Thermochimica Acta, 193 (1991) 135–143
Elsevier Science Publishers B.V., Amsterdam

On-line estimation of biological oxygen demand using direct calorimetry on surface attached microbial cultures

P. Weppen [a,1], J. Ebens [b], B.G. Müller [a] and D. Schuller [b]

[a] *Gesellschaft für biotechnologische Forschung mbH, Biochemical Engineering Department, Mascheroder Weg 1, D-3300 Braunschweig (Germany)*

[b] *Universität Oldenburg, Fachbereich Chemie, Arbeitsgruppe Ökochemie und Umweltanalytik, Postfach 2503, D-2900 Oldenburg (Germany)*

(Received 22 November 1990)

Abstract

Microbial cultures were cultivated on the surface of glass beads inside the measuring cell of an isoperibolic twin-cell calorimeter in order to monitor microbial activity continuously. The system was applied to obtain an on-line estimation of the biological oxygen demand (BOD) of aqueous samples. Characteristics of this system are discussed concerning the feasibility of continuous calorimetric determinations of BOD. Growth kinetics and mass balances of attached microbial growth are too complicated to allow the assumption of a simple stoichiometric metabolism of a particular substrate as expected from the concept of the fully established BOD_5-assay.

INTRODUCTION

Estimation of biological oxygen demand (BOD) and other characteristics of wastewaters is routinely performed to monitor wastewater treatment plants and to avoid undesired effects to the aquatic environment. The standardized 5-day BOD (BOD_5) assay [1] provides the data with serious delay. An accidential discharge of hazardous effluents cannot be detected in time in order to protect the plant or the receiving water course. On-line monitoring of BOD therefore is a useful technique for process control by feedback strategies as a result of dramatically decreasing the typical delay of BOD_5 data.

Several authors have designed methods for on-line estimation of BOD related values in order to automatically improve the performance of large scale biotechnologies such as sewage plants. An oxygen electrode coated with immobilized microorganisms has been used to measure BOD_5 related

[1] To whom correspondence should be addressed at: Christian-Albrechts-Universität Kiel, Biologiezentrum, Forschungsstelle für Ökosystemforschung und Ökotechnik, Olshausenstraße 40, W-2300 Kiel 1, Germany.

values [2]. The system responds within 20 min and shows a life time of about 3 weeks. A respirometric activity monitor has been developed to optimize an activated sludge wastewater treatment plant by control engineering [3]. Riegler [4] describes a commercially available continuous short-time BOD-measuring technique based on oxygen consumption by a fluidized bed of immobilized microorganisms, which was operated at constant and low BOD load by means of a control loop.

The systems mentioned above derive their measuring values from microbial respiration rates using polarographic oxygen sensors and need to be calibrated against well known BOD_5 data in order to provide reliable estimations of BOD_5. In contrast to the monitoring of total or dissolved organic carbon (TOC/DOC) or chemical oxygen demand (COD) the biological probes (BOD) facilitate a prediction of the biological availability of the organic constituents in a particular water. That particular prediction enables an operator to assess the response of microbial populations in sewage plants or the receiving water course. The probes can also be used to identify toxic compounds using slightly modified procedures [4].

Microbial conversion of organic compounds is accompanied by an enthalpy of reaction ΔH_r, which depends on the products and educts of the reaction but not on the microbial pathway.

$$\text{Educts(red)} \overset{\text{Biocatalysis}}{\rightarrow} \text{Products(ox)} + e^-$$

$$e^- + \text{Oxidant} \overset{\text{Biocatalysis}}{\rightarrow} \text{Oxidant(red)}$$

accompanied by ΔH_r of -110 kJ mol^{-1} electrons.

It is well known that oxidative degradation reactions show a constant reaction enthalpy [5] independent of the kind of carbon substrate when looking at electron transfer to the oxidant. The reaction enthalpy (the so called oxycaloric coefficient) averages a value close to -100 kJ mol^{-1} electrons. From this aspect the close relation of oxygen consumption and metabolic heat production becomes evident. Therefore much more has been published on the bioenergetics of aerobic metabolism using data based on oxygen consumption (indirect calorimetry) than on applications of direct calorimetry. Principally, aerobic microbial metabolism seems to similarly affect respiratory rates and heat evolution, but these statements should not be generalized to anoxic metabolic pathways [6].

On-line monitoring of BOD load using direct calorimetry appears to be a good alternative to oxygen consumption measurements, which are scientifically judged by the oxycaloric coefficient. This allows a distinct design of sensor having a different set of advantages. A commercially available Picker microcalorimeter was used to determine biological substrate conversion activities of suspended biomass [7]. The suspended culture was kept outside the calorimeter and substrates or toxic compounds were added periodically.

In this study the methods of biomass immobilization and direct calorimetry were combined to evaluate the feasibility of a calorimetric on-line BOD monitor.

MATERIALS AND METHODS

The calorimetric device

A twin cell calorimeter was designed for continuous operation of microbial cultures under aerobic conditions in the measuring cells and allowing for a continuous media supply [8,9]. The bioreactors were fully integrated into the measuring system as proposed elsewhere [10]. The measuring cells had a volume of 140 ml and were equipped with a gas sparger, feeding and effluent lines, calibrating heaters, and a packed bed of spherical glass beads of diameter 8 mm. Calorimetric characteristics were determined by electric calibration using a built in Pt_{100} resistor. Data listed in Table 1 were used to calculate the heat dissipation rate from temperature readings (ΔT between measuring and (identical) reference cell). The heat flux calorimeter was always operated isoperibolically [11] at a surroundings temperature of 298 K. Deconvolution of the power–time curves obtained was performed by means of the TIAN equation [12]

$$dQ/dt = \left(\lambda_I + \dot{V}_L \lambda_L\right)\Delta T + d\Delta T/dt \cdot C_{op} \quad (1)$$

$d\Delta T/dt$ terms were calculated by floating averages of the slope of the $\Delta T(t)$ function. In eqn. (1) λ_I indicates the heat conductance of the instrument and $\dot{V}_L \lambda_L$ is the heat conductance resulting from media flow. The left term provides the part of power dissipation related to an averaged value of ΔT, whereas the right term provides the contribution of power dissipation related to non-steady ΔT values.

TABLE 1

Physical characteristics of the twin vessel heat-conduction calorimeter (experimentally derived data)

Working volume of vessel (ml)	V_I	140
Heat conductance (static) (W K^{-1})	λ_I	0.262
Mass of glass beads (g)		190
Water content (ml)	V_L	66–72
Operational heat capacity (J K^{-1})	C_{op}	775 ± 25
Range of medium flow rate (ml h^{-1})	$\dot{V}_L$	0–250
Flow-induced heat conductance (water, 298 K) (W h K^{-1} ml^{-1})	λ_L	0.00121
Decay time constant (s)	τ_{op}	2960–1370
Noise (short term) (mW)		0.5–2.5

Immobilized microbial cultures

To develop a biofilm on the glass beads a synthetic culture medium was used containing minerals and 0.75 mmol l^{-1} D-glucose (final concentration) as sole carbon source. Glucose was fed into the calorimetric vessel by a thermostated feeding line. A second feeding line served to supply equal amounts of mineral medium or the introduction of an inoculum culture. The mineral medium was composed of (final concentrations) 6.55 mmol l^{-1} NaH_2PO_4, 3.42 mmol l^{-1} KH_2PO_4, 3.78 mmol l^{-1} $(NH_4)_2SO_4$, 0.068 mmol l^{-1} $CaCl_2$, 0.406 mmol l^{-1} $MgSO_4$ [9]. Trace element solution [13] and Fe–EDTA complex [13] (1 ml l^{-1} of each) were added, the pH was 7.1 ± 0.1, and feeding rates were as mentioned in the experiments. Media were fed to the calorimeter with dilution rates of between 0.100 and 1.400 h^{-1} related to the total reactor volume. With regard to the volume of the bulk liquid phase V_L, mean residence times t_L ranged from 4 to 0.33 h in the calorimetric measuring vessels. Activated sludge from a laboratory scale sewage plant served as an inoculum for the system. A rigid biofilm developed within several days.

Calorimetric BOD determinations were made with diluted samples prepared from synthetic wastewater, that contained bacto-peptone (1.92 g l^{-1}), meat-extract (Merck) (1.22 g l^{-1}), urea (360 mg l^{-1}), NaCl (1.44 mmol l^{-1}), $CaCl_2$ (0.33 mmol l^{-1}), $MgSO_4$ (0.100 mmol l^{-1}) and $FeCl_3$ (0.041 mmol l^{-1}). Fe–EDTA complex and traces were added as described above. COD data were determined by a standard procedure [14].

Effluent water from the secondary settler of a laboratory wastewater treatment plant was used to perform on-line measurements. The plant consisted of an aeration tank (900 ml) and two settlers (350 ml) which were assembled in series. The plant was operated with synthetic wastewater (see above) at a hydraulic load of 0.2 h^{-1}. The distance of 30 m between the laboratory activated sludge plant and the calorimetric monitoring system was bridged by teflon tubing (diameter 3 mm). A high flow rate was provided by a peristaltic pump (Masterflex) in order to minimize the lag time in the line.

EXPERIMENTAL RESULTS

The principal disadvantage of an isoperibolic calorimeter regarding the temporal resolution of details of any heat evolving process, was sorted out by the deconvolution algorithm. Figure 1 shows that heat-flux calibrations were fully resolved if heat dissipation was constant for about 2 h. Calibration experiments using rectangular patterns of heat dissipation showed decreasing resolution of transformed data when the frequency exceeded 2 cycles h^{-1}. These results were satisfactory in relation to values of the time constant τ_{op} of the instrument.

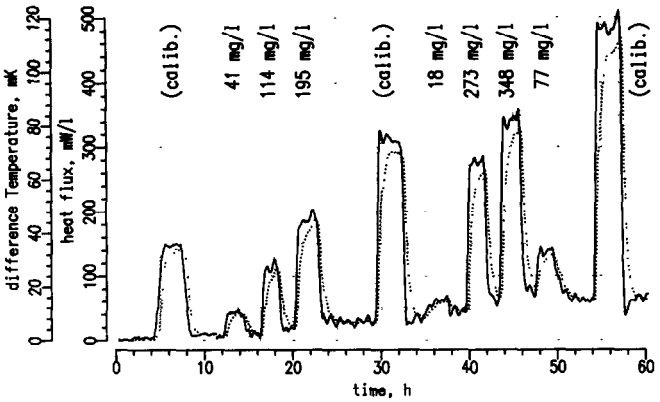

Fig. 1. Response of a readily established biofilm to BOD load temporarily fed to the calorimetric monitor. Electric calibration shows linearity and temporal resolution of the instrument (143 mW l^{-1}, 285 mW l^{-1} and 428 mW l^{-1}. Dotted line shows calorimetric readings of difference temperature, the solid line shows $\dot{Q}$-values obtained by the deconvolution procedure according to eqn. (1).

After the measuring vessel had been inoculated with activated sludge a surface attached biofilm developed during continuous feed of glucose media within 3 days. The microbial population showed structures which were comparable to a flocculating activated sludge, including *Zoogloea* sp. and several genera of protozoa, which were also present in the biocoenosis of the laboratory plant.

The biofilm was loaded with various media flow rates and the heat flux showed an approximation to steady values (Fig. 2), when substrate feeding rate was increased. At low hydraulic load $\dot{V}_L/V_L$ steady state values established readily within 20 h. The biofilm supplied with the media dissipated 86–50% of the heat of reaction calculated from heat of combustion of the

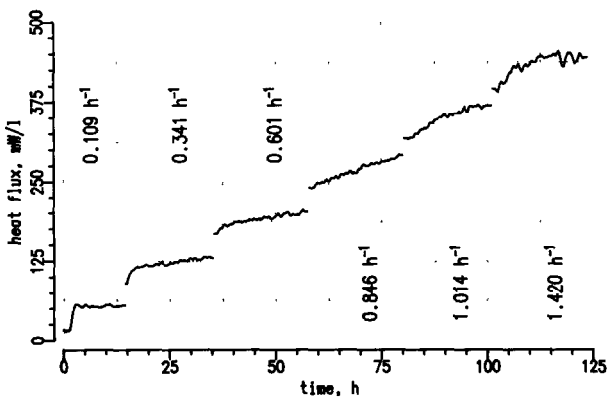

Fig. 2. Heat flux generated by an immobilized biofilm under the regime of increasing substrate and hydraulic load.

TABLE 2

Continuous heat production of surface attached microbial populations at various feeding rates of 0.75 mmol l^{-1} in glucose/mineral medium [a]

Feed (ml h^{-1} l^{-1})	Mean res. time t_L (h)	BOD feed (μmol s^{-1} l^{-1})	$\dot{Q}_{Theory}$ (mW l^{-1})	$\dot{Q}_{measured}$ (mW l^{-1})	Theory (%)
109	4.3	0.141	−66	−54– −57	82–86
341	1.38	0.461	−215	−119– −129	55–60
601	0.78	0.786	−367	−181– −217	49–59
846	0.56	1.105	−515	−252– −279	49–54
1014	0.46	1.317	−615	−326– −366	53–59
1421	0.33	1.85	−862	−409– −451	47–52

[a] Data were normalized to 1 l reactor volume. Mean residence time t_L was calculated assuming a porosity of 47% under aeration. Theoretical power dissipation $\dot{Q}_{Theory}$ was calculated from heat of combustion of glucose (−2800 kJ mol^{-1}) instead of the oxycaloric coefficient. Values of $\dot{Q}_{measured}$ were determined by calorimetry.

glucose. The percentage of heat dissipation decreased with increasing hydraulic load (Table 2).

Figure 1 shows data from short term feeding experiments using synthetic wastewater diluted to various COD concentrations. Heat output was in the range 49–32% of the theoretical value. $\dot{Q}_{Theory}$ values were calculated from COD data (which were assumed to be identical with BOD_t in the case of easily degradable substrates) and the oxycaloric coefficient. The observed heat of reaction decreased slightly within the range 18–350 mg COD l^{-1}. Table 3 lists calculated values regarding this experiment.

The combination of the calorimetric system with the secondary settler of a laboratory sewage works showed the principal capability of this system for in-plant monitoring of biologically degradable carbon sources (Fig. 3). The maintenance of our laboratory plant required daily disconnections of the calorimetric system for about 1–2 h (marks in the plot). During servicing time the heat flux decreased but remained far above the baseline.

DISCUSSION

Belaich [15] showed that microbial metabolism dissipated much less heat of reaction due to biosynthesis (anabolism) than oxidation of the substrates (catabolism). He calculated $\Delta H_{anab.}$ to be close to zero considering commonly used substrates; $\Delta H_{catab.}$ of aerobic metabolism equals $\Delta H_{combust.}$ when products are CO_2 and water. Heat-flux and also O_2-consumption monitoring systems indicate catabolic turnover but scarcely anabolic reactions. BOD_5-related monitoring requires the substrate to be mineralized quantitatively or at least to a certain degree. Unfortunately microorganisms show increasing anabolic yields with increasing growth rate. This effect is

TABLE 3

Heat production of a nutrient starved biological film when diluted OECD wastewater was fed to the fixed bed reactor; flow rate was maintained constant throughout the experiment ($\dot{V}_{Lsamp} = 700$ ml h^{-1} l^{-1}); data are normalized for unit volume

Sample [a] (mg COD l^{-1})	COD input [b] (μmol s^{-1} l^{-1})	$\dot{Q}_{Theory}$ [c] (mW l^{-1})	$\dot{Q}_{measured}$ [c] (mW l^{-1})	Heat/COD [d] (kJ mol^{-1})	Theory (%)
18.5	0.112	−49.9	−24.3	−216.9	48.7
41	0.249	−110.6	−35.3	−141.8	31.9
77	0.467	−207.7	−78.4	−167.9	37.7
114	0.692	−307.6	−109.8	−158.7	35.7
195	1.184	−526	−178	−150.3	33.8
273	1.658	−736	−238	−143.5	32.3
348	2.114	−939	−297	−140.5	31.6

[a] Analytically estimated COD values of diluted artificial wastewater samples.

[b] COD load to the system measured as μmol O_2 s^{-1} l^{-1} reactor volume.

[c] $\dot{Q}_{Theory}$ and $\dot{Q}_{measured}$ are, respectively, the calculated and calorimetrically determined heat-flux values normalized for a unit volume.

[d] Ratio of measured heat production to maximum heat production calculated from COD data and a theoretical value of −444 kJ mol^{-1} oxygen (Prochazka et al. [5]).

ascribed to the maintenance rate (m) of microbes [16], which diminishes the energy conservation by anabolic reactions. The maintenance rate itself depends on the physiological state of the cells [17] and affects the well known Monod kinetics of microorganisms when substrate uptake is assumed to be the limiting metabolic step. A logistic pattern of growth rate versus substrate concentration may result [18].

Experimental values of heat dissipation due to maintenance energy are respectively about −0.55 W $g^{-1}_{Biomass}$ and −0.286 W $g^{-1}_{Biomass}$ when chemostat

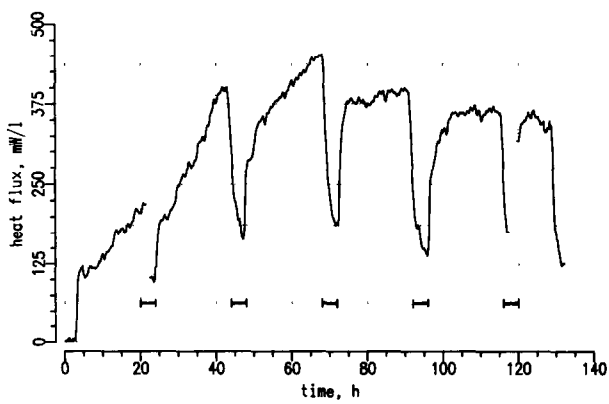

Fig. 3. On-line monitoring of the effluent from a laboratory sewage plant. COD of the effluent was 270 ± 20 mg l^{-1} throughout the experiment (average from daily samples). Bold bars indicate periods of water supply during servicing work on the plant.

cultures of *Escherichia coli* or *Acinetobacter calcoaceticus* are considered [19]. These strains show a theoretical maximum value of energy conservation (Y_{eT}) of 0.63 $\Delta H_{combust.}$ of total amount of substrates consumed. The observed fraction of energy conservation Y_e decreases with decreasing growth rate.

Applying these concepts to the immobilized biofilm a complicated mass and energy balance resulted until an equilibrium of substrate supply and substrate consumption was established being dominated by catabolic reactions. Presupposing substrate supply was the limiting factor given by the experimental conditions, it becomes evident that the biofilm grew until the maintenance term predominated. The increase of biomass lead to an increasing portion of catabolic substrate conversion and consequently to decreasing growth efficiency and hence an approximation of the reaction enthalpy to values of complete oxidation of the BOD supply (Table 2). Of course growth of the biofilm was certainly also controlled by diffusion limitation, or on occasion of its removal from the solid support by shear forces.

Monitoring of BOD samples prepared from artificial wastewater indicated the feasibility to develop a flow injection analysis design for continuous measurements. The batch operation guaranteed reliable baseline estimates due to the resting state of the biofilm. The low recovery of theoretically expected ΔH_r from COD determinations (Table 3) indicates incomplete adaptation of the biofilm to the particular constituents and therefore minor consumption. Alternatively a high degree of anabolic utilization or mass transfer limitations should also result in low recovery of theoretically expected heat flux.

When linking the biofilm calorimeter to a laboratory sewage plant it became evident that a lack of adaptation was responsible for unsteady estimates of ΔH_r during the first 2 days (Fig. 3). The system showed increasing heat-flux values and later it responded instantly when servicing of the secondary settler was finished. The performance of the laboratory plant remained constant during that experiment.

The on-line measuring technique was found to be complicated since metabolism of the biomass is divided into catabolic reactions accompanied by large enthalpy changes and anabolic reactions which show relatively small enthalpy changes. Furthermore a biofilm shows endogenic metabolism yielding a steady increase of the baseline with increasing amounts of immobilized biomass on the surface of the glass beads. The contribution of maintenance energy rate or endogenous metabolism may be responsible for the steady increase of heat production (Fig. 2) under steady COD load. Unfortunately mass balances of an immobilized biofilm are the result of complicated interactions permitting no further discussion here [20].

In general we assume that our considerations on microbial mass or energy balances are valid for calorimetric monitors as well as oxygen based systems. The most important differences between BOD_5 and this short term assay

result from the different approaches: mineralization in the long term and less or more productive microbial growth in the short term.

REFERENCES

1 OECD, Test-guideline C 117/79/GER, 1979.
2 S.E. Strand and D.A. Carlson, Rapid BOD measurement for municipal wastewater samples using a biofilm electrode, J. Water Pollut. Control Fed., 56 (1984) 464–467.
3 T. Takamatsu, S. Shioya, K. Morisaki and D. Ihara, On-line monitoring and control of an activated sludge process for waste water using NMOUR, Eur. J. Appl. Microbiol. Biotechnol., 14 (1982) 187–192.
4 G. Riegler, Kontinuierliche Kurzzeit-BSB-Messung, Korrespondenz Abwasser, 31(5) (1984) 369–377.
5 G.J. Prochazka, W.J. Payne and W.R. Mayberry, Calorific content of certain bacteria and fungi, J. Bacteriol., 104(2) (1970) 646–649.
6 E. Gnaiger, Direct calorimetry in ecological energetics: long term monitoring of aquatic animals, in E. Marti, H.R. Oswald and H.G. Wiedemann (Eds.), Angewandte chemische Thermodynamik und Thermoanalytik, Birkhäuser Verlag, Basel, 1979, pp. 155–165.
7 J.-L. Fortier, B. Reboul, P. Philip, M.-A. Simard, P. Picker and C. Jolicoer, Calorimetric studies of biodegradation processes in biological wastewater treatment, J. Water Pollut. Control Fed., 52 (1980) 89–97.
8 P. Weppen and D. Schuller, Microcalorimetric studies of the mode of action of environmental chemicals on continuous microbial cultures, Thermochim. Acta, 72 (1983) 95–102.
9 P. Weppen, Calorimetric studies on the usability of energy-balances of microbial populations for an ecotoxicological testing strategy, Thermochim. Acta, 94 (1985) 139–149.
10 Y. Ishikawa and M. Shoda, Design and performance of a new microcalorimetric system for aerobic cultivation of microorganisms, Biotechnol. Bioeng., 23 (1981) 2629–2640.
11 W. Hemminger and G. Höhne, Calorimetry — Fundamentals and Practice, Verlag Chemie, Weinheim, 1984.
12 S. Randzio and J. Suurkuusk, Interpretation of calorimetric thermograms and their dynamic corrections, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, New York, 1980, pp. 311–341.
13 R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman and R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of *Cyanobacteria*, J. Gen. Microbiol., 111 (1979) 1–61.
14 Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (DEV H 41), Verlag Chemie, Weinheim, J. Lieferung, 1981.
15 J.P. Belaich, Growth and metabolism in bacteria, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, New York, 1980, pp. 1–42.
16 S.J. Pirt, The maintenance energy of bacteria in growing cultures, Proc. R. Soc. London, Ser. B, 163 (1966) 224–231.
17 S.J. Pirt, Maintenance energy: a general model for energy limited and energy sufficient growth, Arch. Microbiol., 133 (1982) 300–302.
18 C. Qiwu, G.W. Lawson and G. Deen, Study of models of single populations: development of equations used in mircoorganism cultures, Biotechnol. Bioeng., 29 (1985) 682–686.
19 P. Weppen, Kalorimetrische und analytische Untersuchungen zur differenzierenden Bewertung des Einflusses von Umweltchemikalien auf die Energetik des Wachstums von *Escherichia coli* und *Acinetobacter calcoaceticus*, Thesis, Faculty of Chemistry, University of Oldenburg, 1986.
20 G.M. Vaugham and G.A. Holder, Substrate removal in the trickling filter process, J. Water Pollut. Control Fed., 56 (1984) 417–423.