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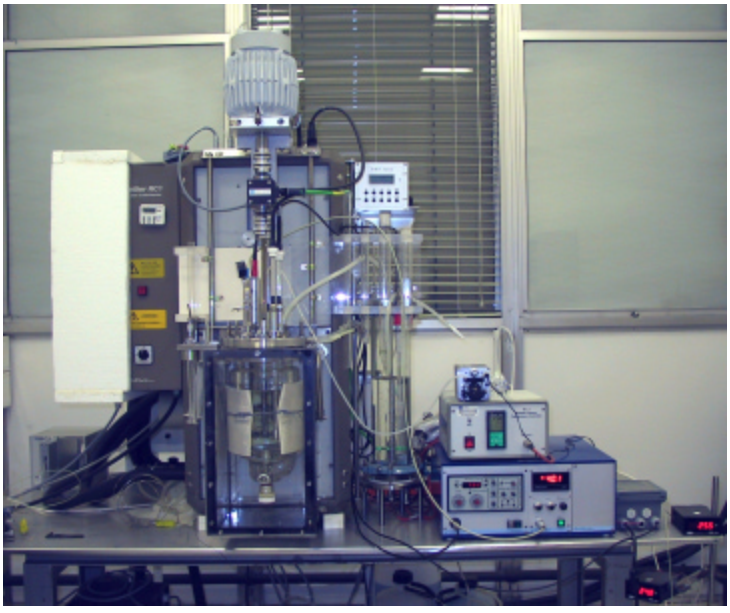
Project TOWEF0

Simultaneous calorimetric and respirometric measurements for textile wastewater characterisation

Emilio Daverio and Jos Ligthart



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Contents

Cover illustrations – from right to left

Rotifer in a sample of activated sludge; clarifiers at the "Alto Seveso" wastewater treatment plant (Fino Mornasco, Italy), courtesy of Lariana Depur s.p.a.; Gram stained filamentous bacteria in a sample of activated sludge (1000 x, immersion oil).
The Bio-RC1 calorimeter at the JRC Ispra site (Institute for Environment and Sustainability, Inland and Marine Waters Unit).

TOWEF0 (Towards Effluent Zero)

Evaluation of the effect of the IPPC application on the sustainable wastewater management in textile industries

Contract No: EVK1-CT-2000-00063

Deliverable: report on the application of simultaneous calorimetry and respirometry in investigating biodegradability and treatability of textile effluents in aerobic activated sludge processes.

Objective: application of calorimetry in investigating and better understanding biodegradability and treatability of textile effluents in aerobic activated sludge processes. Comparison between calorimetry and established respirometric techniques for wastewater characterisation.

Summary of workpackage 5 project proposal: a strategy will be developed to support the decision for the best (pre) treatment scenario out of a given set of available scenarios. The aim will be to meet effluent standards and to obtain optimum recovery of specific compounds and re-use of process water in order to realise sustainable waste management. Existing wastewater characterisation techniques will be improved and adapted to the measurement of textile process wastewater streams. The techniques will enable the on-line measurement of key variables for the biological and physico/chemical treatment of the wastewater such as toxicity, nutrient deficiency and degradability. The techniques will be based in the first instance on respirometry, i.e. the measurement of the oxygen uptake rate of biomass in contact with wastewater under well defined measuring conditions. A comparison with micro-calorimetry will also be undertaken. From the comparison of thermograms evaluated in both the absence and the presence of particular compounds, it will be possible to reach a better comprehension of the effect of toxicity, nutrient deficiency and degradability on the metabolic activities of microorganisms (activated sludge). Moreover, in

WP5 a protocol will be developed to determine the optimal composition of the wastewater produced in the factories in terms of treatment performance. To fully develop the "waste design" concept for finishing textile industries, a network of "on-line" sensors will be designed, in order to allow a real time monitoring and control of wastewater characteristics. The task of the "on-line" sensor is to inform waste producers, treatment plant and industrial operators when and how to discharge into the sewer or a specific pre-treatment or, alternatively, continue to re-use water in production process. The protocol will be based on the on-line information and should also include the means of feeding back the information on the optimal composition to the production units. In collaboration with WP4 the Water Pinch technology approach will be integrated with the characterisation and design of wastewater as far as treatability is concern. Treatability will be used as an index for understanding how many recycling loops wastewaters can undergo before being discharged from the process and disposed of at satisfying grade of treatability.

Simultaneous calorimetric and respirometric measurements for textile wastewater characterisation

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Abstract

Isothermal direct calorimetry has been applied to investigate aerobic biodegradability of mixed final effluents from two textile factories. For this purpose, a bench scale calorimeter (Bio-RC1), especially modified for biological studies and equipped with a dissolved oxygen (DO) probe, has been used. Through pulses of selected substrates (textile wastewaters, acetate or ammonia), aerobic metabolic activity has been assessed for heterotrophic and autotrophic nitrifying bacterial populations in sludge samples from wastewater treatment plants treating domestic and/or textile effluents. Calorimetric data (thermograms) have been compared to DO measurements simultaneously carried out (respirometry), so that wastewater stream degradation has been characterised in terms of microbial heat dissipation and short term biological oxygen demand. A satisfactory agreement was observed between OUR (Oxygen Uptake Rate) profiles and power-time curves confirming that, under strictly aerobic conditions, calorimetry and respirometry provide the same information. Oxycaloric equivalent, defined as the amount of heat released by the culture per mole of oxygen consumed, was estimated when textile wastewaters were the sole exogenous substrate. The values obtained are close to the theoretical value for heterotrophic aerobic metabolism (460 kJ mol O_2^{-1}) and to literature data. Additionally, toxicity effect on microbial communities due to raw wastewater additions has been investigated.

Keywords: calorimetry; oxycaloric equivalent; respirometry; textile wastewater; toxicity

INTRODUCTION

Heat generation is a final by-product of all metabolic processes and its measurement provides an overall estimation of the biological activity of the system under observation. Calorimetry, as well as respirometry, can provide valuable information for modelling and control of aerobic bio-processes as heat production and respiration are linked to two important biochemical processes in wastewater treatment plants (WWTPs): biomass growth and substrate consumption (Spanjers *et al.*, 1998). For heterotrophic aerobic growth, the heat mainly results from degradative reactions by cells (catabolism), since the enthalpy change associated to anabolism is low or may even be negligible compared to that of catabolic processes (Battley, 1987).

Unlike dissolved oxygen (DO) measurements, calorimetry is a technique universal and non-specific. In fact, the same experimental approach, based on the measurement of the heat released by the microbial culture, can be used to investigate autotrophic or heterotrophic metabolism under aerobic, anoxic and anaerobic conditions. At present, biological calorimetry is mainly applied to pure culture studies. The major development of calorimetric techniques in this field was initially strictly related to basic investigation of microbial growth energetics and to the determination of cooling requirements for industrial-scale bioreactors. More recently, calorimetric measurements application has also been extended to bioprocesses and bioreactors modelling and control, and to process development work, such as medium optimisation and growth kinetics identification (von Stockar and Marison, 1989).

In activated sludge processes, heat measurements make possible to assess the overall bacterial activity of sludge samples immediately, continuously and without interfering with the reaction system as well as to derive useful information on the biodegradability or toxicity of the influent. Moreover, the interpretation of power-time curves (thermograms) describing microbial metabolism is a powerful tool to identify significant or critical and even unexpected metabolic events occurring in a biological system, such as shifts from one substrate to another or from one catabolism to another (e.g. from oxidative to fermentative) (von Stockar and Birou, 1989), toxicity and inhibitory effects and nutrient deficiencies.

Heat production rate is related to the conversion rates of substrates or products by the definition of heat yields $Y_{Q/i}$ that represent the heat released per unit amount of converted reactant or generated product. In particular, it has been theoretically demonstrated

(Minkevich and Eroshin, 1973; Birou *et al.*, 1987) that the heat dissipated per mole of oxygen consumed (oxycaloric equivalent, $Y_{Q/O}$) under strictly aerobic conditions, is approximately the same (460 kJ mol O_2^{-1}) for all heterotrophic growth processes regardless of microbial strain and nature of substrates or products. Constancy of this quantity is based on the regularity for the heat of combustion of organic substrates (Thornton, 1917; Kharasch, 1929) and dried biomass per 1 gram equivalent of oxygen consumed (Minkevich and Eroshin, 1973).

The linear correlation between heat production rate and OUR (Oxygen Uptake Rate) has been experimentally verified mainly on pure cell cultures. Several works are reported in literature concerning the evaluation of oxycaloric equivalent for mammalian cells, muscle tissues, brown adipose tissues, aquatic animals and, especially, for bacteria and yeasts.

In their pioneering investigation, Cooney *et al.* (1968) observed, for the oxycaloric equivalent, an average experimental value of 518 ± 12 kJ mol O_2^{-1}. Experiments were performed using bacteria (*E. coli* and *B. subtilis*), a yeast (*C. intermedia*) and a mould (*A. niger*) aerobically grown on glucose, molasses and soy bean meal. A dynamic calorimetric technique was used to evaluate the heat production rate during fermentation by recording the temperature rise of fermentor broth in a 14-liter insulated vessel when temperature controller was turned off. The heat accumulation measured in this manner was corrected for heat losses and gains terms (evaporation, agitation and heat losses to the surroundings) and was compared to oxygen consumption rate determined by measuring the gas flow rate and the oxygen concentration in the exit gas stream. Loung and Volesky (1980), using a similar approach, determined the rate of heat evolution and the rate of oxygen consumption for pure cultures of *A. niger*, *E. coli*, *C. lipolytica*, *C. intermedia* and *C. utilis* grown on ethanol, glucose, sucrose, n-dodecane and n-hexadecane. Based on the experimental data, the correlation between heat dissipation and oxygen consumption was determined as 461 ± 84 kJ mol O_2^{-1}, whereas data ranged from 384 to 600 kJ mol O_2^{-1}. Erickson *et al.* (1978) investigated the lysine synthesis process by cultures of *Brevibacterium* grown on molasses and corn extract. $Y_{Q/O}$ (mean value for three experiments) was estimated to be 26.5 kcal equiv O_2^{-1} (444 kJ mol O_2^{-1}). Birou *et al.* (1987), working with a modified BSC 81 isothermal heat flux calorimeter and with different strains of bacteria (*E. coli*, *Ent. cloacae* and *M. methylotrophus*) and yeasts (*C. boidinii*, *C. lipolytica*, *C. pseudotropicalis*, *C. utilis* and *K. fragilis*) and various substrates with reduction degree from 3 (citric acid) to 6.13

(hexadecane), found an average value, for 12 determinations, of 440 ± 33 kJ mol O_2^{-1}. Randolph *et al.* (1990) working with pure cultures of *S. cerevisiae* CBS 426, cultivated with glucose as the sole carbon source in a Mettler-Toledo RC1 calorimeter modified for investigating biological processes, observed an oxycaloric equivalent of 461 kJ mol O_2^{-1}. The possibility of continuously measuring the heat generated by microorganisms in a standard laboratory fermentor, by means of an accurate energy balance around the system, was studied by van Kleeff *et al.* (1993). Oxygen consumption measurements were coupled to heat production rate data during growth of *C. utilis* on glucose. In this case, $Y_{Q/O}$ was estimated to be 457 ± 12 kJ mol O_2^{-1}. In the nineties, Johansson and Wadsö (1999), using an isothermal microcalorimetric perfusion vessel equipped with a Clark cell O_2 electrode, investigated the aerobic growth of *E. coli*. The mean value (4 experiments) obtained for oxycaloric equivalent was 442 ± 15 kJ mol O_2^{-1}. Recently, Dejean *et al.* (2001) studied, with a TAM flow-through microcalorimeter, heat dissipation and oxygen consumption of resting and growing (lactate) cultures of *S. cerevisiae* W303-1a. Oxycaloric equivalents were found to be 439 ± 10 kJ mol O_2^{-1} and 378 ± 7 kJ mol O_2^{-1} respectively.

The relationship between oxygen consumption and heat production is poorly documented for activated sludge systems. In fact, very few papers exist dealing with simultaneous calorimetric and respirometric measurements applied to investigate wastewater treatment biological processes. Beaubien and Jolicoeur (1985), operating with a twin cell Picker flow microcalorimeter (SODEV Inc.), assessed biomass activity in sludge samples from a lab-scale bioreactor fed on a synthetic medium containing beef extract, bacto peptone and soy broth. During comparative respirometry-calorimetry studies, the oxycaloric equivalent was determined to be 345 kJ mol O_2^{-1}. Aulenta *et al.* (2002) report for $Y_{Q/O}$ a value of 470 kJ mol O_2^{-1}, measured for activated sludge samples fed on acetate in a Mettler Toledo Bio-RC1 calorimeter. To authors knowledge, no explicit values for oxycaloric equivalent are reported in literature for experiments related to raw wastewater degradation by biomass from activated sludge WWTPs.

The direct relationship between OUR and heat flux suggests that, for purely aerobic metabolism, calorimetric measurements may substitute oxygen measurements (indirect calorimetry) or vice versa (Gustafsson, 1991). Moreover, coupling of calorimetric and respirometric measurements may be an interesting tool in biotechnological studies and applications. In fact, complementary information can be extracted from the combination of

these two techniques in the presence of mixed respiratory-fermentative metabolisms, enhanced futile substrate cycling and uncoupling of oxidative phosphorylation (Gnaiger and Kemp, 1990; Kemp, 2000). In these cases, the oxycaloric equivalent experimentally estimated is higher than the theoretical value. Volesky *et al.* (1982) observed an oxycaloric equivalent as high as 1710 kJ mol O_2^{-1} for growth of *S. cerevisiae,* when respiration was partially repressed by a glucose effect and ethanol was formed via the fermentative pathway. For human cells, under strictly controlled aerobic conditions, there have been reports of $Y_{Q/O}$ as highly exothermic as that presented by Schön and Wadsö (1986) for T-lymphoma cells (1100 kJ mol O_2^{-1}) or by Eftimiadi and Rialdi (1982) for neutrophils (590 kJ mol O_2^{-1}). Considerably exothermic $Y_{Q/O}$ values indicate the intensity of anaerobic pathways operating simultaneously with aerobic reactions. Reasons for accumulation and excretion of anaerobic glycolytic endproducts such as lactate, pyruvate and succinate by cells under normoxic conditions can be related to the need for biosynthetic precursors from catabolic substrates in deficient media or to an ATP demand that exceeds mitochondrial capacity, resulting in the reduction of pyruvate with conservation of NAD^+ (Kemp, 2000).

The same approach used for heterotrophic growth can be, in principle, applied also to autotrophic aerobic metabolism. In particular, the linear relationship between OUR and heat production rate has been verified on nitrifying biomass from a full-scale activated sludge plant (Daverio *et al.*, 2003). Oxycaloric equivalent was estimated to be 151 ± 4 kJ mol O_2^{-1} for nitrite oxidisers and 189 ± 13 kJ mol O_2^{-1} for ammonia oxidisers. The finding that $Y_{Q/O}$ for nitrifiers is lower than for heterotrophs is an expected result since the energy sources for the two bacterial populations are different. In fact, the stoichiometric oxidation of ammonia to nitrate theoretically dissipates a significantly lower amount of heat (180 kJ) per mole of oxygen consumed than the combustion of organic compounds (mean value of 444 kJ mol O_2^{-1} as reported by Thornton (1917)). Indeed, the linear correlation between heat flux and OUR for heterotrophic and nitrifying populations, with $Y_{Q/O}$ for heterotrophs approximately 2.5 times higher than for nitrifiers, suggests that the evaluation of $Y_{Q/O}$ variations during aerobic biodegradation of wastewater containing both organic matter and ammonia can be a powerful tool to identify and discriminate the biological activity of the two trophic groups. In Figure 1 (Daverio, 2002), the thermogram and the respirogram acquired after a synthetic wastewater pulse (mixture of biogenic carbonaceous compounds and ammonia) to an activated sludge in endogenous conditions are presented. Immediately after the spike, a

sudden increase in both heat production rate and OUR was detected and three regions characterised by different $Y_{Q/O}$ (Figure 2) can be identified. In zone A ammonia and carbonaceous substrates are simultaneously consumed. After the exhaustion of readily biodegradable organic matter (RBCOD), ammonia oxidation becomes the prevailing oxygen consuming bioreaction (B). A clear decoupling between heat flux and OUR was observed and $Y_{Q/O}$ approached the value related to autotrophic nitrification. Region C refers to the consumption of storage polymers accumulated during RBCOD conversion and $Y_{Q/O}$ is close to the theoretical value for purely heterotrophic metabolism.

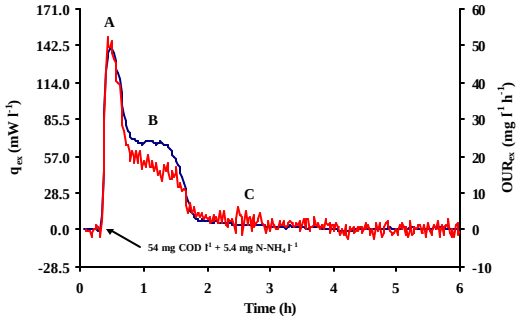

Figure 1. Thermogram and respirogram recorded after synthetic wastewater pulse to an activated sludge; - q_{ex}; - OUR_{ex}.

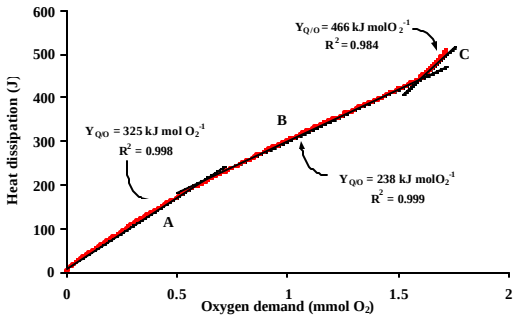

Figure 2. Oxycaloric equivalents estimated from data in Figure 1.

An isothermal bench-scale calorimeter (Bio-RC1), especially suited for biological studies and equipped with a DO probe, has been used to investigate aerobic biodegradability of mixed final effluents from two textile factories (named “wastewater A” and “wastewater B” respectively). Sludge samples from WWTPs treating domestic and/or textile effluents have been considered and, for each sample, bacterial activity of heterotrophic aerobic and autotrophic nitrifying populations has been assessed. Calorimetric profiles (thermograms) have been compared to OUR measurements simultaneously carried out (respirometry) so that it has been possible to estimate the oxycaloric equivalent when textile wastewater was the sole exogenous energy source and to evaluate effluent biodegradability. Experimental results obtained working on wastewater A and wastewater B are presented and discussed separately. In particular, the toxicity effect of wastewater A has been evaluated on aerobic bacterial communities and a comparison between biomass activity from a full-scale activated sludge WWTP and a membrane bioreactor (MBR) pilot plant fed exclusively on wastewater B is presented.

MATERIALS AND METHODS

The calorimeter

Due to the fairly low heat exchanges accompanying biological phenomena compared to chemical reactions, very sensitive calorimetric devices are required to correctly evaluate heat generation by bacterial cultures. The Bio-RC1 (Figure 3), developed by Mettler-Toledo AG, Schwerzenbach, Switzerland for investigating chemical reactions and subsequently adapted for biological studies, is a heat flux bench-scale calorimeter (Marison *et al.*, 1998).

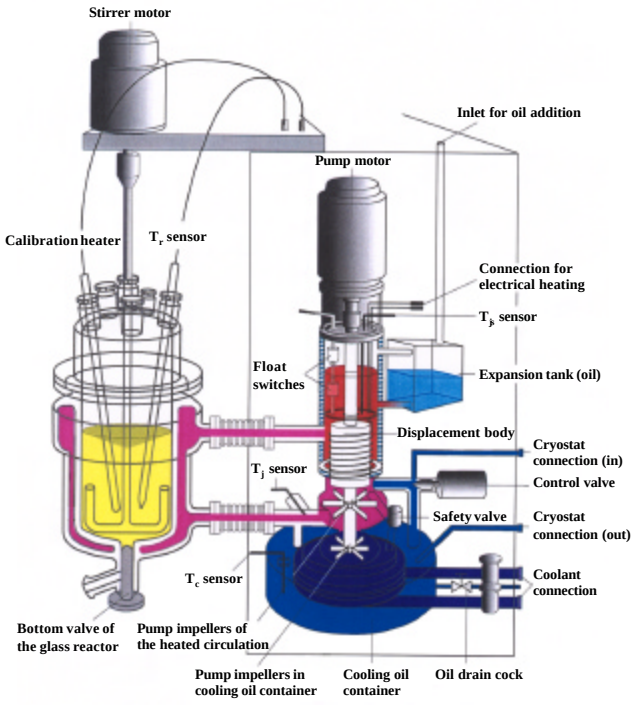

Figure 3. Schematic overview of the Bio-RC1 (adapted from Mettler Toledo RC1e Operating Instructions).

The Bio-RC1 possesses a standard 2-liter jacketed glass reactor and can be operated in isothermal, isoperibolic (constant jacket temperature) or adiabatic conditions. In the isothermal mode, in order to maintain constant the temperature of the reactant medium (Tr) by a proper control of the jacket temperature (Tj), a low-viscosity silicone oil is pumped at high rate (2 l s^{-1}) through the reactor jacket. The jacket temperature is carefully controlled by blending oils from a 'hot' and a 'cold' oil circuit, via an electronically controlled metering valve. Therefore, when a process dissipates or takes up heat, Tj respectively

decreases or increases. The resulting temperature gradient across the reactor wall is directly proportional to the thermal power liberated or absorbed by the process (Qr) according to:

$$Qr = UA \cdot (Tr - Tj) \quad (1)$$

where U is the overall heat-transfer coefficient (W m^{-2} K^{-1}), A the heat transfer surface (m^2) and (Tr-Tj) the temperature difference (K) between the reactor contents and the jacket oil. When a new sludge sample is put into the calorimeter, the UA factor has to be experimentally determined using an internal electrical calibration heater of known power output.

For biological studies, the calorimetric resolution has been improved by decreasing the full range of temperature, thermostatting the temperature signals, increasing the Analog/Digital board resolution and by implementing a PI (proportional-integral) controller for the reactor temperature, instead of the P (proportional) controller of the standard RC1. Some additional upgrades were necessary in order to increase the stability and the sensitivity of the system. In particular, a thermostatted top-plate to reduce evaporation effects, and a bubble column for pre-saturating and thermostatting the inlet gas stream were added. Therefore, by changing both the hardware and the software of the original instrument, the system resolution has been increased to 5-10 mW l^{-1} (Figure 4b) and the stability of the reaction volume temperature to ± 1 mK for several hours (Figure 4a).

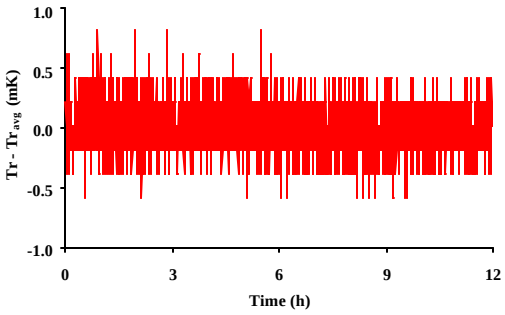

Figure 4a. Stability of the reaction volume temperature during isothermal operation.

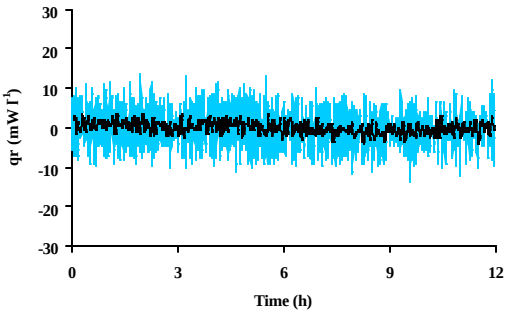

Figure 4b. Heat flux baseline measurement; - qr, - qr 25 per. moving average.

The Bio-RC1 does not require to compute any energy balance for the system, so that the quality of heat measurements does not depend on the accuracy of secondary heat effects

estimation. In fact, heat production rate due to biological exogenous reactions (Q_{ex}) is simply equal to the measured heat flux (Qr) corrected for the baseline contribution, assumed to be constant throughout the test, which is the sum of two terms: the external heat loss and gain terms (e.g. due to stirring, evaporation and condensation effects, heat losses to the environment) and the thermal power dissipation related to microbial endogenous metabolism.

DO concentration and pH are on-line acquired through specific probes directly inserted into the reaction volume and processed by LabVIEW (National Instruments) together with temperature and heat production rate signals.

Biological OUR was calculated taking into account the oxygen mass transfer coefficient (K_{La}), determined before each substrate pulse and considered constant during the test (Roš *et al.*, 1988).

Analytical methods

COD and ammonia analyses were carried out by means of a spectrophotometric test (Spectroquant, Merck). Total (TSS) and volatile (VSS) suspended solids were determined according to *Standard Methods* (1995).

WASTEWATER A

Simultaneous calorimetric and respirometric measurements have been applied to characterise biodegradability and toxicity of the final mixed effluent of an Italian textile factory (Towef0 code: I-06). Raw wastewater characteristics are summarised in Table 1.

Table 1. Raw wastewater A characteristics.

WASTEWATER A	
COD (mg l^{-1})	1230
N-NH_4 (mg l^{-1})	0.5
TSS (g l^{-1})	0.186
VSS (g l^{-1})	0.166
% VSS	89.2

BIOMASS SOURCE AND EXPERIMENTAL DESIGN

Experiments with raw wastewater A have been performed using four different biomass samples. The first sludge sample was collected at the "Alto Seveso" WWTP (Fino Mornasco, Italy), that treats 30 % domestic wastewater and 70 % textile industry wastewater and receives the final effluent of the I-06 factory. 1.4 l of sludge (VSS = 1.81 g l^{-1}, TSS = 2.47 g l^{-1}) were placed into the calorimeter. Temperature was set to 25 °C and agitation to 150 rpm, aeration was ensured by bubbling air. Before the addition, wastewater samples were accurately thermostatted (25 °C) and intensively aerated. Calibration procedure for overall heat transfer factor determination was performed at the beginning and at the end of each experiment. During data processing, UA changes (about 5 % rise) due to volume increase after wastewater addition were taken into account. The total heat dissipation for each calorimetric peak was simply evaluated as the area under the thermogram.

In order to evaluate biomass adaptation to wastewater consumption and the toxicity effect of the effluent, two heterotrophic aerobic bacterial cultures were set up in 2-liter lab-scale bioreactors. The inoculum was sampled at the "Varese lago" WWTP (Gavirate, Italy). Working volume was 1.5 l. pH and temperature were fixed at 7.5 and 25 °C respectively. The reactors were operated using intermittent feeding. The first culture was fed with acetate as the sole carbon and energy source. 150 ml feed were added 1 time per day so that the organic loading rate was 0.390 g COD l^{-1} d^{-1}. Feed solution contained per litre: 5.56 g CH_3COONa, 2 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.055 g $CaCl_2 \cdot 2H_2O$, 0.44 g K_2HPO_4, 0.34 g KH_2PO_4, 0.002 g $FeCl_3 \cdot 6H_2O$, 0.003 g Na_2EDTA, 1 ml trace element solution. Trace element solution contained per litre: 100 mg $ZnSO_4 \cdot 7H_2O$, 30 mg $MnCl_2 \cdot 7H_2O$, 300 mg H_3BO_3, 200 mg $CoCl_2 \cdot 6H_2O$, 10 mg $CuCl_2 \cdot 2H_2O$, 20 mg $NiCl_2 \cdot 6H_2O$, 30 mg $NaMoO_4 \cdot 2H_2O$. The effluent was taken up each day at the end of the cycle from the stirred and aerated mixed-liquor. Thus, sludge age was correspondent to HRT (Hydraulic Retention Time) and was 11 days. The same operational parameters were used for the second culture but 350 ml l^{-1} wastewater A were added to feed solution.

For calorimetric tests, the mixed liquor (0.7 l) was sampled from the reactor just before a new feed addition. Sludge was diluted with a solution containing non-limiting N and P concentrations (same ratio used for culture medium) until a volume of 1.4 l was reached and

was put into the calorimeter. Nitrification was inhibited by adding allyl-thiourea (10 mg l^{-1}). VSS concentration was 0.43 g l^{1} (TSS = 0.48 g l^{-1}) for the experiments with biomass cultivated on acetate and 0.29 g l^{-1} (TSS = 0.32 g l^{1}) in the case of biomass cultivated on acetate and wastewater. Additionally, in order to evaluate wastewater A toxicity effect on nitrifying population, a sludge sample from the "Varese lago" WWTP, that includes nitrification, was used as inoculum for an enriched nitrifying culture. Total volume was 8 l and feed was added four times per day. Volumetric loads were: 100 mg $N\text{-}NH_4$ l^{-1} d^{-1}, 250 mg COD l^{1} d^{-1}, 800 mg $NaHCO_3$ l^{-1} d^{-1}. Yeast extract was used as carbon source for heterotrophic population. Once a day the sludge was settled and 5 l of supernatant were removed and replaced with the same volume of tap water (HRT = 16 days). The culture was aerated, agitated and kept at room temperature (20 to 25 °C). In the calorimeter temperature was fixed at 25 °C and pH was controlled to 8.0 ± 0.2 by adding a 0.5 M NaOH solution. Biomass concentration was 1.36 g VSS l^{1} (TSS = 1.52 g l^{-1}).

RESULTS AND DISCUSSION

The thermogram and the respirogram acquired during an experiment with "Alto Seveso" biomass are reported in Figures 5 and 6 respectively. At t = 1.5 h, the endogenous sludge was spiked with 100 ml wastewater. Wastewater degradation is characterised by an immediate increase in heat production rate and in oxygen uptake rate, probably due to the presence of a small amount of readily biodegradable COD, and by a quite long tail. Wastewater additions were repeated 5 times. A satisfactory reproducibility was observed for both oxygen and heat measurements. During the 5 tests, endogenous respiration dropped from 12 to 7 mg l^{1} h^{-1} and a progressive decrease was observed also in the maximum exogenous heat production rate and in the maximum exogenous OUR (from 42 to 17 mg l^{1} h^{-1}) resulted from repetitive wastewater pulses. This can be mainly attributed to a toxicity effect due to wastewater additions. The short term biological oxygen demand, evaluated as the area under the respirogram for each wastewater pulse, was almost constant. For 100 ml wastewater addition and a total reaction volume of 1.5 l the average value was 15 mg l^{1} (Coefficient of Variation = 11 %).

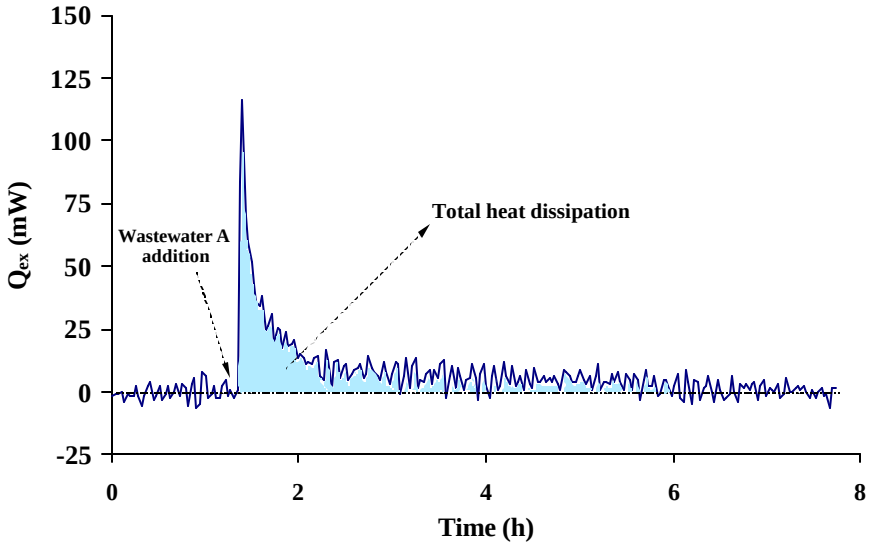

Figure 5. Thermogram acquired during wastewater A degradation ("Alto Seveso" biomass).

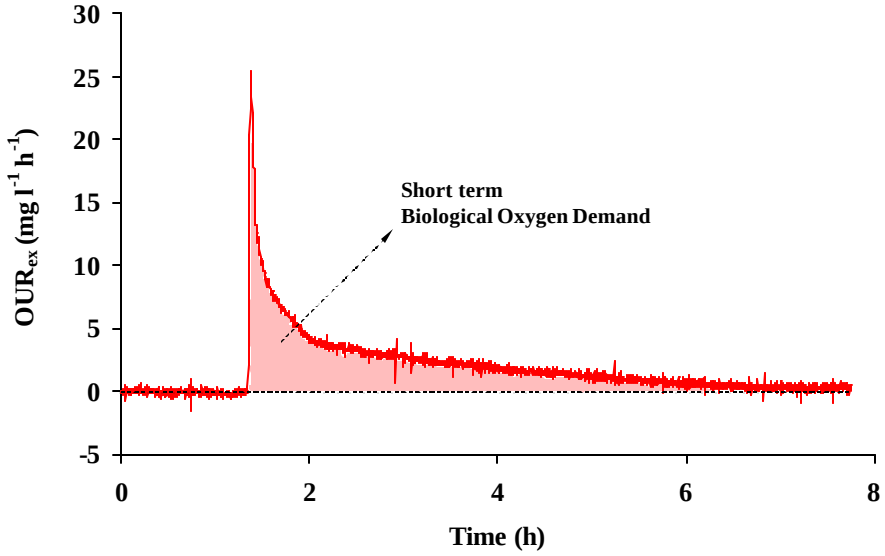

Figure 6. Respirogram acquired during wastewater A degradation ("Alto Seveso" biomass).

By plotting the volumetric heat dissipation, calculated as the area under the thermogram (Figure 5), versus the oxygen demand (area under the respirogram in Figure 6), it is possible to estimate the oxycaloric equivalent as the slope of the linear regression on the experimental data (Figure 7). $Y_{Q/O}$ resulted to be 403 kJ mol O_2^{-1}. This value is in good agreement with the theoretical value for he terotrophic aerobic metabolism (460 kJ mol O_2^{-1}). Besides the absolute value of the slope, one may note that a satisfactory linear

relationship (R^2 = 0.995) between heat production rate and oxygen uptake rate was obtained for a biomass sample from a full-scale WWTP fed on a real raw wastewater stream. The slight deviation from the calculated value at the end of wastewater degradation (upper points in Figure 7) can be related to the difficulties in evaluating exogenous heat production rate and OUR when they approach zero. These inaccuracies are due to the small differences in oxygen concentration at the end of the test (DO measurements) and to possible shifts in the thermal baseline value (heat measurements).

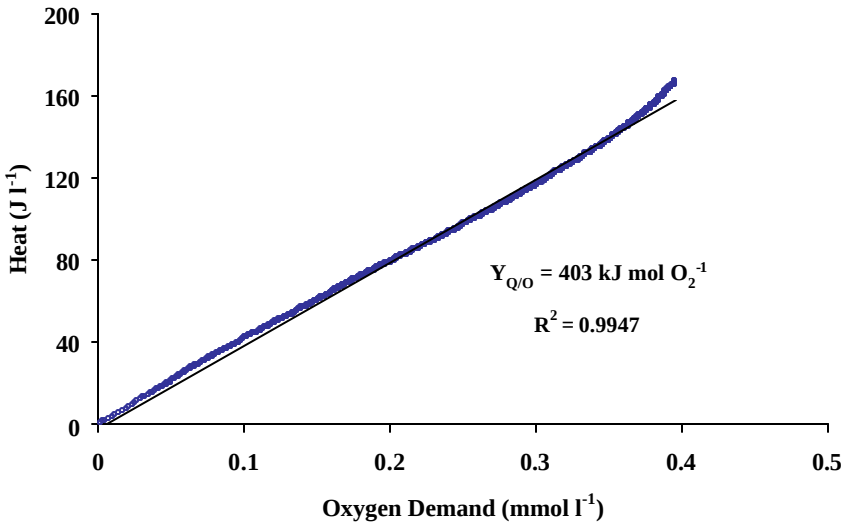

Figure 7. Oxycaloric equivalent estimated for wastewater A degradation.

Additionally, in order to evaluate both autotrophic nitrifying and heterotrophic activity, a new sample (VSS = 2.28 g l^{-1}; TSS = 3.20 g l^{-1}) of "Alto Seveso" sludge was spiked with ammonia and acetate, selected as reference readily biodegradable carbonaceous substrate. The thermal response to wastewater addition was compared to acetate and ammonia consumption. In Figure 8, the respirogram obtained after substrate pulses is reported. Peak A refers to 100 ml wastewater addition, peak B to acetate (10 mg COD l^{1}) and peak C to ammonia (3.3 mg $N\text{-}NH_4$ l^{-1}). Endogenous activity at the beginning of the experiment was 10 mg l^{-1} h^{-1} (4.4 mg g VSS^{-1} h^{-1}) and slightly decreased during the test. Exogenous OUR on acetate was 30 mg l^{1} h^{-1} whereas only 7 mg l^{1} h^{-1} was the maximum oxygen uptake rate after wastewater pulse. Respirogram acquired for ammonia has an uncommon shape, characterised by a slow OUR decrease that starts immediately after the maximum is reached. More research is needed to explain this phenomenon. Heat flux profiles (Figure 9)

confirmed the results obtained with DO measurements. The maximum exogenous heat production rate for wastewater degradation was 36 mW. The ratio between maximum OUR and maximum heat production rate, that provides a rough estimate of $Y_{Q/O}$, is similar for acetate and wastewater (394 and 320 kJ mol O_2^{-1}), whereas becomes significantly lower (165 kJ mol O_2^{-1}) for ammonia consumption.

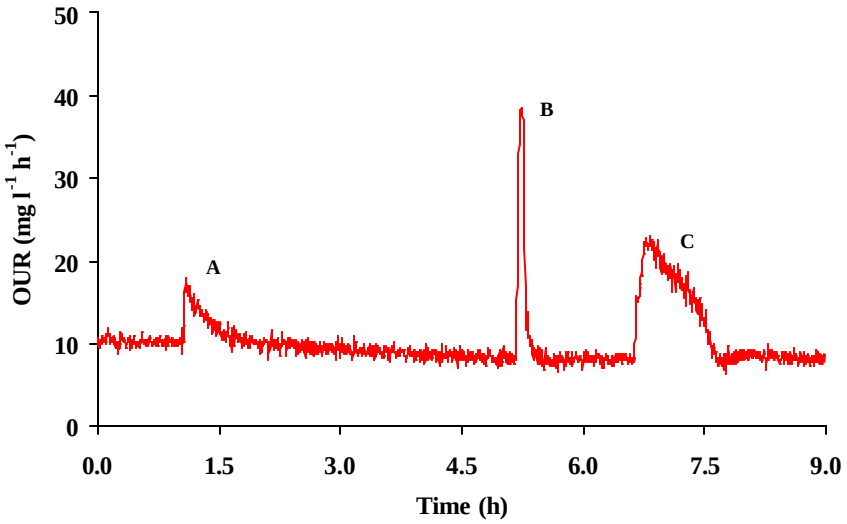

Figure 8. Respirogram acquired after substrate spikes to "Alto Seveso" WWTP biomass.

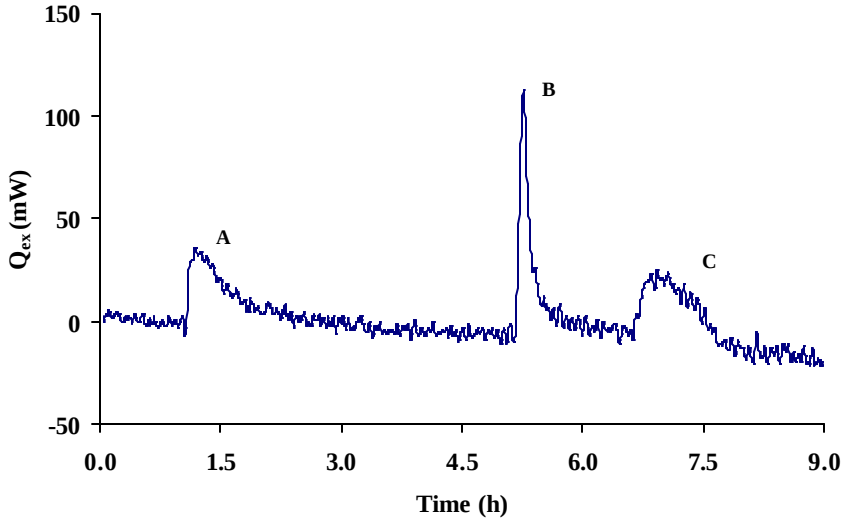

Figure 9. Thermogram acquired after substrate spikes to "Alto Seveso" WWTP biomass.

Similar results were obtained with a sample of the same sludge and identical substrates by Bisschops (2002). The respirometer used was a Manotherm RA-1000. Respirograms related to acetate, 20 mg COD (A1) and 40 mg COD (A2), ammonia, 3.5 mg N (N1) and 1.75 mg N (N2) and raw wastewater, 50 ml (WW1) and 100 ml (WW2) are presented in Figure 10. Also in this case, an inhibitory effect of wastewater on biomass activity was identified. In fact, a significant decrease of the maximum OUR related to acetate degradation was observed after wastewater exposure.

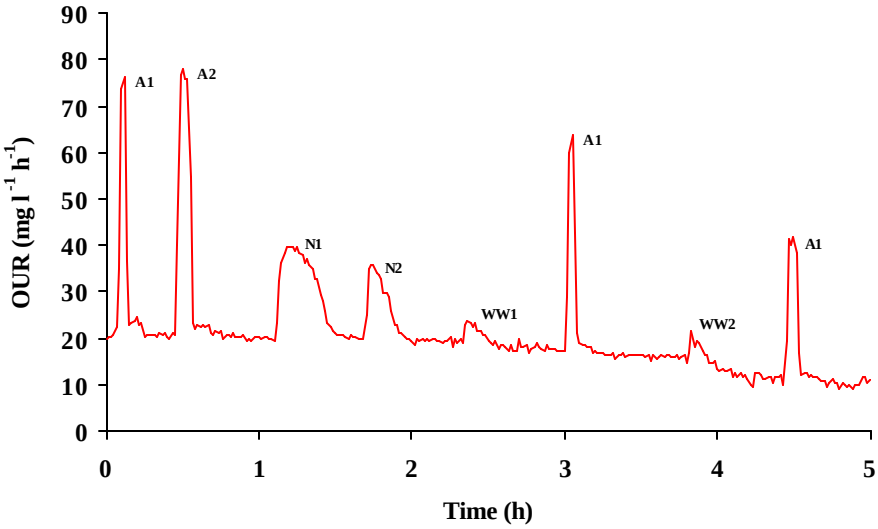

Figure 10. Respirogram acquired with a RA-1000 respirometer after substrate pulses to "Alto Seveso" sludge. (Bisschops, 2002).

Subsequently, the toxicity effect of wastewater A was evaluated on cultivated biomass. In Figures 11 and 12 the results related to biomass fed on acetate as the sole carbon and energy source are presented. Thermograms and respirograms A1 and A2 refer to acetate consumption (200 mg COD), thermogram and respirogram WW to wastewater degradation (100 ml). C is the electrical calibration for UA determination after wastewater addition, when the reaction volume increased from 1.4 to 1.5 l. Biomass inhibition has been calculated by comparing the maximum heat production rate acquired before and after biomass exposure to wastewater according to the following equation:

$$I\% = 100 \cdot (Q_{ex} - Q_{ex}') / Q_{ex} \quad (2)$$

where Q_{ex} is the maximum exogenous heat production rate related to acetate degradation before wastewater exposure and Q_{ex}' is the maximum exogenous heat production rate related to acetate degradation after wastewater exposure.

The maximum heat production rate for acetate consumption decreased from 375 mW to 330 mW after wastewater exposure (12 % inhibition), whereas the total heat dissipation remained almost constant (650 J) since, at each spike, the same amount of acetate was added to the system. The percentage inhibition estimated from OUR profile compares well with the one calculated through heat measurements (Figure 12). In fact, after wastewater exposure, endogenous OUR measured before acetate pulses dropped from 2.62 mg g VSS^{-1} h^{-1} to 2.26 mg g VSS^{-1} h^{-1} and maximum OUR during acetate degradation decreased from 193 mg g VSS^{-1} h^{-1} to 169 mg g VSS^{-1} h^{-1} (12 % inhibition). As reported in the insert in Figure 12, the sole acetate consumption was related to an oxygen demand of around 55 mg O_2 and $Y_{Q/O}$ was estimated to be 370 kJ mol O_2^{-1}. This value is in good agreement with those observed by Beaubien and Jolicoeur (1985) working with activated sludges (345 kJ mol O_2^{-1}) and by Birou *et al.* (1987) on pure cultures of *C. utilis* growing on acetate (385 kJ mol O_2^{-1}). In thermograms A1 and A2, the peak related to primary substrate consumption is followed by an exothermic tail representing the degradation of intracellular storage polymers, mainly poly-beta-hydroxybutyrate (PHB), accumulated during acetate depletion. This phenomenon has been typically observed when biomass grows under transient conditions, for example when a substrate concentration gradient is produced, leading to successive periods of feast and famine, like in intermittently-fed reactors or when biomass is subjected to alternating aerobic, anoxic or anaerobic conditions, like in nutrient removal processes (Majone *et al.*, 1999; Beun *et al.*, 2000). Taking into account also PHB consumption the total heat released for acetate degradation was 1170 J and the whole oxygen demand around 100 mg O_2.

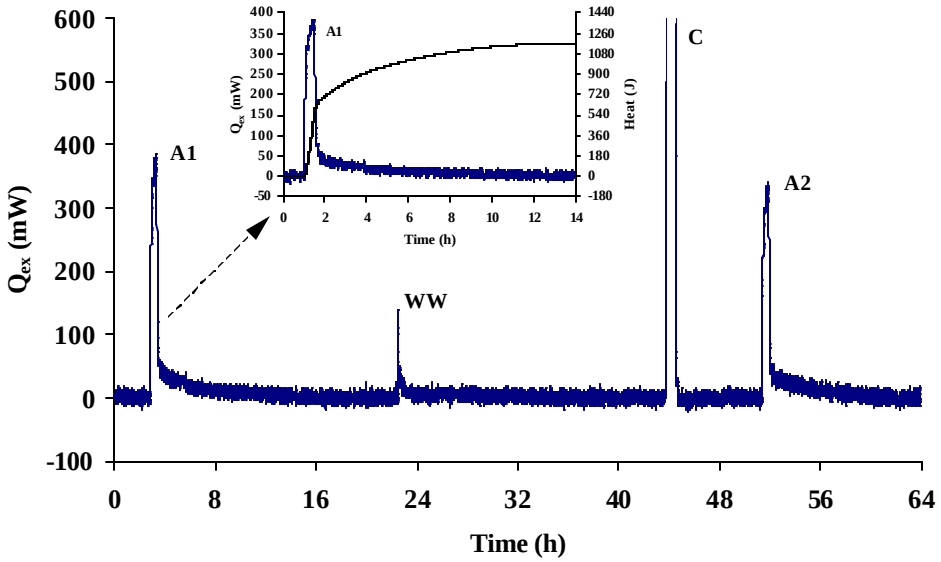

Figure 11. Wastewater A toxicity effect: thermogram related to the experiment with biomass fed exclusively on acetate. In the insert: acetate degradation and calculated heat dissipation (— q_{ex}, — heat dissipation).

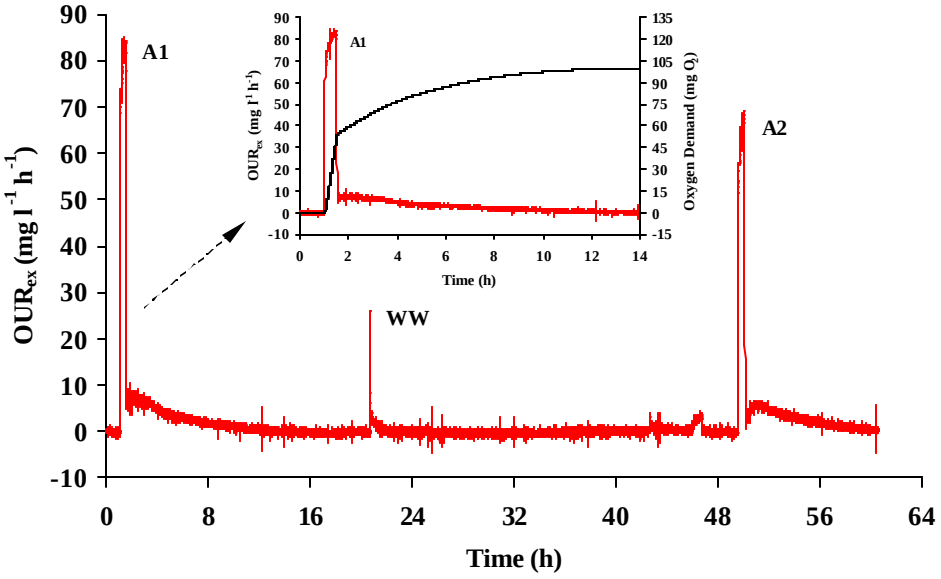

Figure 12. Wastewater A toxicity effect: respirogram related to the experiment with biomass fed exclusively on acetate. In the insert: acetate degradation and calculated oxygen demand (— OUR_{ex}, – – Oxygen Demand).

Similar results were obtained for the culture fed on acetate and wastewater. In Figure 13, thermograms A1, A2 and A3 refer to acetate consumption (200 mg COD) and thermogram WW1 and WW2 to wastewater degradation (100 ml). C is the electrical calibration. At t = 66 h the sludge was settled and 100 ml supernatant were withdrawn. Subsequently, wastewater and acetate pulses were repeated. The maximum heat production rate for acetate degradation decreased from 378 mW to 328 mW after the first wastewater exposure (13 % inhibition) and to 282 mW (25 % inhibition) after the second pulse. Notwithstanding biomass had been exposed to wastewater during cultivation (5 weeks), the degree of inhibition was not significantly different than the one detected for biomass fed exclusively on acetate. Also wastewater degradation was not affected by the adaptation period.

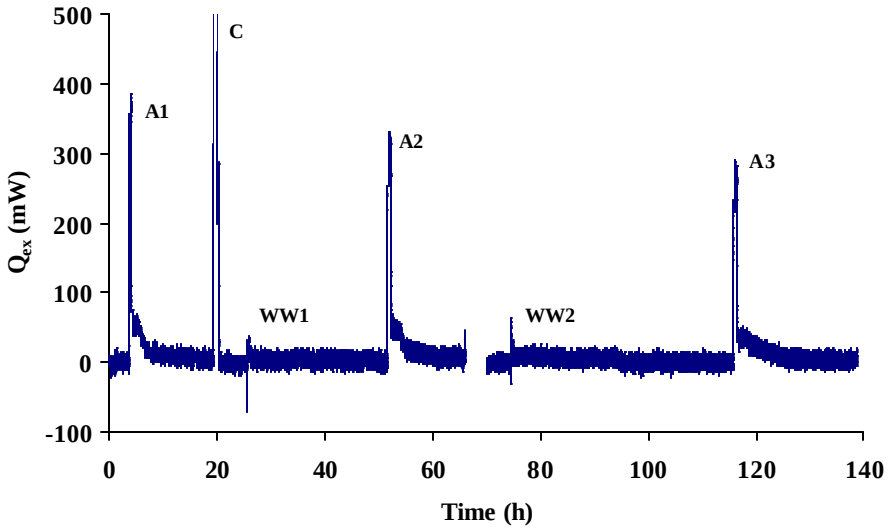

Figure 13. Wastewater A toxicity effect: thermogram related to the experiment with biomass fed on acetate and wastewater.

In order to verify that the decrease in heat production rate was mainly related to wastewater inhibitory effect and not to biomass decay occurred between the first and the second acetate addition, acetate pulses were repeated on a sample of the same biomass used for the previous test without adding wastewater. In Figure 14, the thermograms related to acetate degradation are presented. Only 3 % decrease in the maximum heat production rate was observed after the second acetate pulse (A2) that was performed 47 hours after the first addition (A1). Therefore, in the time frame of a single batch test, biomass decay had only a minor effect on the observed decrease in biological activity.

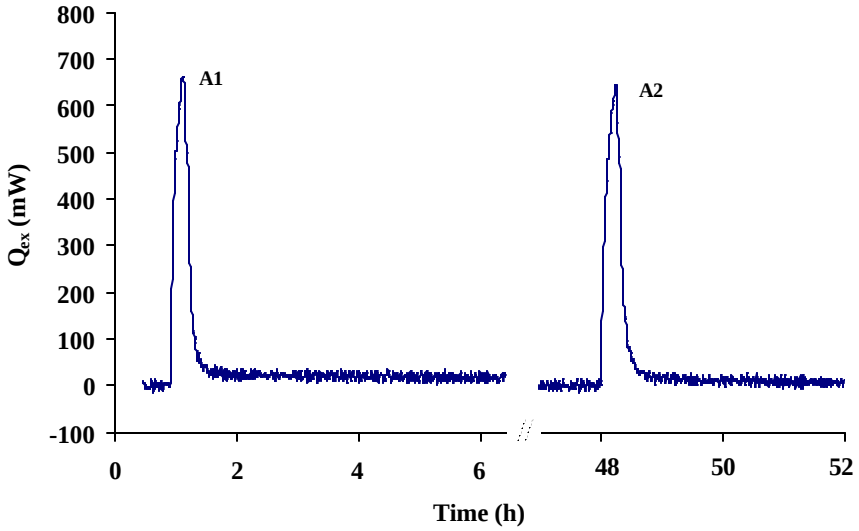

Figure 14. Acetate additions to biomass cultivated on acetate and wastewater: cell decay effect investigation.

Finally, wastewater A inhibitory effect was evaluated on the enriched nitrifying culture (Figure 15). Thermograms N1, N2 and N3 refer to ammonia pulses (20 mg N-NH_4) and thermogram W to wastewater addition (100 ml). The maximum heat production rate for ammonia degradation decreased from 227 mW to 165 mW after wastewater exposure (27 % inhibition). The percentage inhibition estimated for nitrifiers was more than two times higher than the one for the heterotrophs. This was expected since nitrifying bacteria are recognised to be, among the aerobic microbial populations, the most sensitive to toxicity effects, especially in activated sludge processes (Kroiss *et al.*, 1992). Nevertheless, a recover of microbial activity was observed when ammonia pulse was repeated (N3) without new wastewater additions. In fact, in this case, maximum heat flux increased up to 205 mW (10 % inhibition). The total heat released was almost the same for each ammonia peak (mean value = 496 J; CV = 4 %), thus confirming a good signal reproducibility also for nitrification experiments.

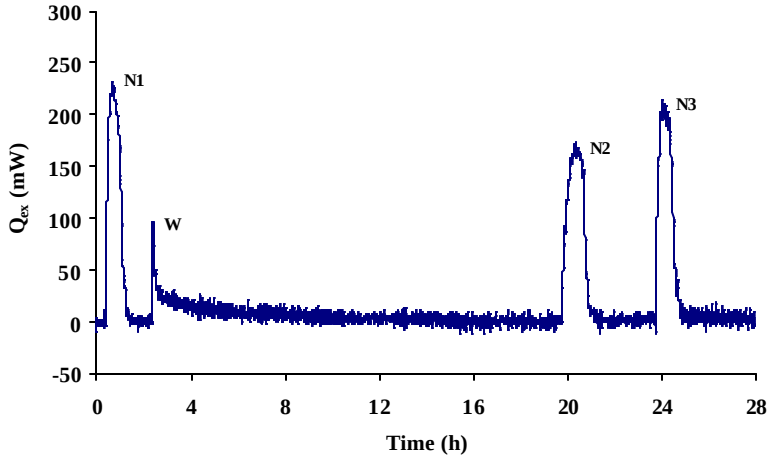

Figure 15. Wastewater A toxicity effect: thermogram related to the experiment with the enriched nitrifying culture.

WASTEWATER B

Calorimetric and respirometric measurements have been carried out to investigate the aerobic biodegradability of the final effluent (wastewater B) of a polyester textile finishing factory. Wastewater B is treated in an activated sludge (AS) WWTP located at the factory. The treatment steps are: neutralisation/equalisation, extended aeration, secondary settling with aerobic digestion of wasted sludge. An additional aerobic MBR (Membrane BioReactor) pilot plant operating on wastewater B was installed in parallel with the full-scale WWTP (Malpei *et al.*, 2002). The MBR was a ZeeWeed®-10 bench test unit (Zenon Environmental Inc., Oakville, Canada) made of a hollow fibre module submerged in a 200 l aeration tank. Characteristics of raw wastewater B and operational parameters for the two plants are summarised in Table 2 and Table 3 respectively.

Table 2. Raw wastewater B characteristics.

WASTEWATER B	
COD (mg l^{-1})	3916
N-NH_4 (mg l^{-1})	5.8
TSS (g l^{-1})	1.87
VSS (g l^{-1})	1.64
% VSS	88

Table 3. WWTPs operational parameters.

	Full scale (AS)	MBR
Aeration tank volume (m^3)	1500	0.22
Influent ($m^3 d^{-1}$)	440	0.07
HRT (d)	4	3.3
F/M ratio (g COD g TSS^{-1} d^{-1})*	0.05 - 0.13	0.017 – 0.36
TSS range (g l^{-1})	7.5 - 15.7	4.4 - 23.5
% VSS range	87 - 90	84 - 90

* Food over biomass ratio

BIOMASS SOURCE AND EXPERIMENTAL DESIGN

A preliminary experiment was performed to evaluate the biodegradability of raw wastewater B. The sludge was directly sampled at the AS plant. 700 ml of mixed liquor were diluted with 700 ml demineralised water so that reaction volume was 1.4 l and biomass concentration in the calorimeter was 5.87 g VSS l^{-1} (TSS = 7.03 g l^{-1}). Temperature set point was 25 °C, agitation 150 rpm and pH 7.8 ± 0.2. After reaching a stable Qr baseline (Qr endogenous), the sludge was spiked with a known amount (100 ml) of raw wastewater.

In order to compare the biological activity of the MBR biomass with the one observed during the test on AS plant sludge, calorimetric experiments have been repeated under the same experimental conditions. Three exogenous substrates were used: raw wastewater B (100 ml), ethanol (30 mg COD) and ammonia (3 mg $N\text{-}NH_4$). The initial biomass concentration in the calorimeter for MBR sludge was 4.71 g VSS l^{-1} (TSS = 5.50 g l^{-1}).

Additionally, wastewater B was separated into two fractions: the supernatant (filtered at 0.45 µm) and the solid fraction that was obtained by centrifuging raw wastewater (5000 rpm, 5 min), discharging the supernatant, and resuspending in demineralised water. For this purpose, 500 ml wastewater were used and the procedure was repeated 3 times. At the end of the process, the solid fraction was resuspended in 100 ml demineralised water. MBR biomass was used to evaluate biodegradability of the two fractions separately.

RESULTS AND DISCUSSION

In Figure 16, the thermogram and the respirogram simultaneously acquired after raw wastewater addition to AS plant biomass, are reported.

Immediately after substrate spike (t = 1.8 h), a sudden increase in both heat production rate and OUR was observed. The maximum heat production rate was 80 mW l^{1} and the associated maximum OUR was 20 mg l^{1} h^{-1}. Immediately after the maximum was reached, OUR and heat flux started to slowly decrease. Subsequently (t = 3 h), the depletion of the ready biodegradable fraction of the effluent was clearly indicated by a fast drop in both heat production rate and OUR followed by an exothermic tail related to the consumption of a slowly biodegradable substrate, probably storage polymers.

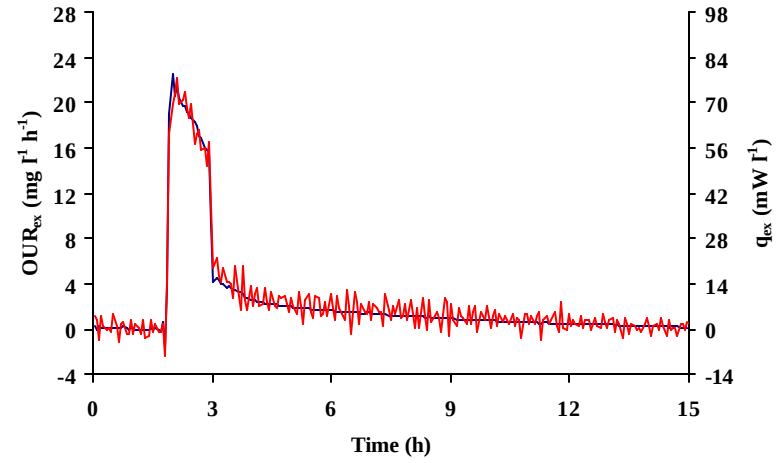

Figure 16. Comparison between OUR and heat production rate profiles simultaneously acquired (raw wastewater B, AS plant biomass). — q_{ex}, — OUR_{ex}.

By plotting the specific heat dissipation, expressed in J l^{1}, versus the oxygen consumption (mmol O_2 l^{-1}) it has been possible to derive, from the slope of the linear regression on the experimental data, the oxycaloric equivalent (Figure 17). Two slightly different oxycaloric equivalent values were found for the RBCOD (400 kJ mol O_2^{-1}) and for the slowly biodegradable fraction of the wastewater (468 kJ mol O_2^{-1}). When different substrates are used as energy source by the same bacterial population, a slight difference in oxycaloric equivalent is expected. This phenomenon has been widely observed on pure cultures (Birou *et al.*, 1987) and can provide useful information to identify shifts from one substrate to another during wastewater consumption.

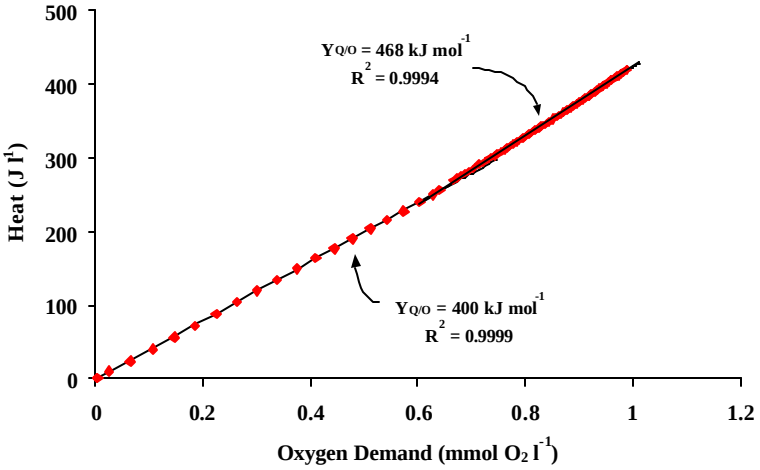

Figure 17. Oxycaloric equivalent for raw wastewater B (AS plant biomass).

The exogenous heat production rate per unit amount of volatile suspended solids (q_{ex}) was derived from calorimetric measurements in order to compare biological activity of MBR and AS plant biomass. A respirometric approach can be used for the same purpose, by evaluating the Specific Oxygen Uptake Rate (SOUR), expressed as the oxygen consumption rate per gram of volatile suspended solids. As for OUR profiles, three variables related to power-time curves may be taken into account. The duration and the maximum height of the exothermic peak provide an indication of the overall biomass activity, whereas the kinetics of the initial thermal response to substrate addition can be related to the degree of adaptation of biomass to the selected substrate (Redl and Tiefenbrunner, 1981). In Figure 18, respirograms (a) and thermograms (b) recorded after raw wastewater addition (100 ml) to the two sludges are presented. As expected, both MBR and AS plant biomass were well adapted to raw wastewater degradation (immediate thermal response to wastewater pulse). The maximum specific heat production rate was 44 % lower for MBR biomass and the time required to degrade the ready biodegradable fraction was significantly higher (4.3 h instead of 1.6 h). Similar results were obtained by means of respirometric measurements.

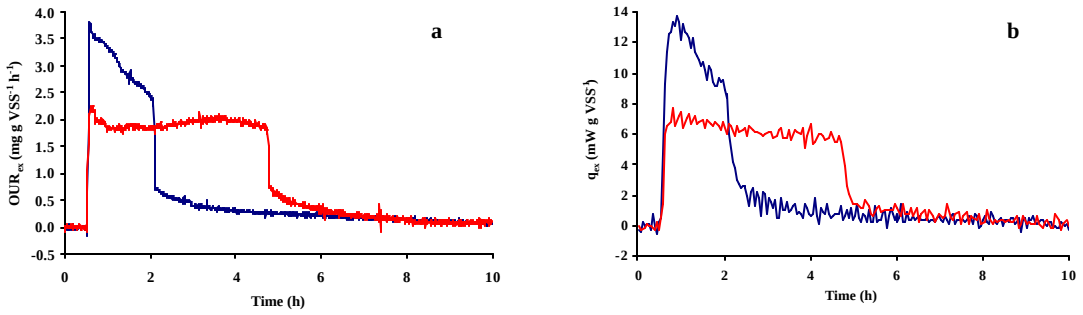

Figure 18. Respirograms (a) and thermograms (b) related to raw wastewater B degradation: comparison between AS plant (-) and MBR (-) biomass.

Analogously, during ethanol degradation (Figure 19), selected as readily biodegradable reference compound, the specific activity of MBR microorganisms was lower compared to AS plant biomass. OUR profiles (a) were in satisfactory agreement with power-time curves (b) and oxycaloric equivalents were similar for the two sludges (470 kJ mol O_2^{-1} for MBR biomass and 456 kJ mol O_2^{-1} for AS plant biomass). Finally, ammonia was added to investigate the biological activity of nitrifying population in MBR and AS plant sludge

samples (data not shown). In both cases, nitrifying activity was not detected either in terms of oxygen consumption or in terms of heat dissipation.

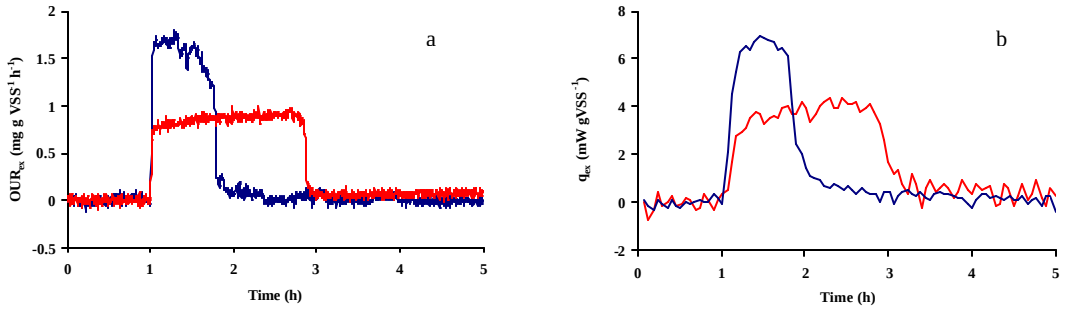

Figure 19. Respirograms (a) and thermograms (b) related to ethanol degradation: comparison between MBR (-) and AS plant (-) biomass.

A detailed summary of respirograms and thermograms characteristics for ethanol and wastewater degradation is reported in Table 4. SOUR has been shown to be a good indicator of microbial viability of activated sludges (Huang and Cheng, 1984) and an analogous meaning can also be attributed to q_{ex}. Notwithstanding SOUR is affected by many factors, in particular by mean cell residence time and raw wastewater characteristics, the values observed for both sludges are significantly lower than those typically reported in literature (Huang and Cheng, 1984; Henze *et al.*, 1987; Jørgensen *et al.* 1992; Kristensen *et al.*, 1992). To authors best knowledge, no explicit literature data exist for q_{ex} in activated sludge, however, also in this case, the experimental values are lower than those obtained in our laboratory with different biomass samples and substrates (ranging from 60 to 140 mW g VSS^{-1}). Moreover, during plants monitoring, the apparent yield (i.e. cumulative increase of mixed liquor TSS with respect to the cumulative COD removed) for both MBR and AS plant resulted to be higher than the values of net yield usually observed in conventional activated sludge plants and in MBR plants operating the same food over biomass ratio (Malpei *et al.*, 2002). This phenomenon can be explained taking into account that an appreciable concentration of suspended solids (0.6 - 1.8 g VSS l^{-1}) entered the aeration tanks together with raw wastewater. This seems to be related to the fact that part of the effluent from the dewatering process of wasted sludge was mixed with factory wastewater into the neutralisation/equalisation basin. On the contrary, the contribution of poorly biodegradable polyester fibre fragments on solid concentration is probably negligible. The high supply of

solids with wastewater stream also affects the specific sludge activity since it causes an increase in VSS concentration that is not proportional to the increase in active biomass.

Table 4. Comparison between MBR and AS plant biomass.

	AS plant	**MBR**
q_{ex} max raw wastewater (mW g VSS^{-1})	13.8	7.7
q_{ex} max ethanol (mW g VSS^{-1})	6.9	4.0
$SOUR_{end}$ (mg g VSS^{-1} h^{-1})	0.69	0.23
$SOUR_{ex}$ max ethanol (mg g VSS^{-1} h^{-1})	1.7	0.9
$SOUR_{ex}$ max raw wastewater (mg g VSS^{-1} h^{-1})	3.8	2.3
Peak duration raw wastewater (h)	1.6	4.33
Peak duration ethanol (h)	0.8	1.9

In order investigate the short term biodegradability of the solids contained in wastewater B, raw wastewater was divided into supernatant and solid fraction and the MBR biomass was used to test the two fractions. In Figure 20, power-time curves acquired after substrate pulses to the MBR sludge and calculated total heat dissipation are reported. Raw wastewater dose led to a total heat production of 785 J whereas only 233 J were dissipated after solid fraction addition. By taking into account the heat released per unit amount of COD added it is possible to evaluate and compare the short term biodegradability of the different fractions. The specific heat dissipation was 4.5 kJ g COD^{-1} for filtered wastewater (supernatant), 2.0 kJ g COD^{-1} during raw wastewater consumption and only 0.23 kJ g COD^{-1} when the solid fraction was dosed as exogenous substrate. These findings indicate that suspended solids contained into wastewater B are poorly biodegradable. OUR measurements (data not shown) were in good agreement with calorimetric profiles. Data are summarised in Table 5.

Table 5. Data related to wastewater B fractions degradation.

	Raw wastewater	**Supernatant**	**Sediment**
Volume (ml)	100	100	500
COD (mg l^{1})	3916	1384	10100
Heat dissipation (J)	785	626	233
Specific heat dissipation (J mg COD^{-1})	2.0	4.5	0.23
O_2 demand (mg)	65	47	23
Specific O_2 demand (mg mg COD^{-1})	0.16	0.34	0.02

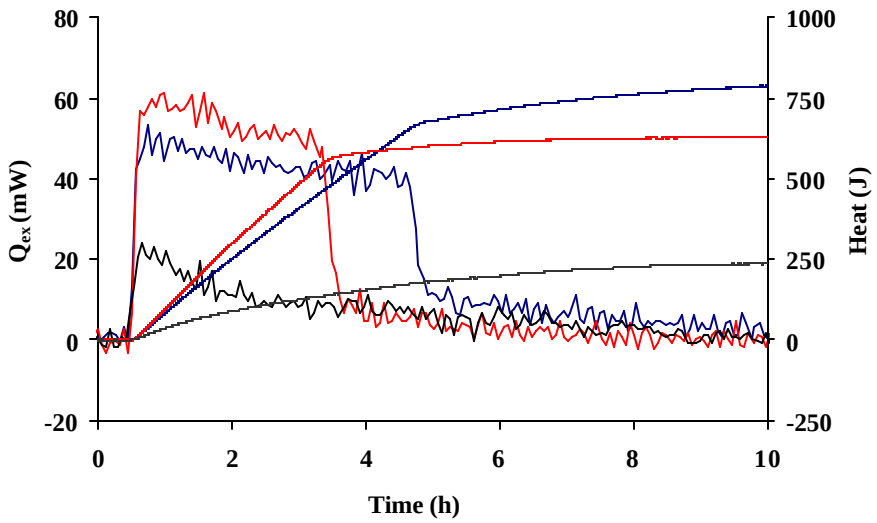

Figure 20. Degradation of wastewater B fractions and calc ulated heat dissipation (- raw wastewater B, - supernatant, - sediment). (Thermograms related to MBR biomass).

CONCLUSIONS

A bench scale isothermal calorimeter (Bio-RC1), especially modified for biological studies has been used to characterise aerobic biodegradability of final effluents from two textile factories. Moreover, calorimetric profiles have been compared to DO measurements simultaneously carried out. The following conclusion can be drawn:

- Wastewater stream degradation has been characterised in terms of microbial heat dissipation and short term biological oxygen demand. A linear correlation between calorimetric and respirometric data was observed in aerobic batch tests with biomass samples from full-scale WWTPs fed on textile effluents confirming that, under strictly aerobic conditions, calorimetry and respirometry provide the same information.

- Oxycaloric equivalent has been evaluated for two textile wastewaters. $Y_{Q/O}$ was estimated to be 403 kJ mol O_2^{-1} for wastewater A. Two slightly different $Y_{Q/O}$ have been detected for the easily biodegradable fraction (400 kJ mol O_2^{-1}) and for the slowly biodegradable fraction (468 kJ mol O_2^{-1}) of wastewater B. All these values are in good agreement with those reported in literature for pure microbial cultures and with the theoretical value of 460 kJ mol O_2^{-1} valid for heterotrophic aerobic metabolism.

- Calorimetric measurements, as well as respirometry, have been successfully applied to detect the toxicity effect on aerobic microbial populations (heterotrophs and nitrifiers) due to wastewater addition and to evaluate and compare biomass activities from different WWTPs treating the same textile effluent.

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