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Mesophilic and thermophilic activated sludge post treatment of anaerobic effluent

Sludge and Wastewater Characterisation Using Batch Experiments

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Abstract

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

Abbreviations: ASM1 – Activated Sludge Model No. 1, BOD – Biochemical Oxygen Demand, BOD-st – short term BOD (mg O_2 l^{-1}), COD – Chemical Oxygen Demand, f_p – fraction of inert COD in heterotrophic biomass, S_s – readily biodegradable substrate (mg COD l^{-1}), S_I – initially present inert soluble COD, SMP – soluble microbial products, VFA – volatile fatty acids, S_p – soluble inerts generated from biomass decay, X_p – particulate inerts generated from biomass decay, X_s – slowly biodegradable substrate of particulate nature, X_I – initially present inert particulate COD

Introduction

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

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

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

Materials and methods

Wastewater description

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

Mesophilic and thermophilic sludge

Mesophilic and thermophilic seed sludge for the batch experiments was obtained from two lab-scale activated sludge reactors operating under semi-steady state conditions at 30 and 55 °C (Vogelaar et al. 2002). Both reactors had a sufficiently long temperature adaptation period for the sludge to acclimatize to the specific temperature conditions. Both systems were operated at a 20 days sludge retention time (SRT).

Batch respirometric experiments – short term BOD

Readily biodegradable BOD was determined by measuring the short term BOD of the wastewater with mesophilic and thermophilic sludge (Spanjers et al. 1994). The readily biodegradable BOD is defined as the oxygen consumption of activated sludge caused by readily biodegradable substrate. Batch respirometric experiments were performed with a modified version of a Manotherm RA 1000 respirometer (Spanjers et al. 1994). The WTW DO probe was replaced with a heat resistant Mettler Toledo Inpro 6000 probe, measured DO values were logged and the respiration rate was estimated using the analytical method as described by Spanjers (1993). The respirometer was connected to a temperature controlled 4 liters batch vessel. The pH was kept between 7.1 and 7.6. Evaporation was negligible due to a condenser placed on top of the batch vessel. Aeration and mixing of the vessel contents took place by sparging of pressurized air.

BOD5 tests

BOD-5 experiments of fractionated wastewater samples were used as a measure for the slowly biodegradable substrates. Experiments were conducted using WTW Oxitops®. 300 ml Schott bottles were filled with 40 ml wastewater and seeded with 0.25 ml thermophilic or mesophilic sludge from the continuous activated sludge reactors. Four different wastewater fractions were used: raw, paper filtered, membrane filtered (Whatman GF/F) and a centrifuged particulate fraction of the wastewater re-suspended in a nutrient medium. Sulphide was removed in advance from all the samples by stripping the solution with nitrogen gas. A 100 % sulphide removal was verified experimentally. All samples were taken from one batch of wastewater collected at the mill. The contents of the bottles were mixed by magnetic stirrers. Incubation took place at 30 or 55 °C, three bottles were used for each temperature. The decrease in the headspace pressure due to oxygen consumption was measured every 20 minutes for 5 days. Carbon dioxide was fixed using sodium hydroxide pellets. Oxygen consumption was calculated using the gas law and Henry's law and taking the water vapor pressure at 30 and at 55 °C into account. Before and after the experiment, the COD of 4 different fractions (total, suspended, colloidal, soluble) was measured to establish a COD balance. Nitrification was inhibited by addition of allylthiourea (ATU, 5 mg l^{-1}).

Biodegradability tests

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

Analytical methods

Chemical Oxygen Demand (COD) was determined according to APHA (1995). COD was measured over

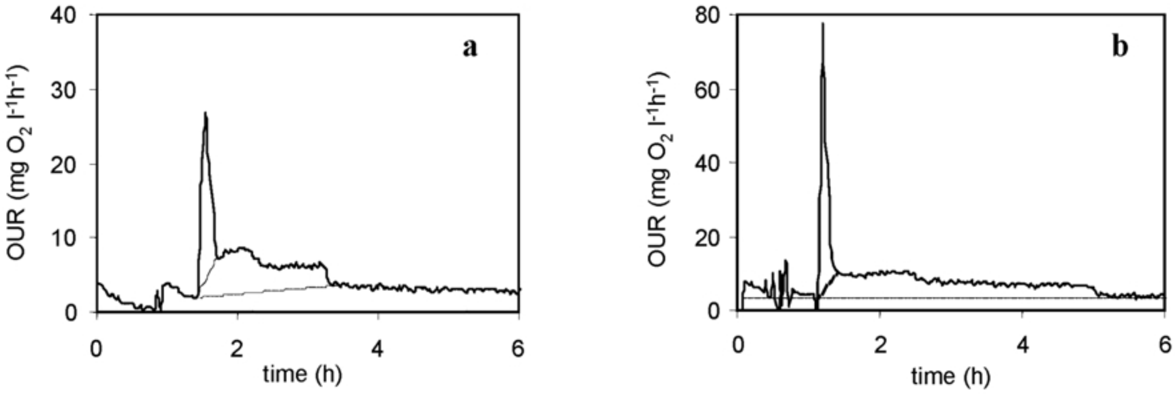

Figure 1. Wastewater short term BOD of: (a) T = 30 °C, mesophilic sludge, (b) T = 55 °C, thermophilic sludge.

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

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

four wastewater fractions: total, suspended, colloidal and soluble. Paper filtration (Schleicher and Schuell $595\frac{1}{2}$ folded paper filters, pore size 4.5 μm was used to distinguish between the total and the suspended fraction. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The colloidal fraction was calculated as the difference between the soluble fraction and the paper filtered fraction. Mixed liquor total- and volatile suspended solids were measured according to the Dutch normalized standards (NEN 6621). Sulphate and thiosulphate were determined on a Dionex ionchromatograph with an anion exchange column (Dionex, IonPac AS17) using a hydroxide gradient. Eluent was made on-line with an EG-40 eluent generator using de-ionized water as carrier. Ions were detected using suppressed conductivity. The presence or absence of sulphite was determined using Merckoquant sulphite test strips (Merck, Germany). Sulphide was determined according to Trüper & Schlegel (1964).

Batch respirometric experiments – short term BOD

In total 10 BOD-st measurements have been performed, 5 with mesophilic sludge and 5 with thermophilic sludge. Figure 1 shows two representative respirograms of these tests. The short term BOD of the wastewater was calculated as the area under the curve after substrate addition. Correction took place for the endogenous respiration rate (the grey line in Figure 1). Short term BOD was in both cases very low (average values 89 ± 34 and 96 ± 34 mg $O_2\ l^{-1}$ at 30 and 55 °C). Results were susceptible to a large variation. Additional BOD-st experiments were performed with wastewater containing no sulphide due to preliminary stripping of the solution with nitrogen gas. In these respirograms there was no sharp peak as was found in the foregoing with sulphide.

Additions of a pure sulphide solution to the sludge (Figure 2) gave only a sharp peak with little or no tailing. The first peak in the wastewater respirograms could thus be identified as the biological oxidation of sulphide into sulphate or elemental sulphur.

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

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

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

Increasing the sulphide dosage to demineralized water led to an increased oxygen uptake rate (Figure 2a, b; first two peaks). Additions of sulphide to activated sludge under both temperature conditions (Figure a,b; last two peaks) did not result in a production of sulphate either just as when adding sulphide to demineralized water. However, monitoring the influent and effluents of both lab-scale activated sludge reactors for sulphide and sulphate showed that under continuous reactor operation a complete oxidation of sulphide took place to sulphate.

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

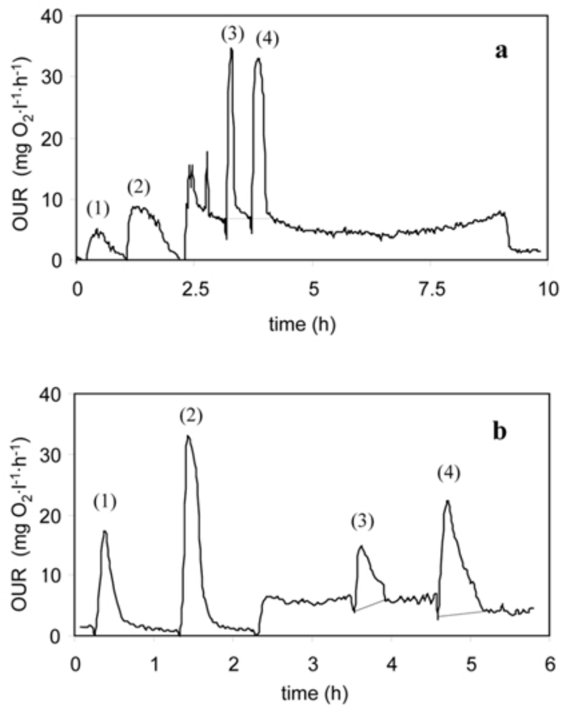

Figure 2. Sulphide additions (150 mg l^{-1}) to: (a) T = 30 °C, demineralized water and mesophilic sludge, (b) T = 55 °, demineralized water and thermophilic sludge.

BOD5

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

Figures 3 and 4 show a series of BOD experiments with different wastewater fractions. This particular batch of wastewater contained a higher amount of easily biodegradable substrate as the anaerobic pretreatment system was overloaded at that moment.

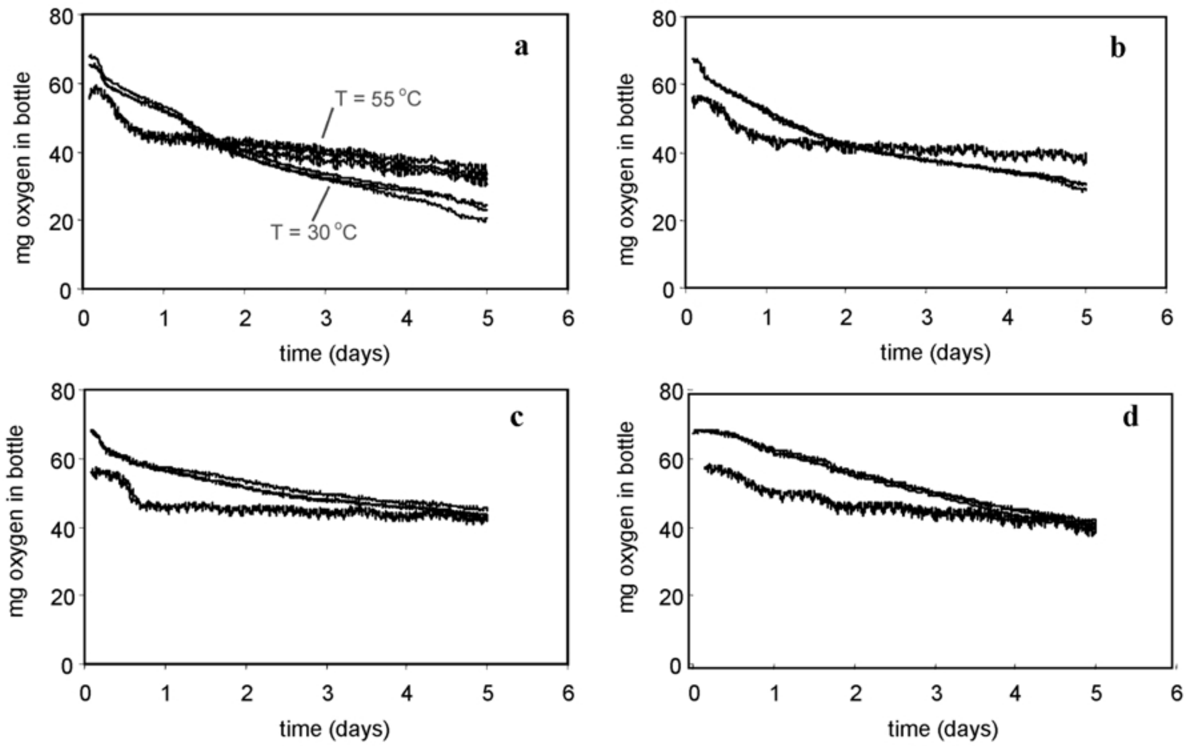

Figure 3. Amount of oxygen present in the BOD bottles over time. (a) Raw wastewater, (b) paper filtered, (c) membrane filtered, (d) re-suspended centrifuged fraction. (—) T = 30 °C, (***) T = 55 °.

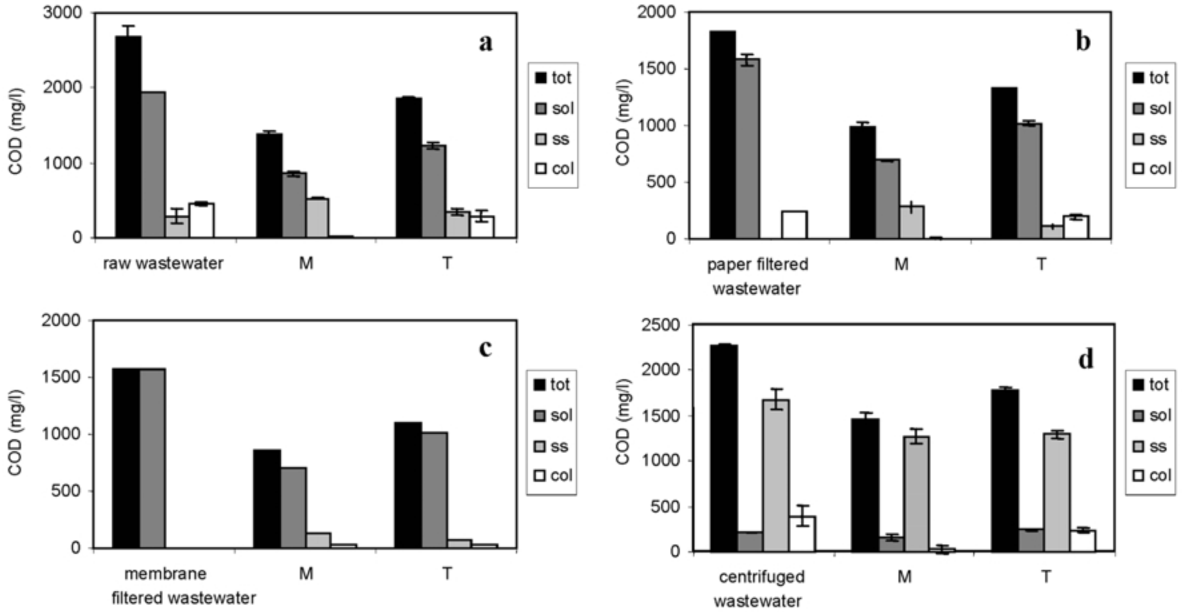

Figure 4. Distribution of COD fractions before and after a 5 day biodegradation (BOD) test. (a) Raw wastewater, (b) paper filtered fraction, (c) membrane filtered fraction, (d) re-suspended centrifuged fraction.

Therefore the data of this experiment can not be compared directly to data of the other batch experiments. Figure 3 shows the amount of oxygen present in the BOD bottles as a function of time. At 55 °C, the initial oxygen consumption rate was higher compared to 30 °C but then leveled off much quicker compared to the 30 °C bottles. Ultimately, the total amount of oxygen consumed over a 5 days period was lower under thermophilic conditions as compared to mesophilic conditions.

Figure 4 presents the distribution of different COD fractions before and after a 5 days BOD test with different wastewater fractions. A COD balance was established over the bottles. The average deficit in the balance was 8% with a maximum of 19%.

Three main observations can be made from Figure 4: (1) There was a distinct difference in removal of soluble COD between both temperature conditions (Figure 4a, b, c). (2) The batch test with the membrane filtered wastewater showed a slight production of colloidal COD both at 30 and at 55 °C that was not significantly different. (3) At 30 °C, all of the colloidal COD present in the wastewater is absorbed to the sludge and partly hydrolyzed and oxidized while at 55 °C a large colloidal fraction remained unaffected (Figure 4d). The difference in COD removal in Figure 4d was approximately similar to the amount of colloidal COD that remained in the liquid at 55 °C while it was removed at 30 °C. The rate at which colloidal and suspended COD was oxidized in the test was lower under thermophilic conditions compared to mesophilic conditions, as can be seen from the decrease in the oxygen concentration in time (Figure 3d).

Biodegradability test

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

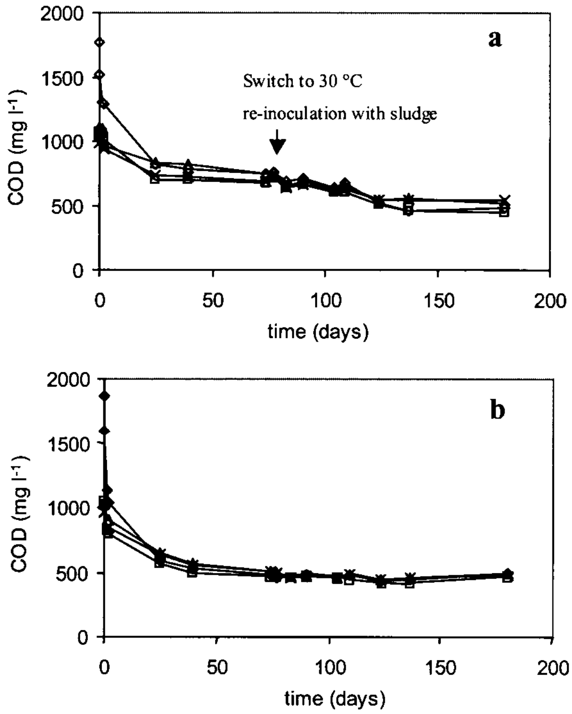

Figure 5. (a) Thermophilic biodegradation of the wastewater, subsequently followed by mesophilic degradation (and re-inoculation with mesophilic sludge). (b) Mesophilic biodegradation. Batch 1, COD_{tot} (♦); Batch 1, COD_{sol} (□); Batch 2, COD_{tot} (△); Batch 2, COD_{sol} (*times*).

Figure 6 depicts two diagrams showing the relative distributions of the different wastewater fractions for both temperatures. Average short term BOD values are converted into readily biodegradable COD by assuming a yield factor of 0.5 for both sludges. The BOD5 is converted into the sum of the slowly biodegradable COD and the readily biodegradable COD by assuming a yield factor of 0.3. Inert COD was the nonbiodegradable fraction after 83 days of biodegradation (Figure 5) and the very slowly biodegradable fraction was calculated from the balance of the total wastewater COD and the before mentioned COD fractions. The diagram shows that under thermophilic conditions, the inert COD fraction was higher and slowly and very slowly biodegradable COD fractions were lower as compared to mesophilic conditions.

Table 2. Wastewater characterization in terms of biodegradable and non-biodegradable fractions according to the biodegradability test after 85 days.

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

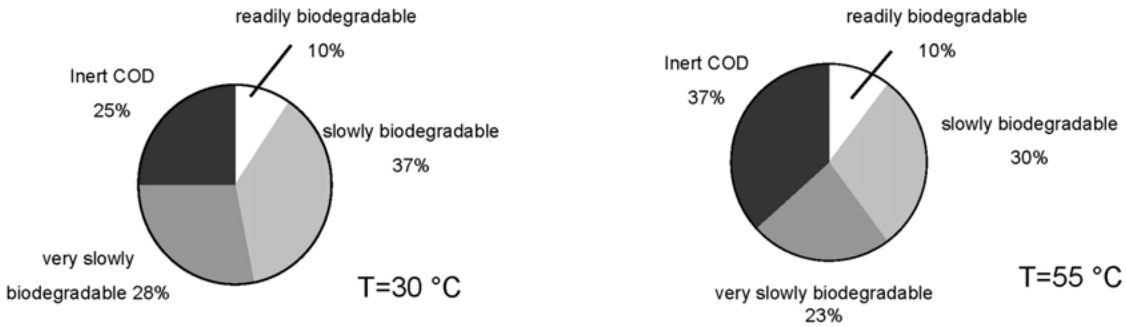

Figure 6. Wastewater fractions at 30 and 55 °C.

Discussion

Batch respirometric experiments – short term BOD

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

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

The results of Figure 2a, b concerning the oxidation of sulphide show that a competition took place between an abiotic and a biotic reaction. Steudel (2000) reports that metal ions can catalize the chemical oxidation of sulfide to elemental sulphur following overall reaction (1). Trace amounts of metals like iron could have been present in the reaction vessel, catalyzing the chemical oxidation. Removal of sulphide by precipitation with metals can be ruled out since the metal concentrations were too low to make a significant contribution possible.

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

The increase in the reaction rates with an increasing sulphide dosage supports this hypothesis since the chemical reaction as described above would increase in rate as the concentrations of the reactants increase. Also, the abiotic reaction rate increases as expected with temperature and from the rate increase an approximate doubling of the reaction rates for each 10 °C can be calculated. These results show that as temperature increases, the importance of abiotic reactions increases significantly. Interestingly, in the mesophilic experiment, an increase in the sulphide dosage to the activated sludge did not increase the reaction rate thus apparently under these conditions, the amount of biocatalyst is controlling the conversion rate. Addition of sulphide to both mesophilic and thermophilic sludge did not result in a significant sulphate production (measured directly after the respiration rates dropped after the sulphide additions in Figure 2a, b) while under continuous reactor operation, for both systems, virtually all influent sulphide could be found back in the effluent as sulphate. Probably, at 30 °C, sulphide is partially oxidized by biological means and stored as elemental sulphur granules inside the cells that can later on be oxidized into sulphate. It is known that *Thiothrix* spp. are able to store elemental sulphur in granules inside the cells as an energy source (Nielsen et al. 2000). Under thermophilic conditions, the first partial oxidation takes place to some extent by abiotic means, and elemental sulphur in the liquid phase or inside the cells is later on oxidized into sulphate. Somewhat similar results were obtained by Bérubé & Hall (2000) who studied the removal of hydrogen sulfide from an evaporator condensate in a thermophilic aerobic membrane bioreactor (60 °C). All hydrogen sulfide was removed from the mixed liquor but the authors could not distinguish between the biological and the abiotic reaction. They suspect, based on their results, that the abiotic reaction contributes significantly to the overall sulfide removal due to the high temperature conditions. Stripping of hydrogen sulfide with the off-gas was probably negligible since no sulfide smell was ever detected. We also found no evidence for an increased volatilization of H_2S with the off-gas at 55 °C although the solubility of hydrogen sulphide decreases with temperature. With increasing temperatures, the equilibrium between HS^- and H_sS at pH 7 shifts more to HS^-so possibly this effect is counteracting the lower solubility at higher temperatures.

BOD5 tests

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

In contrast to these results, the rate of oxygen uptake in the bottles containing only suspended and colloidal COD was higher at 30 °C compared to 55 °C (Figure 3d). Since these bottles contained virtually no soluble COD, oxygen consumption could only take place after hydrolysis of suspended and colloidal material. Normally, higher hydrolysis rates are expected under thermophilic conditions. Two possible explanations are postulated: (1) Possibly not the full diversity of enzymes was available at 55 °C since the biomass might simply not be able to synthesize these enzymes or synthesis takes place after a certain lag phase. LaPara et al. (2000) found that gelatin and α-lactose were biodegraded at 55 °C but not simultaneously in contrast to bioconversion at 22 °C. At 55 °C, lactose was only converted after nearly all gelatin was removed. Similar results were obtained by Kosseva et al. (2001) who also found a non-simultaneous degradation of protein and other carbonaceous compounds from Stilton whey at 65 °C.

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

The non flocculating behavior of the colloidal fraction under thermophilic conditions is of utmost importance for practical applications since under continuous operation, influent colloidal COD would wash through the reactor system, deteriorating the effluent quality. This was indeed found to be the case under continuous reactor operation (Vogelaar et al. 2002). In case the influent contains virtually no colloidal material, the thermophilic effluent was also found to be clear. This in accordance with the results of the BOD test with a membrane filtered wastewater fraction (Figures 3, 4c): hardly any colloidal COD was produced and the liquid remained transparent.

Biodegradability test

Inert COD remaining in the wastewater after the biodegradation test presumably consists of lignin like components. Lignin is present in wood pulp that is made by non-chemical pulping methods and is thus also returned in the paper mill process water by the recycled wastepaper. Lignin polymers are hardly biodegradable under aerobic conditions (Kortekaas 1998). Franta et al. (1994) used pyrolysis GC/MS to characterize residual COD from a treated paper mill wastewater and found indeed that most of the higher weight organic compounds were derived from lignins.

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

SMP production by biomass can take place by decay processes (modeled in Activated Sludge Model No. 1 as cryptic growth) and by substrate metabolism itself (Barker & Stuckey 1999). SMP are mostly complex molecules that are still to a large extent biodegradable. Significant production of SMP from substrate metabolism mainly occurs when sludge is heavily overloaded resulting in an excretion of complex intermediates that can later on still be degraded to a large extent as observed by Schiener et al. (1998) in a heavily loaded anaerobic baffled reactor. SMP might be produced during the initial stage of the batch test but can still be converted during the long time course of the experiment.

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

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

Differences in remaining inert COD levels between mesophilic and thermophilic treatment have also been found by other researchers (Tripathi 2000; LaPara et al. 2000, 2001). LaPara et al. (2001) explain their results by production of SMP, containing a higher recalcitrant fraction at 55 °C as compared to 30 °C. They came to this conclusion from batch experiments with lactose and gelatin as sole carbon source giving a higher residual COD level at 55 °C compared to 25 °C (LaPara et al. 2000). Furthermore, subsequent treatment of a pharmaceutical wastewater at 55 and at 30 °C did not yield the same COD removal as 30 °C treatment alone (LaPara et al. 2001). The major difference between their work and the current findings is the time scale of the experimental work: 25 to 48 hours in their experiments compared to 35–85 days in the current research. In the glucose biodegradation experiment as described above, residual COD levels at 55° remained higher compared to the 30 °C batch vessel for a 20 days period after which they dropped to a similar level during the end of the experiment. It thus seems plausible that thermophilic biomass produces more SMP, but given a sufficient time period, these can still be degraded. Summarizing: the difference in remaining COD levels in Figure 5 is most likely the result of the inability of the thermophilic biomass to degrade the whole range of components that can be degraded under mesophilic conditions.

In Figure 6 the wastewater characterization is summarized by fractionating the total COD into readily-, slowly, very slowly biodegradable and inert COD. Based on the current results and these diagrams, it is proposed that readily biodegradable COD, comprising simple soluble substrates, is to a similar extent biodegradable under both temperature conditions but as substrates become more complex in nature, their biodegradability decreases to a larger extent under thermophilic conditions compared to mesophilic conditions.

Conclusions

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

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