Calorimetric assessment of activity in WWTP biomass

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Abstract A heat flux bench-scale calorimeter (Bio-RC1) has been used to assess the metabolic activity of microbial populations involved in wastewater treatment biological processes under aerobic, anoxic and anaerobic conditions. Under strictly aerobic conditions, a linear correlation was observed between oxygen uptake rate and heat flux for heterotrophic and nitrifying bacterial populations. Using the same calorimetric approach and the same apparatus, toxicity and biodegradability of a pesticides factory wastewater were investigated. The activity of heterotrophic and nitrifying aerobic communities was monitored considering both oxygen consumption and heat dissipation, whereas, under anoxic conditions, calorimetric data were compared to the traditional NUR (nitrate uptake rate) test. Heterotrophic activity was found to be 52% inhibited after toxic wastewater exposure under both aerobic and anoxic conditions and 30% inhibition was observed on autotrophic ammonia oxidation. Additionally, calorimetric measurements have been successfully applied to investigate anaerobic digestion. The thermal response of a mesophilic granular sludge to repetitive glucose pulses has been evaluated and a toxicity test has been performed by exposing the biomass to increasing concentrations of formaldehyde.

Keywords Aerobic; anaerobic; anoxic; calorimetry; toxicity; wastewater treatment

Introduction
Heat measurements can provide a global evaluation of the overall metabolic activity of living organisms and, in particular, of the various microbial populations involved in wastewater treatment biological processes under different metabolic conditions. Most of the techniques suitable for WWTPs monitoring and control purposes, such as respirometry and titrimetric sensors, are specific for one or more bacterial communities and cannot be operated for every biological process. On the contrary, calorimetric techniques are universal and non-specific. Therefore, 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. Calorimetric measurements are non-invasive, do not need sample pre-treatment and biomass activity can be derived immediately, continuously and without interfering with the reaction system. When a bioreaction takes place, the measured net heat flux may always be directly related to the conversion rate of reactants or to the generation rate of the products. Therefore, calorimetric data can provide useful information on the overall bacterial activity but also on the biodegradability or toxicity of the influent. Moreover, the interpretation of power-time curves (thermograms) related to microbial exogenous metabolism is a powerful tool to identify significant or critical and unexpected biological events such as shifts from one substrate to another and nutrient deficiencies.

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. Biological calorimetry is widely employed for studies of microbial growth energetics on pure cultures, whereas little work is present in the literature regarding the application of heat measurements to assess biomass activity in WWTPs. In the eighties, a flow-through microcalorimetric system (SODEV) was used to evaluate the influence of organic shock loads or toxic contaminants, such as heavy metals and aliphatic
alcohols, on heterotrophic aerobic biomass (Fortier et al., 1980; Beaubien et al., 1985; Beaubien and Jolicoeur, 1985; Jolicoeur et al., 1988). In the nineties, flow microcalorimetry was applied to investigate the toxic effect of phenol on biological nitrification (Ramos-Prieto et al., 1997). Under anaerobic conditions, a LKB drop ampoule microcalorimeter was used to assess hydrolytic biological activity and to evaluate the biodegradability of specific substrates such as skimmed milk, olive oil and starch (Redl and Tiefenbrunner, 1981). Later, acidogenic processes in a lab-scale fermentor fed on lactose and in a UASB (Upflow Anaerobic Sludge Blanket) reactor fed on a cheese factory wastewater were studied (Jolicoeur et al., 1988). Recently, anaerobic digestion of heavily polluted wastewaters was monitored by means of an isothermal heat conduction microcalorimeter (Menert et al., 2001). Moreover, an on-line estimation of the BOD of aqueous samples was performed using direct calorimetry on surface attached cultures (Weppen et al., 1991).

In this work, a high-resolution bench-scale calorimeter (Bio-RC1) has been used to investigate biomass activity in sludge samples drawn from full-scale wastewater treatment plants. Toxicity and biodegradability of a pesticides factory wastewater are evaluated under aerobic (autotrophic and heterotrophic populations) and anoxic conditions. At the same time, a comparison between heat measurements and established OUR (oxygen uptake rate) and NUR (nitrate uptake rate) measurements is reported. Preliminary results on anaerobic digestion monitoring, including sludge adaptation to glucose and inhibition to formaldehyde additions, are also presented.

**Methods**

**The calorimeter**

Direct biological heat flux calorimetry is based on the quantification of the heat flow rate that has to be removed from a bioreactor in order to maintain the isothermal conditions. The Bio-RC1 is a bench-scale calorimeter by Mettler-Toledo AG (Schwerzenbach, Switzerland), initially developed to study chemical reactions. In order to investigate biological processes, the sensitivity of the standard RC1 has been increased up to 5–10 mW l⁻¹. The Bio-RC1 consists of a 2-litre jacketed glass vessel with a design comparable to a lab-scale bioreactor. A low-viscosity silicon oil is pumped at high rate through the jacket. Operating in isothermal mode, when a bioprocess dissipates or takes up heat, the control algorithm (PI control) adapts the circulating oil temperature ($T_j$) in order to maintain the temperature of the reactor contents ($T_r$) constant. The desired $T_j$ value is obtained by mixing warm and cold oil amounts through an electronically controlled metering valve. Both $T_r$ and $T_j$ are accurately measured and their difference is directly proportional to the heat flux through the jacket ($Q_r$) according to:

$$Q_r = UA \cdot (T_r - T_j)$$

where $T_r$ and $T_j$ are expressed in K, $Q_r$ in W and $UA$ is the overall heat transfer coefficient expressed in W K⁻¹.

The $UA$ factor is determined for each sludge sample, before starting the experiment, through a calibration heater of known power output. The system is also equipped with a DO probe for OUR determination and with a pH probe directly inserted into the reaction vessel. A detailed description of the equipment is reported by Marison et al. (1998) and Aulenta et al. (2002).

**Biomass source and experimental design**

The mixed culture used for aerobic and anoxic calorimetric experiments was sampled at the “Varese lago” WWTP. A preliminary calorimetric test was performed, under aerobic

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conditions, by adding a pulse of a synthetic wastewater to the sludge (1.4 l). The initial VSS concentration was 2.36 g l⁻¹. Wastewater contained a mixture of biogenic compounds (acetate, ethanol, glucose, L-glutamic acid, peptone, yeast extract) and ammonium chloride. Afterwards, toxicity and biodegradability of a pesticides factory wastewater were evaluated both under aerobic and anoxic conditions. Before each test, the sludge was cultivated for one week under aerobic or anoxic conditions inside a 2-litre lab-scale bioreactor. Working volume was 1.5 litres. Before inoculating the bioreactor, the sludge was settled in order to reach a VSS concentration of about 5 g l⁻¹ and 750 ml of concentrated sludge were mixed with 750 ml of a diluted wastewater solution (1:100 dilution). pH and temperature were fixed at 7.5 and 25°C respectively. The reactor was operated using intermittent feeding. For the heterotrophic aerobic culture, 5 ml feed were added 4 times per day so that the organic loading rate was 0.2 g COD l⁻¹ d⁻¹. Acetate was the sole exogenous energy source. Feed solution contained per litre: 39.08 g NaCH₃COOH, 14.06 g (NH₄)₂SO₄, 0.70 g MgSO₄ · 0.35 g CaCl, 3.09 g K₂HPO₄, 2.39 g KH₂PO₄. For the anoxic cultivation, nitrogen was continuously bubbled in the reactor and sodium nitrate was added to feed solution at a concentration of 50 g l⁻¹. The same approach was used for the nitrifying culture. Two times per day the sludge was fed with 5 ml of a solution containing per litre: 45.85 g NH₄Cl, 30 g yeast extract and 96 g NaHCO₃. Ammonia and COD loading rate were 0.08 g N-NH₄ l⁻¹ d⁻¹ and 0.1 g COD l⁻¹ d⁻¹.

The sludge (1.4 l) was sampled from the reactor just before a new feed addition and put into the calorimeter. The initial VSS concentrations for the experiments on heterotrophic aerobic, nitrifying and denitrifying biomass were 1.81 g l⁻¹, 1.76 g l⁻¹ and 1.75 g l⁻¹ respectively. For the test on heterotrophic aerobic biomass, nitrification was inhibited by adding allyl-thiourea (10 mg l⁻¹). After reaching a stable Qr baseline (Qr endogenous), the sludge was spiked with a known amount of a specific substrate (acetate, 120 mg COD or ammonia, 15 mg N). Under anoxic conditions, a non-limiting nitrate amount (100 mg N-NO₃) was dosed before each substrate addition. After endogenous conditions were reached again, 100 ml wastewater (1:2 dilution) were added. Before the addition, the solution was pre-thermostatted and saturated with the same gas mixture used for sludge aeration in the calorimeter and pH was adjusted with H₂SO₄. After wastewater degradation, acetate or ammonia pulses were repeated. Calibration procedure for overall heat transfer factor determination was performed at the beginning and at the end of each experiment with a new sludge sample. During data processing, UA changes (about 5% rise) due to volume increase were taken into account. Biomass inhibition due to toxic shock load has been calculated by comparing the maximum heat production rate acquired before and after biomass exposure to wastewater. The total heat dissipation for each calorimetric peak was simply evaluated as the area under the thermogram. OUR was calculated taking into account the oxygen mass transfer coefficient (KLa), determined before each substrate pulse and considered constant during the test (Roš, 1993). NUR was calculated from the slopes of the nitrate utilisation profile. According to Henze (1986), the nitrate utilisation profile was built by adding 60% of nitrite concentration to nitrate concentration, considering that the reduction of 1 g N-NO₂ to 1 g N₂ gaseous needs the same amount of electrons as the complete reduction of 0.6 g N-NO₃ to N₂.

A mesophilic granular sludge drawn from a UASB digester fed on condensate from a waste broth evaporator (DSM Gist-Brocades, Casteggio, Italy) was used for the calorimetric experiments under anaerobic conditions. The influent of the full-scale plant consisted of a mixture of VFAs (volatile fatty acids). The sludge was washed with a mineral solution including macronutrients and trace elements. The medium contained per litre: 340 mg NH₄Cl, 18 mg MgSO₄ · 4H₂O, 16 mg CaCl₂ · 2H₂O, 74 mg KH₂PO₄, 2 mg FeCl₃ · 4H₂O, 2 mg CoCl₂ · 6H₂O, 0.5 mg MnCl₂ · 4H₂O, 0.03 mg CuCl₂ · 2H₂O, 0.05 mg
ZnCl₂, 0.05 mg H₃BO₃, 0.09 mg (NH₄)₆Mo₇O₂₄·4H₂O, 0.1 mg Na₂SeO₃·5H₂O, 0.05 mg NiCl₂·6H₂O, 1 ml HCl (36%), 1 mg EDTA, 0.5 mg Resazurin, 2 g NaHCO₃. The same solution was also used to dilute the sample until a reaction volume of 1.25 l and a biomass concentration of 19.7 g VS l⁻¹ were reached. After inoculating the calorimeter, strictly anaerobic conditions were initially ensured by bubbling in the reactor a 1:1 N₂ and CO₂ mixture. The temperature of the reaction vessel was fixed at 35°C. Once a stable heat flux baseline in endogenous conditions was reached, a known amount (800 mg COD l⁻¹) of glucose from a concentrated stock solution was added to the sludge. Glucose pulses were repeated until a reliable and reproducible thermal response was recorded. Afterwards, a toxicity test was performed by adding increasing concentrations of formaldehyde (200 to 7,200 mg l⁻¹).

Analytical methods
Nitrate and nitrite determinations were performed by ion chromatography. A Dionex DX500 Ion Chromatograph (column: Dionex IonPac AG; eluent: Na₂CO₃ 3.5 mmol l⁻¹ and NaHCO₃ 1.0 mmol l⁻¹; flow rate: 1.2 ml min⁻¹) was used for the analysis. Total and volatile solids determinations were performed according to Standard Methods (1995).

Results and discussion
Under strictly aerobic conditions, a linear correlation was observed between OUR and heat flux for heterotrophic and nitrifying populations, suggesting that simultaneous calorimetric and respirometric measurements may be an interesting tool in biotechnological studies and applications. In Figure 1, the thermogram and the respirogram acquired after a synthetic wastewater pulse (54 mg COD l⁻¹ and 5.4 mg N-NH₄ l⁻¹) are presented. Immediately after the spike a sudden increase in both heat production rate and OUR was observed due to carbonaceous substrates and ammonia oxidation. After the ready biodegradable organic matter was completely depleted, ammonia oxidation became the prevailing oxygen consuming bioreaction. The different heat released per mole of oxygen consumed (oxycaloric equivalent) in heterotrophic and autotrophic aerobic bioprocesses resulted in a decoupling between heat flux and OUR (region of 0.8 to 1.6 hours) which makes it possible to discriminate the biological activity of the two trophic groups. The oxycaloric equivalent should be nearly the same for all heterotrophic aerobic growth processes regardless of bacterial culture and nature of carbonaceous substrate and its theoretical value is 460 kJ mol O₂⁻¹ (von Stockar and Marison, 1989). Oxycaloric equivalent experimentally determined on nitrifying biomass was 151 kJ mol O₂⁻¹ for nitrite oxidisers and 189 kJ mol O₂⁻¹ for ammonia oxidisers (Daverio et al., 2003).

Afterwards, the biodegradability and toxicity of a pesticides factory wastewater was tested

![Figure 1](https://iwaponline.com/wst/article-pdf/48/3/31/423224/31.pdf)
under aerobic and anoxic conditions using the same biomass source. Initially, the aerobic heterotrophic bacterial population was considered. In Figure 2, thermograms and respirometry A and C refer to acetate consumption and thermogram and respirogram B to wastewater degradation. The maximum heat production rate for acetate degradation decreased from 250 mW to 120 mW after wastewater exposure (52% inhibition), whereas the total heat dissipation remained constant (670 J). A good agreement was observed between heat flux and OUR profiles simultaneously acquired. The oxycaloric equivalent was 360 kJ mol O$_2^{-1}$. This value is in good agreement with the one reported by Beaubien and Jolicoeur (1985) working with activated sludges (345 kJ mol O$_2^{-1}$) and with the one observed by Birou et al. (1987) on pure cultures of *Candida utilis* growing on acetate (385 kJ mol O$_2^{-1}$). In thermograms A and C, the peak related to primary substrate consumption is followed by an exothermic tail representing the degradation of intracellular storage polymers, mainly 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, as in intermittently fed reactors or when biomass is subjected to alternating aerobic, anoxic or anaerobic conditions, as in nutrient removal processes (Majone et al., 1998). Wastewater degradation lasted 16 hours. Immediately after the addition, heat flux rose up to 90 mW (36% of the maximum heat flux during acetate degradation before toxicant addition). Subsequently, it started to slowly decrease down to 15 mW and then remained constant until endogenous conditions were reached. The total heat dissipation was 1,070 J.

The same procedure was repeated in order to evaluate the effect of the wastewater on the functional microbial groups involved in nitrogen biological removal (nitrifiers and denitrifiers). In Figure 3 the calorimetric profile acquired during the anoxic test is presented. Thermograms A and C are related to acetate consumption, whereas thermogram B refers to wastewater degradation. Heterotrophic biomass response to toxic shock load was similar to the one observed under aerobic conditions. In Figure 4, a comparison between thermogram A and nitrate and nitrite concentration profiles is presented. After acetate spike, heat dissipation rate increased up to 390 mW then, after acetate depletion, immediately decreased to 80 mW and then slowly to the endogenous baseline. Anoxic accumulation and degradation of storage polymers led to two different nitrate utilisation rates and heat production rates. In fact, maximum NUR was 19.8 mg N g VSS$^{-1}$ h$^{-1}$ during acetate consumption, whereas it dropped from 3.7 to 1.26 mg N g VSS$^{-1}$ h$^{-1}$ during the exothermic tail. Wastewater degradation generated a maximum thermal power of 110 mW (28% of the one observed after the first acetate spike) and the associated NUR was 6.5 mg N g VSS$^{-1}$ h$^{-1}$. The total heat dissipation was 400 mW pointing out that, probably, with respect to aerobic conditions, only a partial degradation occurred. After the second acetate spike, heat flux increased up to 190 mW (51.6% inhibition) and NUR was 10.9 mg N g VSS$^{-1}$ h$^{-1}$.
In Figure 5, the thermograms and the respirograms acquired during the test on nitrifying biomass are reported. In this case, profiles A and C refer to ammonia consumption and profile B to wastewater degradation. A good agreement between thermograms and respirograms was again evident but one can easily observe that the amount of heat released per mole of oxygen consumed during ammonia oxidation is low compared to heterotrophic wastewater degradation leading to a clear uncoupling between the respirografic and the calorimetric profiles during substrate consumption. After wastewater exposure, nitrifying biomass was 30% inhibited. Wastewater consumption by heterotrophic population grown on yeast extract led to a total heat dissipation of 980 J and to a maximum heat production rate of 100 mW.

Under anaerobic conditions, the adaptation to glucose degradation of a granular sludge from a full-scale UASB reactor was investigated. In Figure 6, two thermograms acquired after a pulse of glucose are presented. Thermogram A has been recorded after the first glucose spike to the endogenous non-adapted sludge. Initially, a lag phase (region of 2 to 9 hours) characterised by a low exogenous heat production rate (4 mW) was observed. Heat flux started to increase exponentially only 7 hours after glucose addition and the maximum (120 mW) was reached after 11 hours. The long lag phase followed by an exponential rise in heat flux probably indicates the growth of glucose-metabolising bacteria initially present at low concentration in the sludge sample rather than enzymes induction in resident organisms present in high numbers (Chynoweth and Mah, 1977). Immediately after the maximum was reached, the thermal power quickly fell down due to the primary substrate limitation and the exothermic peak was followed by an endothermic region related to aceticlastic methanogenesis. This peculiar net heat uptake associated with aceticlastic methanogenesis has been experimentally verified in pure cultures of *Methanosarcina barkeri* (Liu *et al.*, 2001). Thermogram B was obtained after several glucose spikes and biomass adaptation is clearly indicated by the sharp peak. The thermal response of the enriched culture was immediate and the heat production rate increased up to 610 mW.
When a reliable and reproducible thermal response (reference thermogram), after biomass adaptation was finally obtained, a toxicity test was performed by exposing the biomass to formaldehyde. In Figure 7, the reference thermogram (A) related to glucose degradation (1,600 mg l–1) is compared to the ones acquired after biomass exposure to increasing formaldehyde concentrations (B, 200 mg l–1; C, 600 mg l–1; D, 1,800 mg l–1; E, 3,600 mg l–1 and F, 7,200 mg l–1). Although formaldehyde was readily degraded (data not shown), the first toxicant dose led to 5% decrease in the maximum heat production rate related to glucose consumption. 63% inhibition was observed when formaldehyde concentration was increased up to 3,600 mg l–1. No further biomass activity was detected after 7,200 mg l–1 pulse.

Conclusions
The investigation has shown the following.

• Biomass activity and toxicity effects in sludge samples drawn from full-scale wastewater treatment plants can be calorimetrically investigated using the same experimental approach and the same apparatus.
• A good agreement was observed between calorimetric and traditional OUR and NUR measurements.
• The toxic effect of a pesticides factory wastewater on both nitrifying and denitrifying activity has been evaluated thus showing the potentialities of calorimetry in investigating the whole biodegradation process in activated sludge plants which include nitrogen removal.
• Anaerobic digestion was successfully investigated: through the analysis of heat evolution profiles following substrate addition, biomass adaptation to glucose consumption
has been monitored and the toxic effect of formaldehyde on the enriched culture has been evaluated.

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References


