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ORIGINAL PAPER

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Kinetic comparisons of mesophilic and thermophilic aerobic biomass

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Abstract Kinetic parameters describing growth and decay of mesophilic (30°C) and thermophilic (55°C) aerobic biomass were determined in continuous and batch experiments by using oxygen uptake rate measurements. Biomass was cultivated on a single soluble substrate (acetate) in a mineral medium. The intrinsic maximum growth rate (μ_{max}) at 55°C was 0.71 ± 0.09 h^{-1}, which is 1.5 times higher than the μ_{max} at 30°C (0.48 ± 0.11 h^{-1}). The biomass decay rates increased from 0.004 h^{-1} at 30°C to 0.017 h^{-1} at 55°C. Monod constants were very low for both types of biomass: 9 ± 2 mg chemical oxygen demand (COD) l^{-1}at 30°C and 3 ± 2 mg COD l^{-1}at 55°C. Theoretical biomass yields were similar at 30 and 55°C: 0.5 g biomass COD (g acetate COD)$^{-1}$. The observed biomass yields decreased under both temperature conditions as a function of the cell residence time. Under thermophilic conditions, this effect was more pronounced due to the higher decay rates, resulting in lower biomass production at 55°C compared to 30°C.

Keywords Aerobic · Decay · Kinetics · Respirometry · Thermophilic

Introduction

Thermophilic wastewater treatment has attracted increasing interest in recent years, due mainly to the growing tendency for industrial water system closure and the subsequent need to purify hot wastewater streams [19]. An often-mentioned advantage of thermophilic treatment is a higher conversion rate. Regarding anaerobic conversions at elevated temperatures, data are available that support these higher conversion rates [9, 24]. However, kinetic data for thermophilic aerobic conversions are scarce, to some extent contradictory, and not always reliable when complex wastewaters were used in the studies. Based on a literature review, 3–10 times higher rates are expected in thermophilic treatment systems compared to their mesophilic analogs [19]. Based on more recent experimental data the same authors state that biokinetic advantages are not to be expected at higher temperatures [21].

Another often mentioned advantage is the expected lower sludge production. However, lower biomass yields have been confirmed in only one study [5]. Reasons for the discrepancy between expectations and measurements are related to: (1) the complex nature of the wastewaters used in some experiments [12, 16], (2) production of soluble microbial products (SMP)—from biomass decay and by substrate metabolism [2]—that was not taken into account [20, 31], (3) an experimental set-up that did not provide good parameter identification [12, 16, 21, 31] and (4) some experiments were performed only under thermophilic conditions. The experimental data obtained were compared to literature data of mesophilic conversions but these can also vary to a large extent, making the comparison rather difficult [5, 7, 8, 12, 20, 31].

The variability of kinetic parameter estimates has been recognized previously by other researchers, who proposed that a distinction should be made between extant and intrinsic parameters, in which extant means "currently existing" and intrinsic means "belonging to the essential nature of a thing" [14]. Given the controversy regarding thermophilic kinetics, the aim of this research was to estimate intrinsic kinetic parameters of mesophilic and thermophilic biomass, assuming that

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differences in kinetics are the sole result of the temperature difference. Biomass was cultivated at 30 and 55°C on a single soluble substrate (acetate) in a mineral medium. Experimental data were fitted to a model [13] and the estimated kinetic parameters were evaluated in relation to literature data.

Materials and methods

Reactor description

Two 2.5 l temperature-controlled continuous reactors without biomass recycle were operated at 30 and 55°C. The reactor temperature was maintained by circulating water through the water jackets of the reactors. Both reactors were continuously fed with a non-sterilized synthetic influent. No biomass inoculum was used and temperature-adapted biomass developed spontaneously as a result of the non-sterile operating conditions. Wall growth was controlled by daily reactor cleaning. The pH was controlled at 7.2 ± 0.2. Mixing took place by aeration with pressurized air and by a magnetic stirring device at the reactor bottom. At 55°C, dissolved oxygen (DO) was measured using a Mettler Toledo inpro 6000 probe; at 30°C, DO was measured using a WTW OXY 191. In both reactors, DO values were always maintained above 3 mg O_2 l^{-1}. Evaporation via the exhaust air was negligible because of condensers on top of the reactors. Both reactors were operated at dilution rates (D) of 0.077 and 0.023 h^{-1}, corresponding to hydraulic retention times (HRT) of 13 and 43 h.

The 4-l batch reactor was a temperature- and pH-controlled glass vessel connected to a respirometer. Evaporation in the batch vessel was maintained at a negligible level by cooling the exhaust air, and aeration took place with pressurized air. The respirometer used for oxygen uptake rate (OUR) measurements was a modified version of a Manotherm RA 1000 respiration analyzer [29]. OUR measurements at 55°C were taken by changing the conventional WTW DO probe for a Mettler Toledo Inpro 6000 probe. Every 2 min, a new respiration data point was obtained.

Reactor influent consisted of non-neutralized acetic acid [2.6 g l^{-1} chemical oxygen demand (COD)], NH_4Cl (480 mg l^{-1}), K_2HPO4 (140 mg l^{-1}), $MgSO_4{\cdot}7H_2O$ (50 mg l^{-1}), $FeSO_4{\cdot}7H_2O$ (2.5 mg l^{-1}), $CaCl_2{\cdot}2\ H_2O$ (2.5 mg l^{-1}) and 25 µl trace element solution per liter as described in [34].

Model description

The model [13] assumes that substrate can only be removed by growth, i.e., there is no substrate production from decay, and respiration is associated with both growth and decay. This means that heterotrophic biomass oxidizes itself for maintenance purposes. We assume that the biomass is completely biodegradable, although generally some part is turned into inert material and is thus not oxidized. This, however, was not included in the following model.

Biomass growth

$$\frac{dX_h}{dt} = (\mu - k_d)X_h \tag{1}$$

Substrate removal

$$\frac{dS}{dt} = -\frac{\mu}{Y}X_h \tag{2}$$

Growth rate

$$\mu = \mu_{max}\frac{S}{K_s + S} \tag{3}$$

Oxygen uptake rate

$$OUR = \left(\frac{1-Y}{Y}\right)\mu X_h + k_d X_h \tag{4}$$

in which X_h is the concentration of heterotrophic biomass (g biomass COD l^{-1}), µ the growth rate (h^{-1}), k_d the decay rate (h^{-1}), S the substrate (acetate) concentration (mg COD l^{-1}), Y the theoretical yield [g biomass COD (g substrate COD)$^{-1}$], μ_{max} the maximum growth rate (h^{-1}), K_s the Monod constant (mg COD l^{-1}) and OUR is the respiration rate (mg O_2 l^{-1} h^{-1}).

A distinction is made between the theoretical yield (Y), which is the maximum yield obtained in the absence of decay, and the observed yield (Y_{obs}) which varies depending upon the extent of decay and thus upon the cell residence time. Eq. 5 presents the relation between the observed yield and the theoretical yield, as a function of the cell residence time (θ) and decay rate (k_d).

$$Y_{obs} = \frac{Y}{1 + k_d\theta} \tag{5}$$

Experimental set-up

Temperature-adapted biomass was cultivated in the continuous reactors at 30°C and 55°C and was used for batch experiments. Three types of batch experiments were performed, depending on the ratio of substrate over biomass:

1. Growth experiments to determine the intrinsic maximum growth rate, at a high initial substrate/biomass ratio (varied between 25 and 30 in the experiments).
2. Decay experiments to determine the decay rate, at a high initial biomass concentration and with no additional substrate added.
3. Yield experiments to determine the theoretical yield and the Monod constant at a low initial substrate/biomass ratio (varied between 0.07 and 0.1 in the experiments).

Batch experiments were performed mainly with biomass cultivated at 13 h HRT. Only the yield experiments were conducted with biomass grown at 13 and 43 h HRT. Biomass was transferred directly from the continuous reactor to the batch vessel in order to avoid temperature shocks. In batch experiments 2 and 3, the biomass was substrate-depleted by switching off the acetate supply in the continuous reactors for 10 min, after which the biomass was sampled. Substrate depletion was verified experimentally as OUR measurements showed a constant endogenous rate during the start of the batch experiments.

The continuous reactor experiments were run both for biomass cultivation and to determine the observed biomass yield at both 13 and 43 h HRT. Furthermore, the extant maximum biomass growth rate [14] at 13 h HRT was determined in the continuous reactors by a wash-out experiment and additional estimates of the biomass decay rates were made from the decrease in the reactor biomass contents as a function of HRT.

Parameter estimation

Continuous reactors

The observed biomass yield (Y_{obs}) at both HRTs was obtained directly from a COD balance over the reactors. Biomass COD was measured as the total effluent COD minus the membrane-filtered (0.6 µm) fraction of the effluent. The extant maximum growth rate

(μ_{max}) was estimated from a wash-out experiment [27, pp 33,34]. In this experiment, D was increased beyond the critical dilution rate. The biomass grew at its extant maximum growth rate and as D exceeded μ_{max}, biomass was washed out. From the rate at which the biomass was washed out and the D imposed, the extant μ_{max} of the biomass growing at that moment in the bioreactors could be calculated.

Batch experiments

The intrinsic maximum growth rate (μ_{max}) minus decay rate was estimated from the exponential increase in the OUR in batch experiment 1. The model differential equations were solved analytically and the simulated OUR was fitted to the measured OUR by minimizing the sum of the squared errors. The growth rate was assumed to be the maximum growth rate since substrate concentrations significantly exceeded the K_s.

The decay rate (k_d) was obtained from the exponential decrease in OUR in batch experiment 2, assuming the growth rate to be zero.

The theoretical biomass yield (Y) was estimated from batch experiment 3, in which a known small amount of substrate was added to a high concentration of biomass. As these experiments took place in a short period of time, biomass decay can be assumed to have been zero, indicating that the obtained yield factor is a good estimate of the theoretical yield. Y was calculated from the balance of the cumulative oxygen uptake due to substrate conversion and the amount of substrate added (biomass production is substrate conversion minus oxygen uptake). The cumulative oxygen uptake was obtained by integration of the respiration rate over time after subtraction of the endogenous respiration rate. The initial endogenous OUR was estimated from a period of (relatively) stable OUR before substrate addition.

The Monod constant (K_s) was estimated from batch experiment 3. From the course of the respiration rate, the known initial amount of substrate and the calculated yield, the substrate concentration at each time point could be calculated. The Monod constant was estimated as the substrate concentration at time t, where the respiration rate was half of the maximum respiration rate.

Analytical methods

Mesophilic and thermophilic biomass was determined as COD [1]. COD was measured for two wastewater fractions, total and soluble. The soluble fraction was obtained by membrane filtration (Whatman GF/F, pore size 0.6 μm). The optical density (OD_{600}) was measured on a Milton Roy Spectronic 601 spectrophotometer at 600 nm. Acetate was measured on a Hewlett Packard GC, model 5890 A. The GC was equipped with a 2 m ×2 mm glass column, packed with Supleco port (100–120 mesh) coated with 10% fluorad FC431. Carrier gas was nitrogen saturated with formic acid.

Results

Continuous reactor experiments

Both continuous reactors received the same influent COD, 2,600 mg COD l^{-1}. Effluent acetate concentrations decreased slightly as a function of HRT and were, on average, 6 ± 3 and 4 ± 1 mg COD l^{-1} at 13 and 43 h HRT at 30°C, respectively. Effluent acetate concentrations of the thermophilic reactor were similar: 6 ± 3 and 4 ± 0 mg COD l^{-1} at 13 and 43 h HRT respectively.

Table 1 Average effluent chemical oxygen demand (*COD*) concentrations minus the amount of acetate in the effluent, ± standarddeviations. Numbers in parentheses indicate the number of measurements. *HRT* Hydraulic retention time

HRT (h)	30°C Reactor		55°C Reactor	
	Total COD	Soluble COD	Total COD	Soluble COD
13	1190 ± 89 (6)	107 ± 22 (6)	1000 ± 57 (6)	62 ± 36 (6)
43	997 ± 120 (7)	63 ± 23 (7)	727 ± 49 (4)	39 ± 7 (4)

Effluent COD concentrations, excluding acetate, for both reactors are listed in Table 1. These data comprise (1) biomass COD, which is the total effluent COD minus soluble COD, and (2) slowly biodegradable soluble COD that is not substrate or biomass. This fraction is regarded as SMP [2] or as soluble exopolymeric substances (EPS), which are essentially the same in systems without any input of particulate substrate [23]. At both cell residence times, effluent COD levels for both the soluble and the total fraction were lower at 55°C than at 30°C. Observed biomass yields as calculated from these data decreased as a function of the HRT. This effect was most pronounced at 55°C resulting in a significantly lower observed yield at 55°C [$Y_{obs} = 0.26 \pm 0.01$ mg biomass COD (mg substrate COD)$^{-1}$] than at 30°C ($Y_{obs} = 0.35 \pm 0.04$) both at 43 h HRT. Theoretical and observed yields are listed in Table 2, all expressed as biomass COD substrate COD^{-1}.

Based on these observed yield values at different HRT, an estimation can also be made of the theoretical yield and decay rate according to Eq. 5. This yields: $Y = 0.41$ and $k_d = 0.004$ h^{-1} at 30°C and $Y = 0.45$ and $k_d = 0.017$ at 55°C.

In both continuous reactors, three biomass wash-out experiments were performed. The reactor dilution rate was suddenly increased from 0.077 h^{-1} to 0.65 h^{-1} at 30°C and to $D = 0.98$ h^{-1} at 55°C. The decrease in reactor biomass content over time (measured as OD_{600}) is depicted in Fig. 1. In both cases a 15-min lag phase occurred, after which the biomass content dropped as expected according to the model. Estimated extant maximum growth rates were 0.18 ± 0.05 h^{-1} at 30°C and 0.33 ± 0.07 h^{-1} at 55°C (Table 2).

Table 2 Kinetic constants of mesophilic and thermophilic biomass obtained from batch and continuous experiments. μ_{max} and k_d expressed as h^{-1}, K_s expressed as mg COD l^{-1}, Y and Y_{obs} expressed as biomass COD (acetate COD)$^{-1}$

Parameter	Experiment	Mesophilic (30°C)	Thermophilic (55°C)
μ_{max}–k_d	Batch (1)	0.48 ± 0.11	0.69 ± 0.09
k_d	Batch (2)	0.1 ± 0.02	0.2 ± 0.03
k_d	Continuous	0.004	0.017
μ_{max}–k_d	Wash-out	0.18 ± 0.05	0.33 ± 0.07
K_s	Batch (3)	9 ± 2	3 ± 2
Y		0.50 ± 0.04	0.49 ± 0.09
Y_{obs}	13 h HRT	0.39 ± 0.04	0.37 ± 0.02
Y_{obs}	43 h HRT	0.35 ± 0.02	0.26 ± 0.01

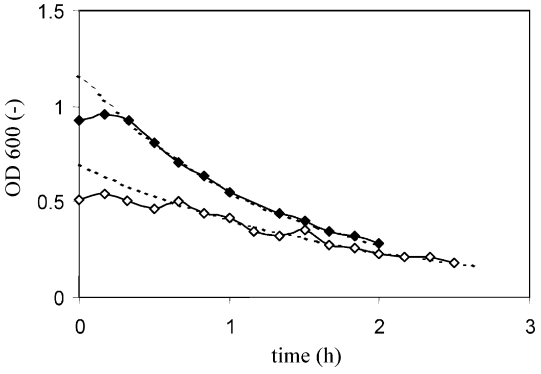

Fig. 1 Biomass washout, measured as optical density (OD_{600}) during a temporary increase in the dilution rate. *Open diamonds* 30°C, *closed diamonds* 55°C. *Dashed lines* represent the model fit to the data

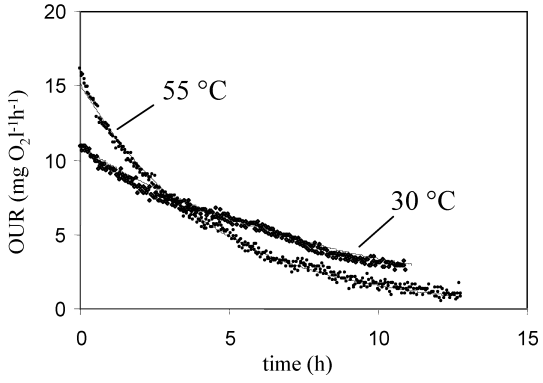

Fig. 3 Exponential decrease in the OUR during a batch decay experiment (batch experiment 2). *Diamonds* 30°C, *circles* 55°C. *Solid lines* Model fit

Batch experiment 1: intrinsic maximum growth rate

In total, four mesophilic and eight thermophilic growth experiments were carried out. Figure 2 presents two representative growth curves, one at each temperature. In both cases, the OUR increased exponentially in the growth phase and dropped sharply when substrate was depleted. Intrinsic maximum growth (minus decay) rates obtained directly from these data were 0.48 ± 0.1 h^{-1} and 0.69 ± 0.09 h^{-1} at 30 and 55°C, respectively. The model could be accurately fitted to the experimental data in each growth experiment. The growth rate was approximately 50% higher under thermophilic conditions compared to mesophilic conditions.

Batch experiment 2: decay rate

In the decay experiments (Fig. 3), the measured OUR decreased exponentially with time. Under each temperature condition, four experiments were conducted, all giving a reasonably good fit of the data to the model. The rate at which the OUR dropped at 55°C was twice the rate at 30°C, resulting in first order decay constants of 0.10 ± 0.02 h^{-1} at 30°C and 0.20 ± 0.03 h^{-1} at 55°C.

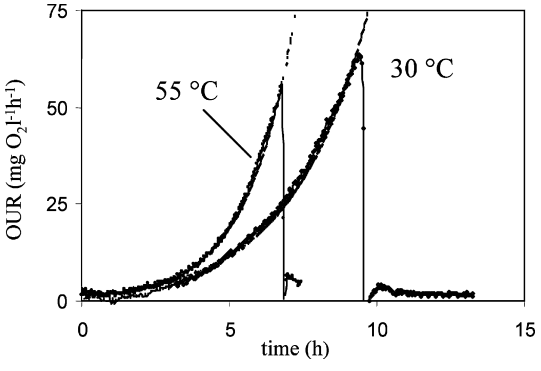

Fig. 2 Two representative curves showing the increase in the oxygen uptake rate (*OUR*) over time in a batch growth experiment (batch experiment 1). *Diamonds* 30°C, *circles* 55°C. *Dotted line* Model fit

Batch experiment 3: theoretical yield and Monod constant

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

Monod constants obtained from the calculated substrate concentrations during the yield experiment (batch experiment 3) were 9 ± 2 and 3 ± 2 mg COD l^{-1} at 30 and 55°C, respectively. The higher values at 30°C were due to the more pronounced tailing in the respirograms after the first sharp peak. If it is assumed that substrate concentrations are already zero at the point of inflection in the respirogram, Monod constants less than 1 mg COD l^{-1} are obtained for both temperature conditions. This assumption can be made since microorganisms generally produce storage products that are subsequently oxidized after substrate depletion, causing tailing in the respirogram [25].

The measured maximum OUR in the yield experiments was calculated per gram biomass COD and per liter volume of the continuous reactor. The maximum OUR per gram biomass COD was slightly higher at 55°C than at 30°C at 213 ± 12 mg O_2 (g biomass COD)$^{-1}$ h^{-1} at 55°C and 194 ± 8 mg O_2 (g biomass COD)$^{-1}$ h^{-1} at 30°C (at 13 h HRT), respectively. However, volumetric OUR per liter reactor was lower at 55°C compared to 30°C due to a lower biomass

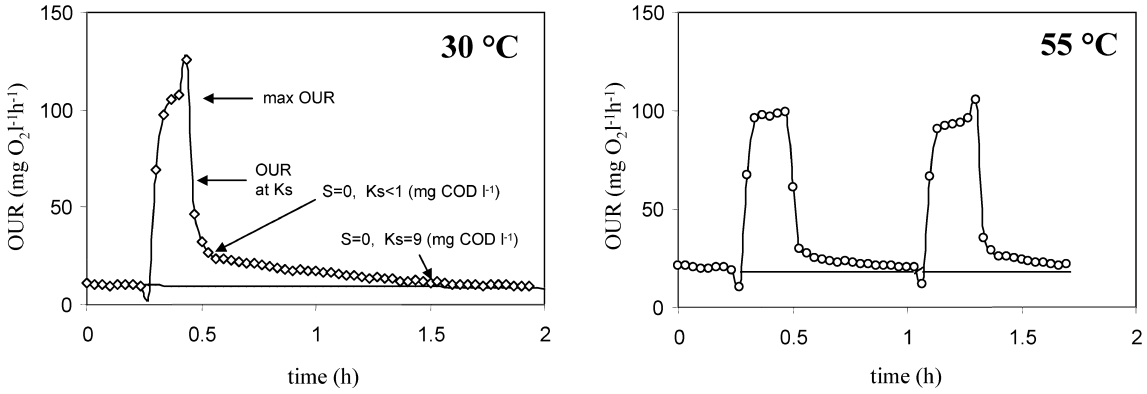

Fig. 4 Course of the OUR during equal additions of acetate to mesophilic and thermophilic biomass (batch experiment 3). *Open diamonds* 30°C, *open circles* 55°C, *solid line* endogenous OUR

concentration in the continuous reactor, although both reactors received the same influent COD under continuous operation. This effect was more pronounced at longer HRT, i.e., at 55°C, the maximum OUR per liter reactor dropped more rapidly as a function of the HRT than at 30°C. At 43 h HRT, maximum volumetric OURs were 136 ± 5 mg O_2 l^{-1} h^{-1} at 30°C and 111 ± 14 mg O_2 l^{-1} h^{-1} at 55°C. Endogenous respiration rates at 55°C were approximately twice the rate at 30°C, 41 ± 0.2 mg O_2 (g biomass COD)$^{-1}$ h^{-1} at 55°C compared to 18 ± 4 mg O_2 (g biomass COD)$^{-1}$ h^{-1} at 30°C.

Discussion

Effluent soluble COD and Monod constant

In both reactor effluents, acetate concentrations were very low. This is well in accordance with the low Monod constants (less than 9 mg COD l^{-1}) estimated from the batch experiments. Most likely, Monod constants for both sludges are in the order of 1 mg COD l^{-1} since the estimations of 9 mg COD l^{-1} were made by neglecting possible oxidation of internal storage products. However, we did not verify this experimentally by measuring the accumulation of these compounds in the biomass. Nevertheless, both estimations predict that, in all cases, very low effluent substrate concentrations are to be expected. Higher Monod constants have been reported [12, 16, 20, 31] but these were confounded by the presence of a non-biodegradable wastewater fraction [12, 16] or by SMP regarded as non-biodegraded substrate [20, 31]. Generally, Monod constants are extremely low [27, p 10] and this study shows that this is also the case for thermophilic microorganisms. One exception is that microorganisms can lose their substrate affinity in order to obtain extremely high growth rates of 3.5 h^{-1} [7, 8, 28] but this phenomenon does not seem to be common [28].

In this study, both reactor effluents also contained SMP/soluble EPS that were formed as a result of substrate metabolism, biomass decay and/or hydrolysis of bound EPS [2, 23]. The effluent soluble COD was found not to be completely inert since the COD concentrations decreased as a function of the HRT. This was expected since soluble EPS is regarded as biodegradable [23].

Under thermophilic conditions the soluble COD fraction was smaller compared to mesophilic conditions at both HRT. This would imply either that biodegradation of this fraction takes place at a higher rate at 55°C or that production via hydrolysis of bound EPS and cell lysis is lower. Cell lysis is not expected to be lower under thermophilic conditions as decay rates increase with temperature (this study) and cells increasingly lose membrane integrity at higher temperatures [17]. Most likely, lower production of bound EPS under thermophilic conditions and possibly a higher conversion rate of the soluble EPS/SMP limits the size of this COD pool. It should also be noted that we used a 0.6-μm filter for effluent fractionation implying that some bacteria were possibly not retained on the filter, especially at the lower D, which may lead to slight overestimation of the effluent soluble COD concentration.

The fact that the concentration SMP/soluble EPS was lower at 55°C than at 30°C is also of interest since, in other studies with complex wastewaters [22, 33], significant differences in removal of soluble COD have been found. It is uncertain whether this was caused by a higher production of inert SMP at 55°C, or whether the thermophilic biomass was unable to convert the same variety of compounds as the mesophilic biomass. Based on these results, the latter explanation seems most likely.

Decay rates

In batch experiment 2, a doubling of the decay rate was found with a temperature increase from 30 to 55°C. This was also in accordance with the specific endogenous respiration rate, which doubled with the same temperature increase. The estimated decay rates calculated from the continuous experiments, however, showed a 4-fold increase in decay with temperature. Furthermore, they were approximately a factor of 10 lower than the decay rates found in the batch experiments (Fig. 3, Table 2), because in batch experiments, the OUR did

not drop as a result of actual decay, reducing the weight of the biomass, but a transition in the metabolic activity of the biomass took place towards starvation conditions, the so-called stringent response process [26]. Under low growth conditions, metabolic and maintenance processes are slowed down, resulting in a lower measured OUR. However, the biomass content did not decrease significantly. This was also clear from Fig. 3: the cumulative oxygen uptake underneath the curves is approximately 50 mg O_2 l^{-1} while an initial amount of 500 mg biomass COD l^{-1} was consumed. This means that during the batch experiment, only 10% of the biomass was actually oxidized, which would, according to the model, result in a 10% decrease in OUR. However, the OUR dropped to almost zero, indicating that other processes than actual decay must have taken place. The decay rates obtained from the continuous experiments are therefore considered to be more reliable in describing the decay process as these were obtained from measurements of the actual biomass content.

The decay rate at 55°C was well in accordance with literature values [16, 31] while others found a 10-fold lower value [20]. Literature data concerning pure cultures of microorganisms mostly report higher decay rates under thermophilic conditions [11, 18, 28] although some do not [3, 6]. Based on the current results and literature data, we believe that bacterial decay is higher under thermophilic conditions compared to mesophilic conditions, but to what extent remains subject to speculation. Increases of between 2- to 4-fold [this study; 17] and approximately 10-fold [19] have been reported. Most evidence however points to an approximate doubling of the decay with a temperature increase from 30 to 55°C.

Theoretical yield and observed yield

From a theoretical perspective, yields are expected to be similar under both temperature conditions. Based on thermodynamic considerations, the theoretical yield depends only on the balance between the Gibbs energy release from the oxidation of acetate with oxygen, and the building of new biomass from acetate and inorganic nutrients [15]. The Gibbs energy release from the oxidation of acetate is slightly affected by temperature and increases at a maximum of 15% with a temperature increase from 30 to 55°C as estimated from the Gibbs-Helmholtz equation [10]. Assuming a similar biomass composition at both temperatures (this is valid when comparing moderate thermophiles and mesophiles [30]) results in similar theoretical yields for both types of biomass: 0.47 g biomass COD (g substrate COD)$^{-1}$ at 30°C and 0.50 g biomass COD (g substrate COD)$^{-1}$at 55°C. It is doubtful whether these differences can actually be measured in practice, also given the assumptions that were made in the theoretical calculations. Nevertheless, the theoretical estimates correspond quite well with the results obtained (0.50 and 0.49 at 30 and 55°C, respectively). The theoretical yield estimations from the continuous culture experiments were slightly lower since they took production of SMP/soluble EPS into account. Also, almost no trend has been observed between theoretical yield and growth temperature [7, 8, 12, 16, 20, 21, 31]. The observed yield was lower at 55°C compared to 30°C due to the higher decay rates (Table 2). The differences became larger with increasing cell residence time. These findings confirm previous research by Bérubé and Hall [5] who also found a decrease in the observed yield as a function of temperature. We believe that theoretical yield factors are similar for both mesophilic and thermophilic aerobic biomass and that the observed yields are different due only to the higher decay rates under thermophilic conditions.

Maximum growth rates

Comparison of the maximum growth rates obtained in batch experiments and in experiments involving continuous wash-out shows a significant difference in their absolute values (Table 2) although the relative differences between 30 and 55°C were quite similar. This clearly shows the difference between intrinsic and extant μ_{max} values. The extant μ_{max} was lower as a result of the low biomass growth rate in the continuous reactors (0.08 h^{-1}) having its effect on the physiological state of the bacteria (relatively low levels of proteins, RNA, DNA). The biomass could not change its physiological state instantaneously into a state of maximum growth and therefore a lower extant μ_{max} was found compared to the intrinsic μ_{max} [14].

The intrinsic μ_{max} was calculated by adding the obtained k_d value from the continuous experiment to the (μ_{max}–k_d) value of batch experiment 1. This yields $\mu_{max} = 0.48 \pm 0.11$ h^{-1} and $k_d = 0.004$ h^{-1} at 30°C and $\mu_{max} = 0.71 \pm 0.11$ h^{-1} and $k_d = 0.017$ h^{-1} at 55°C. This is a 50% increase in the maximum growth rate and an approximate 4-fold increase of the decay rate with temperature. Reported μ_{max} values for thermophilic wastewater treatment processes vary between 0.1 and 0.5 h^{-1} [7, 8, 12, 16, 20, 21, 31]. For pure cultures of microorganisms μ_{max} values of 1.0 h^{-1} have been reported [4] but extremely high growth rates of 3.5 h^{-1} could be obtained only under typical conditions with incomplete substrate utilization [11, 28] or by growth on a complex medium [18]. The intrinsic μ_{max} values obtained in this study correspond well with the reported literature data and, given the reproducibility of the results, can be regarded as accurate estimates of the intrinsic μ_{max} for the specified substrate and temperatures.

Model simulations

In order to estimate the consequences for thermophilic wastewater treatment processes, model simulations were made with the obtained kinetic data. Reactor biomass

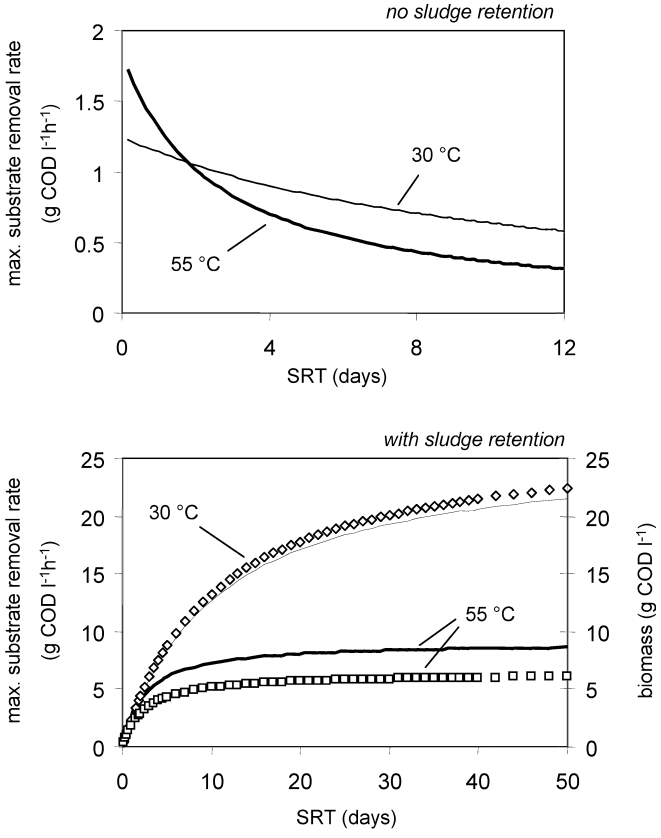

Fig. 5 Model predictions for the maximum volumetric conversion rate as a function of the sludge retention time (*SRT*), at 30 and 55°C, in reactors with and without sludge retention. Reactors with sludge retention are operated at the same hydraulic retention times (*HRT*). *Thin line* Maximum volumetric conversion rate (30°C), *thick line* maximum volumetric conversion rate (55°C), *open diamonds* biomass concentration (30°C), *open squares* biomass concentration (55°C)

concentrations and maximum volumetric substrate removal rates were estimated as a function of the sludge retention time (SRT) for a reactor with and without biomass recycle (Fig. 5). Kinetic parameters used in the simulations are: $\mu_{max} = 0.48\ h^{-1}$, $k_d = 0.004\ h^{-1}$, $Y = 0.5$ g biomass COD (g substrate COD)$^{-1}$ and $K_s = 9$ mg COD l^{-1} at 30°C and $\mu_{max} = 0.71$, $k_d = 0.018$, $Y = 0.5$ and $K_s = 9$ at 55°C. For both reactor configurations, reactor influent COD was 2,600 mg COD l^{-1}; the HRT of the reactors with sludge retention was 12 h for both temperatures.

The simulations show that at low SRT, due to the higher μ_{max}, higher volumetric conversion rates can also be obtained at 55°C compared to 30°C. However, at higher sludge ages the biomass concentration will be lower in the thermophilic reactor compared to the mesophilic reactor, without being fully compensated by the 50% increase in the maximum growth rate. The main point that we address in this paper is that maximum volumetric conversion rates are expected to be lower in a thermophilic bioreactor compared to a mesophilic analog when both systems receive the same organic loading rate.

In these simulations, the intrinsic μ_{max} values were used whereas the wash-out experiment has already shown that extant maximum growth rates decrease as a function of SRT. Maximum substrate removal rates are thus likely to be lower than the estimations shown in Fig. 5. Nevertheless, the same trend of a lower conversion capacity at 55°C than at 30°C at long SRT is expected. This was confirmed in the yield experiments (Fig. 4) with acetate additions to biomass grown at 13 h and 43 h SRT. The maximum volumetric OUR in the 55°C batch experiment was smaller than the OUR in the 30°C experiment, especially with biomass cultivated at 43 h SRT.

However, in practice, the low biomass concentration in a thermophilic bioreactor with sludge retention can be increased by increasing the loading rate on the system. In this sense, biokinetic advantages are to be expected; the same wastewater flow can be treated in a thermophilic bioreactor of approximately half the size as a mesophilic analog with a similar sludge production. It should be noted that these estimations hold only for wastewaters that contain only soluble, completely biodegradable COD. Other prerequisites are a sufficient oxygenation capacity as a larger fraction of the influent is actually oxidized, and an efficient separation of wastewater and sludge in order to maintain biological activity in the reactors. Both these requirements can become limiting factors under thermophilic conditions [19], especially the retention of biomass [32]. To confirm these findings for wastewater treatment applications, additional experiments should be performed with biomass cultivated at higher SRT and reliable data concerning actual decay rates are still scarce.

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