effluent

The experiments were conducted to determine whether the effluent degradation was enhanced with increasing the biomass concentrations of both sludges. The flasks inoculated with pulp mill sludge (2.15 g/L) at 40°C, 50°C and 60°C were able to reduce the COD's to 420 mg/L, 552 mg/L and 580 mg/L, respectively (Fig. 3.6.). The flasks inoculated with pulp mill sludge (3.5 g/L) after 5 days reduced COD's to 397 mg/L, 510 mg/L and 565 mg/L (Fig. 3.7.). The overall % COD removal for pulp mill sludge with a biomass concentration of 2.15 g/L observed was 29.32 - 45% at 40°C, 23.58 - 37.82% at 50°C and 19.74 - 31.41% at 60°C, and with 3.5 g/L % COD removal was 42.6 - 54.26% at 40°C, 32.02 - 40.83% at 50°C and 24.57 - 35.43% at 60°C.

The overall % COD removal for sewage sludge with a biomass concentration of 2.15 g/L demonstrated: 28.93 - 37.18% at 40°C, 20.1 - 33.63% at 50°C and 16.6 - 27.79% at 60°C, with 3.5 g/L demonstrated: 35.9 - 46.59% at 40°C, 26.47 - 36.76% 50°C and 18.06 - 30.98% 60°C (Figs. 3.8. & 3.9.).

Pulp mill sludge showed better effluent degradability as compared to sewage sludge at each of the biomass concentrations investigated (At a biomass of 2.15 g/L pulp mill sludge degradation increased by 7.82% at 40°C, 4.19% at 50°C and 3.62%
at 60°C, while at a biomass concentration of 3.5 g/L pulp mill sludge degradation increased by 7.67% at 40°C, 4.07% at 50°C and 4.45% at 60°C, respectively. It can be concluded that biomass concentration is a critical parameter for the effluent degradation. Improved degradation could be obtained, if the biomass was subjected to a longer acclimatization period. The best effluent degradation was observed at 40°C as compared to 50°C and 60°C with pulp mill and sewage sludge.

Activated sludge plants at UK paper mills typically operate at mean organic loading rates ranging from 0.07 to 0.21 kg BOD/kg mixed liquor suspended solids (MLSS). One critical operational aspects of an activated sludge plant is maintaining a good settling sludge. Poor settlement (bulking) has caused operational problems since the 1920’s and activated sludge plants treating paper mill wastewaters are prone to this. Bulking creates problems in ensuring that the correct amount of sludge is recycled back to the aeration tank to maintain the MLSS at a sufficient concentration to guarantee efficient treatment (Thompson et al., 2001).
Fig. 3.6. COD removal and degradation profiles of batch experiment using pulp mill effluent inoculated with pulp mill sludge having a biomass concentration of 2.15 g/L at thermophilic temperatures.
Fig. 3.7. COD removal and degradation profiles of batch experiment using pulp mill effluent inoculated with pulp mill sludge having a biomass concentration of 3.5 g/L at thermophilic temperatures.
Fig. 3.8. COD removal and degradation profiles of batch experiment using pulp mill effluent inoculated with sewage sludge having a biomass concentration of 2.15 g/L at thermophilic temperatures
Fig. 3.9. COD removal and degradation profiles of batch experiment using pulp mill effluent inoculated with sewage sludge having a biomass concentration of 3.5 g/L at thermophilic temperatures
3.3.3 Effect of aeration on degradation of pulp mill effluent

The percentage COD removed with pulp mill sludge using normal aeration from day 1-5, was 53.01 - 66.47% at 40°C, 32.01 - 55.87% at 50°C and 19.89 - 38.07% at 60°C while the injection of double aeration showed 50.02 - 66.95% at 40°C, 27.78 - 57.78% at 50°C and 22 - 39% at 60°C (Fig. 3.10.). In terms of % COD removed, doubling the aeration had a minimal increase in % COD removal as compared with normal aeration. This implies that the system was not a retardation factor in terms of COD removal. The same trend was true for sewage sludge, having % COD removal with normal aeration observed at: 37.36 - 50.5% at 40°C, 25.77 - 38.6% at 50°C and 20.44 - 31.5% at 60°C, while doubling the aeration showed: 38.99 - 51.91% at 40°C, 26 - 41.77% at 50°C and 21.72 - 33.29% at 60°C (Fig. 3.11.). When comparing both sludge types, it is evident that pulp mill sludge is most suitable for the bleach pulp mill effluent degradation.

From these results, it can be concluded that increasing the aeration rate has a negligible effect on the final COD value. Rozich and Colvin (1997) have recommended utilizing aggressive aeration equipment and greater tank depth to accommodate the potential enormous oxygen requirements of thermophilic treatment processes. The simultaneous requirements for rapid and high efficiency oxygen transfer make the selection of aeration equipment one of the most critical process design choices. However the results obtained on the batch studies are contradictory and this could be due to operating these experiments on such a small scale.
3.3.4 Effect of additional nutrients on degradation of pulp mill effluent

The permeate COD's obtained with double nutrient addition are slightly greater than that obtained with the normal nutrient addition. This indicates that the system was not being limited by nutrients. The COD's obtained using sewage sludge from eThekwini Northerns Waste Water Treatment Works are slightly lower than that obtained with Enstra’s pulp mill sludge, possibly indicating that the inoculum used could have an effect on the degradation. At 40°C, 50°C and 60°C, the degradation obtained with sewage sludge was somewhat lower than that obtained with the pulp mill sludge.
Fig. 3.10. Effect of aeration on COD removal and degradation profiles of pulp mill effluent using pulp mill sludge as inocula at thermophilic temperatures
Fig. 3.11. Effect of aeration on COD removal and degradation profiles of pulp mill effluent using sewage sludge as inocula at thermophilic temperatures.
The experiments were conducted to determine whether the additional nutrients supplementation further enhanced effluent degradation. The overall % COD removal for standard nutrients using pulp mill sludge and sewage sludge obtained was: 51.98 - 68% and 38 - 52% at 40°C; 31.92 - 46.92% and 26.7 - 43.7% at 50°C, 25.05 - 36.01% and 21.24 - 34.95% at 60°C respectively (Figs. 3.12. & 3.13.). With additional nutrient supplementation (i.e. doubling the nutrients) with pulp mill sludge and sewage sludge as inocula, the percentage COD removal observed was: 53.83 - 70.1% and 38.8 - 52.9% at 40°C, 32.25 - 47.31% and 26.95 - 44.09% at 50°C, 25.93 - 37.5% and 21.56 - 36.15% at 60°C. Additional nutrient supplementation had a slight increase in % COD removed as compared to standard nutrients for both sludge types. Although the nutrient supplementation varied, the batch systems at 40°C still performed better as compared to 50°C and 60°C. For future experiments standard nutrients was implemented, as it was not limiting the batch systems. Comparing both sludges in terms of COD removal, pulp mill sludge yet again proved to be the ideal inoculum for the treatment of bleach pulp mill effluent.

The unique nutritional requirements of thermophilic organisms, are likely responsible for the varying success of previous researchers treating different wastes at different temperatures. The researchers reporting good results generally treated complex wastes likely to contain sufficient micronutrients or provided supplemental nutrients. For research purposes, the addition of complex nutrients such as peptone or yeast extract is usually sufficient to enable biodegradation of most readily degradable
compounds. Further work is necessary to identify specific nutrient supplements suitable for full-scale operation.

Fig. 3.12. Effect of nutrient supplementation on COD removal and degradation profiles of pulp mill effluent using pulp mill sludge at thermophilic temperatures
Fig. 3.13. Effect of nutrient supplementation on COD removal and degradation profiles of pulp mill effluent using sewage sludge at thermophilic temperatures.
3.3.5 Batch experiments leading to fed-batch experiments

The batch trials was conducted in order to develop a semi continuous system using pulp mill sludge and sewage sludge as inocula for the degradation of effluent at thermophilic temperatures. Degradation occurred within 2 h, while increased COD removal was observed within 24 h (Fig. 3.14.). But there was no significant increase in COD removal observed after 48 h incubation as compared to 24 h for both sludges. The overall % COD removal for flasks inoculated with pulp mill sludge after 48 h.
Fig. 3.14. COD removal and degradation profiles of batch experiments with pulp mill sludge and sewage sludge as inocula leading to fed-batch experiments
were: 6.5 - 58.69% at 40°C, 3.57 - 42.14% at 50°C and 3.19 - 35.11 at 60°C, respectively; while the overall % COD removal for sewage sludge varied: 4.12 - 41.24% at 40°C, 2.74 - 36.84% at 50°C and 1.04 - 26.04% at 60°C respectively.

Based on the above results, it was deduced that experiments be shifted from a batch to a fed-batch cultivation system, as described in chapter 4.
CHAPTER 4: FED-BATCH AND CONTINUOUS SYSTEMS FOR THE DEGRADATION OF PULP MILL EFFLUENT BY THERMOPHILIC ORGANISMS

4.1 ABSTRACT

The biodegradation of bleach pulp mill effluent was studied under aerobic thermophilic conditions (40°C, 50°C and 60°C) using pulp mill sludge and sewage sludge for the fed-batch systems, while only pulp mill sludge was used as the inoculum for the continuous system. Aeration, biomass concentration and nutrient addition were optimized on batch systems, prior to operating the fed-batch and continuous systems. Effluent treated with pulp mill sludge in the fed-batch system had an average COD removal of 76.9% at 40°C, 45.8% at 50°C and 28.23% at 60°C, while the fed-batch system inoculated with sewage sludge had an average COD removal of 60.69% at 40°C, 39.9% at 50°C and 22.04% at 60°C. The continuous system treated with pulp mill sludge only, had an average maximum COD removal of 84.98% at 40°C, 62.83% at 50°C and 49.35% at 60°C, respectively.
4.2 MATERIALS AND METHODS

4.2.1 Bleach pulp mill effluent

Bleach pulp mill effluent was obtained from SAPPI-Enstra (Springs, Gauteng), and maintained as described in section 3.2.1

4.2.2 Inocula for fed-batch experiments

Pulp mill sludge and sewage sludge inocula was obtained and maintained, as described in section 3.2.2

4.2.3 Inoculum for the continuous experiments

Pulp mill sludge was used as the inoculum in the continuous reactor. The pulp mill sludge was maintained as described in section 3.2.2

4.2.4 Fed-batch experiments

The experiments were conducted in Erlenmeyer flasks (2 L) at various temperatures such as 40°C, 50°C and 60°C. The activated sludge from SAPPI-Enstra plant was used as inoculum. Each flask contained a 2 L volume of effluent (pH adjusted to 7.0 with either concentrated H₃PO₄ AR grade-Unilab or 10 M NaOH, depending on original pH of effluent) together with the pulp mill sludge and sewage sludge, which was inoculated, so as to give an approximate initial biomass concentration of 3.5 g/L. A fish tank pump (Petpro aquarium air pump-Life 104) connected with silicone tubing (4 mm diameter) was used to supply air to the Erlenmeyer flasks (aerated flasks). All flasks were incubated at the specified temperatures in circulating water baths (NewBrunswick, Scientific, USA: dimensions...
of 900 mm x 600 mm). The experimental setup for fed-batch experiments is shown in Fig. 4.1.

The volumes of each flask were adjusted daily with distilled water to maintain a constant volume of 2 L, to overcome evaporation. The contents of each flask was then homogenized on a magnetic stirrer (Snijders hotplate magnetic stirrer) at maximum speed, and 12 mL was then decanted into 50 mL Greiner centrifuge tubes and centrifuged (Eppendorf) for 10 min at 4000 rpm. The supernatant (4 mL) was then added to 16 mL of distilled water (final volume of 20 mL) and then used for COD determinations. The pellet and the remainder of the supernatant were homogenised and returned to the respective flasks.

The suspension was allowed to settle each day (≈ 20 min) and 1 L from each flask was discarded. Fresh effluent (pH 7.0) was then added to each flask to replace the discarded volume. The flasks were then returned to their respective incubation water baths. Due to the poor settleability of the sludge at 50°C and 60°C, 1 L aliquots from each of these flasks were centrifuged at 12 000 rpm for 1 min (supernatant was discarded) and the pellet was reconstituted with fresh pH adjusted effluent (1 L) and returned to respective flasks. After 72 days of incubation, sludge was wasted in each of the flasks to give a final biomass concentration of 3.5 g/L. Samples were then centrifuged immediately after collection to remove biomass, then stored at 4°C prior to analysis.
4.2.5 Continuous experiments

4.2.5.1 Reactor system

The air lift reactor (ALR), used in this study, is illustrated in Fig. 4.2. The reactor was made of glass with a height of 50 cm and a diameter of 6 cm, together with a concentric draft tube of 40 cm in height and a diameter of 2.5 cm with a working volume of 1 L. The reactors were designed and fabricated indigenously at M/s Siegling glass works, Durban, South Africa. Two aeration ports of a fish tank pump (Petpro aquarium air pump-Life 104) connected with silicone tubing (4 mm diameter) were used to supply air to the reactor at a rate of 1.7 l/min. Effluent was fed into the reactor with a peristaltic pump (Watson-Marlow 313S) at a flow rate of 104 ml/hr.

4.2.5.2 Degradation of effluent

The ALR with 1 L of effluent was inoculated with pulp mill sludge to give a final biomass concentration of 3.5 g/L. Experiments were conducted at 40°C, 50°C and 60°C, respectively. Pulp mill sludge was maintained in semi-continuous mode at each of the temperatures prior to the start-up of the continuous experiments. When a steady state was reached, samples were withdrawn from the draft tube for COD analysis every 24 h. The influent was then fed continuously into the reactor through a peristaltic pump. Soya bean oil was used as an anti-foam agent and was added at a level of 0.1% to the feed (influent). The overflow was collected constantly using a peristaltic pump. The reactor was operated for a period of 15 days at each of the above temperatures.
4.2.6 Analytical methods

COD analyses and MLSS were also conducted as described in section 3.1.6.

Fig. 4.1. Experimental set-up for the fed-batch experiments
Fig. 4.2. Schematic diagram of the air lift reactor showing

1 and 4: air pump; 2: feed or influent; 3: peristaltic pump; 5: inlet; 6: outlet;
7: recycle tube; 8: reactor; 9: concentric draft tube; 10: effluent
4.3 RESULTS AND DISCUSSION

4.3.1 Fed-batch experiments

Fluctuations in COD values were evident at all temperatures for a period of 40 days. Thereafter, a relatively stable COD level was maintained. Effluent degradation was prevalent at all temperatures, however, that obtained at 50°C and 60°C was low (Figs. 4.3. -4.5.).

The fed-batch systems using pulp mill sludge at 40°C had an average % COD removal of 76.9 % which was significantly greater than the average % COD removal for sewage sludge (60.69%) at 40°C (Fig. 4.6.). After 40 days, a steady state was achieved with both sludges. As previously described in the batch systems pulp mill sludge was the better inoculum, as was the case in the fed-batch systems. From day 1 to day 40, there are very fluctuating COD values, however this system at 40°C had better % COD removal as compared to 50°C and 60°C (Figs. 4.7. & 4.8.). In this period of time, the feed was very crucial to the % COD removed. The same trend was observed at 50°C and 60°C for both sludge types, however better degradation was observed at 40°C with both sludges. The average % COD removal at 50°C for pulp mill sludge and sewage sludge was 45.8% and 39.9%, respectively, while the average % COD removal at 60°C for pulp mill sludge and sewage sludge was 28.23 % and 22.04%. The fed-batch systems at 40°C also degraded the effluent as optimally as the batch systems and this confirms that 40°C is the desired temperature for efficient degradation, by the thermophilic organisms present in both sludges.
The results of COD removal indicated a poor performance prior to high temperature acclimatization. Increased operating temperature has been suggested to improve the removal of specific compounds like methanol, phthalate and lipids. Low COD removals have been explained by reduced diversity of the thermophilic microbial community and increased part of recalcitrant COD (Suvilampi et al., 2003)

Tripathi and Allen (1999) demonstrated, the results of COD removal indicated a poor performance during high temperature acclimatisation. During acclimatisation the COD removal was 45 – 60% in contrast to 60 – 76% at steady state; the poorer COD removal also coincided with deterioration in sludge settling characteristics during acclimatisation or transient change of temperature. The COD removal % varied from 40 - 76% with steady state averages of 75 ± 9.7%, 73 ± 10%, 62 ± 9% and 63 ± 10% for sequencing batch reactors operating at 35°C, 45°C, 55°C and 60°C, respectively. The COD removal was significantly higher at 35°C and 45°C in comparison with 55°C and 60°C.
Fig 4.3. COD removal using a fed-batch system at 40°C with pulp mill and sewage sludge as inocula
Fig 4.4. COD removal using a fed-batch system at 50°C with pulp mill and sewage sludge as inocula
Fig 4.5. COD removal using a fed-batch system at 60°C with pulp mill sludge and sewage sludge as inocula leading to fed-batch experiments
Fig 4.6. Degradation profiles of fed-batch experiments at 40°C with pulp mill sludge and sewage sludge as inocula.
Fig 4.7. Degradation profiles of fed-batch experiments at 50°C with pulp mill sludge and sewage sludge as inocula.
Fig 4.8. Degradation profiles of fed-batch experiments at 60°C with pulp mill sludge and sewage sludge as inocula.
Fig 4.9. COD removal, pH, MLSS and degradation profiles of the continuous reactor operating at 40°C with pulp mill sludge as inoculum
Fig 4.10. COD removal, pH, MLSS and degradation profiles of the continuous reactor operating at 50°C with pulp mill sludge as inoculum
Fig 4.11. COD removal, pH, MLSS and degradation profiles of the continuous reactor operating at 60°C with pulp mill sludge as inoculum
4.3.2 Continuous experiments

Pulp mill sludge was the inoculum of choice for the continuous experiments, judging its performance on batch and fed-batch systems. From the results, the continuous experiments showed better degradation than the batch and fed-batch systems across all three temperatures. Degradation occurred immediately across the three temperatures and this could be attributed to the acclimatization phenomenon. The reactor at 40°C (average feed of 979 mg/L) had a COD of 430 mg/L with a final value of the 150 mg/L achieved after 15 days (Fig 4.9.). The biomass concentration increased by 1.204g at the end of the experiments. The pH of the reactor at 40°C fluctuated between 7.0 - 8.07.

The reactor at 50°C (average feed of 1103 mg/L) was able to reduce the COD to 650 mg/L after 1 day and after 15 days; the COD’s were reduced to 430 mg/L (Fig 4.10.). An increase in biomass concentration (0.997 g) was also observed. The pH of the reactor at 50°C fluctuated between 6.7 – 7.6.

The reactor at 60°C (average feed of 955 mg/L) was able to reduce the COD to 810 mg/L after 1 day and after 15 days the COD’s were reduced to about 411 mg/L (Fig 4.11.). The biomass concentration increased by 0.565g at the end of the experiments. The pH in the reactor at 60°C fluctuated between 5.02 – 7.99.

The average maximum % COD removal for all three systems was 84.98% at 40°C, 62.83% at 50°C and 49.35% at 60°C. Degradation proceeded rapidly from day 1 as compared to batch and fed-batch systems. Maximum degradation was maintained
after 10 days. The continuous system also degraded the effluent more efficiently at 40°C, which clearly indicates that with all three experimental setups (Batch, Fed-batch and continuous) the continuous reactor at 40°C operated optimally. There was no direct correlation between pH and COD removal as the pH was corrected daily. The biomass grew better at 40°C as compared to the other temperatures, and at this temperature, maximum degradation was also observed. A possible reason for the poorer COD removal in the 50°C and 60°C reactors could be due to an inadequate thermophilic population being prevalent in the systems.

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 (Vogelaar et al., 2002)

Sagastume and Allen (2003) investigated the effects of temperature variations on aerobic biological wastewater treatment with respect to treatment efficiency, solids discharges, sludge physicochemical properties and microbiology. The effects of controlled temperature shifts (from 35°C to 45°C; and from 45°C to 35°C) and periodic temperature oscillations (from 31.5°C to 40°C, 6-day period, over 30 days) were assessed in 4 parallel, lab-scale sequencing batch reactors (SBRs) that treated pulp and paper mill effluent. Overall, the temperature shifts caused higher effluent
suspended solids (ESS) levels (25 –100 mg/L) and a decrease (up to 20%) in the removal efficiencies of soluble chemical oxygen demand (SCOD).

Ahn and Foster (2002) carried out a study to assess the effect of temperature variations, both decreasing and increasing, on the performance of a mesophilic (35°C) and a thermophilic (55°C) upflow anaerobic filter treating a simulated papermill wastewater. The temperature increase had a more severe effect on the mesophilic microorganisms than the temperature decrease. Thermophilic microorganisms appeared to be more resilient to the temperature increase than mesophilic microorganisms.

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 Markl (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.
CHAPTER 5: GENERAL CONCLUSIONS

Bleach pulp mill effluents comprise primarily of chlorinated organic matter, which has attracted considerable concern about the effects of these compounds on the environment. Some of these compounds are known to be toxic, mutagenic, persistent, bioaccumulating and are also thought to cause harmful disturbances in biological systems. Chlorinated organic material cannot be eliminated from bleach plant effluents as there is no alternative to chlorine which is used in the bleaching of pulp, and a ban of the use of chlorine would result in many pulp mills being shutdown which would then finally result in an enormous economic and social effect.

The toxicity of bleach plant effluents have been known for a while, however once the effluent is neutralized and diluted sufficiently with the recipient waters, the toxicity disappears. More stringent regulations on effluent quality entering recipient waters are being faced by the pulp and paper industries worldwide. After complete biological treatment, the pulp and paper mill effluent can contain appreciable concentrations of COD. COD is most often used to determine the amount of pollutants in waste and natural waters and this technique was employed throughout the biodegradation studies.

Thermophilic aerobic wastewater treatment is a new evolving technology that has gained much interest as years go on. This technology is doing so because, the energy and water prices pulp and paper mill industries face are extremely high. The
reuse of process water is of high interest to the industry therefore a thermophilic treatment would be of great benefit.

In chapter 2, a screening procedure determined that the microorganisms from activated sludge (from SAPPI-Enstra plant) was a suitable inoculum for the thermophilic degradation of bleach pulp mill effluent, as it was the only source that had the best growth across the pH and temperature range investigated. However further studies should be conducted on the other sources, as they demonstrated potential for thermophilic degradation, judging by the growth patterns on agar plates. Optimizing growth conditions for the other sources of thermophiles could prove to be beneficial to thermophilic degradation, as nutrients, pH, temperature and oxygen demands varies among microorganisms.

Activated sludge comprises of mixed consortia of microorganisms and as it was already acclimatised to the bleach pulp mill effluent, therefore it was able to grow on the agar plates prepared from the effluent. At the SAPPI-Enstra plant, nutrients (Urea and phosphoric acid) are supplied to the effluent prior to efficient biological treatment. In this study, good growth (activated sludge) was observed on the effluent plates supplemented with the constituents of CDM. This implied that minimal nutrient supplementation enhanced growth. A neutral to basic pH would be suitable for thermophilic degradation.

The batch tests (chapter 3) provided insight to the feasibility of thermophilic degradation of bleach pulp mill effluent. The pulp-mill and sewage sludge treatment
of the effluent did significantly reduce the COD in the batch systems. A higher biomass concentration enhanced degradation in this study, however this cannot be confirmed, as viability tests were not carried out on the sludges. Increasing aeration and nutrient supplementation did not significantly enhance degradation. Probably scaling-up of these batch systems would prove otherwise.

Maximum degradation occurred at 40°C for both sludge types. The higher temperatures did not have a profound effect on COD removal. The maximum permissible or optimal temperature for thermophilic degradation of bleach pulp mill effluent to reduce COD efficiently was 40°C. Pulp mill sludge degraded the effluent much better as compared to sewage sludge. Batch systems demonstrated that an acclimatisation process is required for efficient degradation as well as that the system should be shifted to a fed-batch system to achieve better COD removal.

In chapter 4, the fed-batch and continuous systems replicated similar results in COD removal at the three temperatures, as the batch systems i.e. the optimal temperature for degradation was 40°C, and degradation decreased as the temperature increased. The fed-batch systems confirmed that an acclimatisation process is a prerequisite for efficient thermophilic degradation. In terms of degradation on the fed-batch systems, the pulp mill sludge inoculum was better than the sewage sludge inoculum. Steady state was achieved much faster on the continuous system as compared to the fed-batch system. In comparison to the batch and fed-batch systems,
the continuous system demonstrated maximum COD removal across all three temperatures.

From this study it can be concluded that thermophilic treatment of pulp and paper mill effluent does occur up to 60°C, but the temperature of activated sludge treatment should not exceed 40°C for maximum degradation. A membrane process coupled with the thermophilic biological treatment would be strongly recommended for the higher temperatures, so that better COD removal would be achieved.
APPENDIX 1: MEDIA

1. Chemically Defined Media (CDM) (Redman et al., 1999)
   
   L-Glutamic acid 0.5g 
   Citric acid 0.5g 
   Malic acid (disodium salt) 0.5g 
   Glucose 0.5g 
   Yeast extract 0.1g 
   Agar 15g 
   Distilled Water 1 L 
   
   After autoclaving 10 mL of mineral salts solution (containing 50g K$_2$HPO$_4$ per litre, 10g NaCl per litre, 1 g FeCl$_3$.6H$_2$O per litre, 10 g CaCl$_2$.2H$_2$O per litre and 25 mg MgCl$_2$ per litre) per litre was added to the minimal medium.

2. Potato Dextrose Agar (PDA) Oxoid 
   
   Potato Extract 4.0g 
   Dextrose 20.0g 
   Agar 15.0g 
   Distilled water 1 L 

3. Tryptone Soy Agar (TSA) Biolab
   
   Tryptone 15.0g 
   Soya peptone 5.0g 
   NaCl 5.0g 
   Agar 15.0g 
   Distilled water 1 L
APPENDIX 2 : ANALYTICAL METHODS

1. Determination of Chemical oxygen demand (COD)

Reagents and Standards

a) Standard potassium dichromate solution (0.25N/0.0417M):

A thin layer of potassium dichromate (Merck GR grade) is dried at 100-105°C for 2h and then cooled in a dessicator. Approximately 12.259±0.0005 g of potassium dichromate was dissolved in 400 ml of distilled water in a beaker. The solution is then transferred to a 1L volumetric flask and distilled water was then added to make up the volume. The solution was stable for 3 months.

b) Silver sulphate (AR Grade) powder, AgSO₄

c) Sulphuric acid (Merck GR Grade) 95-97%

d) Sulphuric acid reagent

Approximately 24.86 g ± 0.005 g of the silver sulphate was weighed and added to a 4.59 Kg bottle of Sulphuric acid and then allowed to dissolve.

e) Ferroin indicator (Merck 9161)

f) Ferrous Ammonium Sulphate solution (FAS) 0.1M

Approximately 39.0 g ± 0.05 g Ferrous Ammonium Sulphate (AR Grade) was added to 500 mL of distilled water in a beaker. Thereafter 20 mL of concentrated Sulphuric acid was added and allowed to cool. The solution was then transferred to a 1L volumetric flask and the volume is made up with distilled water. The FAS was stored...
under ambient conditions. The FAS solution must be standardized daily or before use against the standard potassium dichromate solution. Should the normality of the FAS fall below 0.09, then a fresh solution must be prepared. The standardisation was performed in duplicate as follows: 10.0 mL of the standard potassium dichromate was dispensed into a 250 mL Erlenmeyer flask to which 100 mL of distilled water was added. Concentrated sulphuric acid (30 mL) was then added and allowed to cool. The FAS solution (with 2-3 drops of ferroin indicator) was then titrated using a 50 mL burette. The end point was taken as the first sharp colour change from blue-green to reddish brown. The volume of FAS was then recorded and the normality calculated using the formula.

\[
\text{Normality of FAS} = \frac{\text{Volume of 0.25N K}_2\text{Cr}_2\text{O}_7 \times 0.25}{\text{Average volume of FAS}}
\]

- g) Mercuric Sulphate, HgSO₄ (AR grade) crystals or powder

- h) Control Standard-Potassium hydrogen Phthalate (AR Grade)

A thin layer of Potassium hydrogen phthalate (Riedel-de Haen) was dried at 100-105°C for 1h and then cooled in a dessicator. For each litre of solution approximately 0.300 g was weighed and allowed to dissolve in distilled water. This solution has a COD value of 343 mg/L and a shelf life of 3 months when stored under ambient conditions. The COD of the control standard was measured daily to justify the COD’s of the unknown samples.
Equipment

a) Reflux apparatus- consisting of 250 mL round bottom flasks with ground glass necks and jacketed Liebig condensers with ground glass joints to fit the flasks (Fig A).

b) Hot plate (Scientific) to generate sufficient power to reflux the COD mixture.

c) Glass beads

d) Burette

e) Pipettes

f) Volumetric flasks

Sample Preparation

The effluent samples were centrifuged to remove the biomass, and 4 mL of the supernatant was transferred to 50 mL Greiner tubes containing 16 mL of distilled water (1:5 dilution).

Analytical procedure

Mercuric sulphate (approximately 0.4 g) was transferred to a refluxing flask. The sample (20.0 mL) was then added to the flask.

A blank was prepared using 20.0 ml of distilled water instead of the sample and similarly 20.0 mL of the control standard was added to the refluxing flask. 10.0 mL of standard potassium dichromate was added to each flask and approximately 30.0 mL of sulphuric acid reagent was later added. Glass beads (5-10) must be added
Fig. A. Experimental set-up for COD digestion
to the reflux flask to prevent bumping. The contents of each flask was thoroughly
mixed prior to heating on the hot plate. Each flask was then attached to a condenser
and then allowed to reflux for 2h. The condensers was then washed down with 80 mL
of distilled water. The flasks were then allowed to cool to room temperature;
Ferroin indicator (2-3 drops) was then added to the reflux flasks and then titrated with
FAS.

The colour change is sharp, going from blue-green to reddish brown. The volume of
the FAS used for the blank, control standard and samples was recorded.

*Calculation for COD:*

\[
\text{COD} = \frac{(a-b) \times N \times 8000 \times D \text{mgO}_2 / L}{\text{mL sample}}
\]

- a – mL FAS used for blank
- b – mL FAS used for sample
- N – normality of FAS
- D – Number of dilutions of sample
Mixed liquor suspended solids (MLSS) determination

Apparatus:

Whatmann Glass fibre filter (0.45μm pore size)

Membrane filtration unit

Drying oven (operating at 103°C to 105°C)

Desiccator

Procedure:

A sample volume of 10mL was filtered through a pre-weighed glass fibre filter paper. The glass fibre filter paper was placed in the drying oven at 103 to 105°C and left overnight to dry. After 24h it was removed from oven and placed in dessicator. The cooled glass fibre filter paper was then reweighed.

Calculation:

\[
\text{Mg MLSS/L} = \frac{(A-B \times 1000)}{\text{Sample volume (mL)}}
\]

Where: \( A = \text{mass of filter paper + sludge} \)

\( B = \text{mass of filter paper} \)
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