The application of different techniques to determine activated sludge kinetic parameters in a food industry wastewater

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Abstract

In the present work, a continuous-feed system under steady state conditions (classical method) and a respirometric technique based on oxygen consumption measurements, were used to compare microbial parameters using a wastewater model system of a potato processing plant. The effects of the operating conditions in the continuous aerobic reactor on microbial growth and flora composition were also analysed.

Continuous-feed experiments allowed for the calculation of the following biodegradation parameters: maximum substrate consumption specific rate (q_{Smax}) = 0.19 mgCOD $(mgVSS)^{-1}\cdot h^{-1}$, saturation constant (Ks) = 8.3 mgCOD$\cdot\ell^{-1}$, biomass yield ($Y_{X/S}$) = 0.61 mgVSS $(mgCOD)^{-1}$, biomass decay constant (kd) = 0.04$\cdot h^{-1}$ and maximum oxygen consumption specific rate (q_{O2max}) = 0.03 mgCOD $(mgVSS)^{-1}\cdot h^{-1}$.

The respirometric technique als allowed for the calculation of kinetic parameters. However, these parameters depended on dilution rate (D) and/or on dissolved oxygen concentration (DO). Values of q_{O2max} and Ks increased with D; q_{O2max} ranged between 0.05 and 0.13 mgO_2 $(mgVSS)^{-1}\cdot h^{-1}$ and Ks between 2 and 26 mgCOD$\cdot\ell^{-1}$ for D values varying between 0.01 and 0.15$\cdot h^{-1}$.

Microscope observations showed that sludge composition was a function of dilution rate. Low D values (low soluble organic matter (Ss) and high DO concentrations) yielded sludges mainly formed by floc-forming bacteria; high D values (high Ss and low DO concentrations) yielded sludges mainly formed by filamentous micro-organisms.

Since the low substrate/biomass ratios used in the respirometric method limit the growth of micro-organisms maintaining the initial physiological state of the original biomass, the observed differences in the respirometric parameters reflected the actual microbial composition.

Notation

COD	=	chemical oxygen demand (mg$\cdot\ell^{-1}$)
D	=	dilution rate (h^{-1}) = Q/V
DO	=	dissolved oxygen concentration ($mgO_2\cdot\ell^{-1}$)
f_M	=	biomass stirring factor (dimensionless)
kd	=	biomass decay constant (h^{-1})
Ks	=	saturation constant (mgCOD$\cdot\ell^{-1}$)
OC	=	oxygen consumed ($mgO_2\cdot\ell^{-1}$)
OUR	=	oxygen uptake rate ($mgO_2\cdot\ell^{-1}\cdot h^{-1}$)
P	=	microbial product concentration in the reactor (mgCOD$\cdot\ell^{-1}$)
Q	=	continuous flow ($\ell\cdot h^{-1}$)
q	=	measured substrate consumption specific rate (mgCOD $(mgVSS)^{-1}\cdot h^{-1}$)
q_{O2}	=	oxygen consumption specific rate (mgO_2 $(mgVSS)^{-1}\cdot h^{-1}$)
q_{Oe}	=	endogenous oxygen consumption specific rate (mgO_2 $(mgVSS)^{-1}\cdot h^{-1}$)
q_{O2max}	=	maximum oxygen consumption specific rate (mgO_2 $(mgVSS)^{-1}h^{-1}$)
q_{Ot}	=	total oxygen consumption specific rate (mgO_2 $(mgVSS)^{-1}\cdot h^{-1}$)
q_P	=	product formation specific rate (mgCOD $(mgVSS)^{-1}\cdot h^{-1}$)
q_S	=	substrate consumption specific rate (mgCOD $(mgVSS)^{-1}\cdot h^{-1}$)
q_{Smax}	=	maximum substrate consumption specific rate (mgCOD $(mgVSS)^{-1}\cdot h^{-1}$)
r_{Oe}	=	endogenous oxygen consumption rate ($mgO_2\cdot\ell^{-1}\cdot h^{-1}$)
r_{Ot}	=	total oxygen consumption rate ($mgO_2\cdot\ell^{-1}\cdot h^{-1}$)
S	=	substrate concentration in the reactor (mgCOD$\cdot\ell^{-1}$)
Sa	=	substrate concentration before the pulse in the respirometric technique (mgCOD$\cdot\ell^{-1}$)
S_S	=	soluble organic matter concentration (mgCOD$\cdot\ell^{-1}$)
S_o	=	feed substrate concentration (mgCOD$\cdot\ell^{-1}$)
t	=	time (min)
V	=	volume (ℓ)
VSS	=	volatile suspended solids (mg$\cdot\ell^{-1}$)
X	=	biomass concentration (mgVSS$\cdot\ell^{-1}$)
X_r	=	biomass concentration in the respirometer (mgVSS$\cdot\ell^{-1}$)
$Y_{O/S}$	=	oxidation coefficient (mgO_2 $(mgCOD)^{-1}$)
$Y_{P/S}$	=	product yield (mgCOD $(mgCOD)^{-1}$)
$Y_{X/S}$	=	biomass yield (mgVSS $(mgCOD)^{-1}$)
μ	=	specific microbial growth rate (h^{-1})
μ_{max}	=	maximum specific microbial growth rate (h^{-1})

Introduction

Among aerobic biological wastewater treatments, activated sludge systems play an important role. Waste is discharged into large aeration basins in which atmospheric oxygen is diffused by releasing compressed air into the waste or by mechanical surface aerators. Both substrate consumption kinetics and floc-forming capacity of the sludge determine process efficiency. The operation mode of the aeration unit affects the physical properties of the flocs and may

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limit the clarification capacity of the secondary settling tank.

The wastewater of a potato-processing plant includes constituents of the potato that are readily water soluble and appear as dissolved solids, such as solubilised starch, proteins, amino acids, organic acids, and sugars. The organic portion of the waste stream can only be removed by biological treatments (Pailthorp et al., 1975).

Operational problems are known to occur in the activated sludge treatment of potato wastewater, primarily connected with sludge settling in secondary clarifiers. The high carbohydrate waste may be prone to filamentous bacterial growth if the aeration basin environmental conditions are not maintained in the proper ranges (Pailthorp et al., 1975; Sezgin et al., 1978; Wanner, 1994). Considering that biological degradation is the most widely used method to reduce organic loading of wastewater, knowledge of microbial kinetics becomes essential for biological treatment design and to establish the operating conditions that produce sludges with adequate settling characteristics.

Many techniques have been proposed for measuring the kinetics of biodegradation, continuous-feed systems (classical technique) being the most used and accepted one (Pirt, 1975). In these cases, the reactor is fed varying dilution rates. Once the system reaches steady state, the corresponding substrate concentration and biomass are determined. In general, reaching steady state takes considerable time (Williamson and McCarty, 1975) which is its main disadvantage.

Several authors have used respirometric techniques for the determination of microbial kinetic parameters (Cech et al., 1984; Dang et al., 1989; Aichinger et al., 1992; Drtil et al., 1993; Orhon et al., 1995; Ellis et al., 1996; Ubisi et al., 1997), biochemical oxygen demand (Köhne, 1985; Ros, 1993), toxicity of wastewaters (King and Dutka, 1986; Vanrolleghem et al., 1994; Kong et al., 1996) and as an on-line biosensor in wastewater treatment plants (Holmberg et al., 1989; Sollfrank and Gujer, 1990; Vanrolleghem et al., 1994). This method is based on the determination of OUR as related to the biodegradation of a known substrate amount. Although respirometric technique constitutes a rapid and easy method, there are many complicating factors associated with the measurement of kinetic and stoichiometric parameters such as the feed pattern (Chudoba et al., 1985; Beccari et al., 1998), substrate storage (Chudoba et al., 1992; Dircks et al., 1999; Majone et al., 1999) or anoxic-aerobic conditions (Liu et al., 1998; Musvoto et al., 1999; Casey et al., 1999) which restrict or limit its applicability.

The objectives of the present work were to:

- determine and compare microbial kinetic parameters in a continuously stirred activated sludge reactor treating potato processing wastewater by using classical and respirometric techniques; and
- analyse the effect of dilution rate on the microflora modifications and on the respirometric parameters.

Mathematical modelling

Continuous-feed method (classical technique)

Considering a continuously stirred-tank reactor, mass balances for substrate, biomass and product are given as follows:

Mass balance of substrate (completely stirred system)

$$\frac{dS}{dt} = D(S_0 - S) - q_S\ X \tag{1}$$

where:

S = non-degraded substrate concentration in the reactor (mgCOD·ℓ$^{-1}$),
So = feeding substrate concentration (mgCOD·ℓ$^{-1}$),
q_S = substrate consumption specific rate (mgCOD (mgVSS)$^{-1}$·h^{-1}),
D = dilution rate (h^{-1}),
X = biomass concentration (mgVSS·ℓ$^{-1}$).

Under steady state, the substrate mass balance results are:

$$q_S = \frac{D(S_0 - S)}{X} \tag{2}$$

Mass balance of biomass

$$\frac{dX}{dt} = \mu\ X - kd\ X - D\ f_M\ X \tag{3}$$

where:

m = specific microbial growth rate (h^{-1}),
kd = biomass decay constant (h^{-1}),
f_M = biomass stirring factor, defined as the ratio between the biomass concentration in the discharge stream and the biomass concentration in the reactor.

This factor was added to take into account that some microorganisms may attach to the reactor walls. According to Pirt (1975), in continuous-flow cultures of long duration, there may be biofilm formation which varies from a light film of biomass to a massive accretion of it on the vessel surface. The biomass in the reactor is given by the sum of the biofilm formation and the biomass of the discharge stream. Thus, the biomass discharge concentration is always lower than the concentration in the reactor. The laboratory reactor has an f_M of 0.56 determined in a previous paper (Bertola et al., 1999).

Once steady state is reached Eq. (3) becomes:

$$\mu = f_M\ D + kd \tag{4}$$

Balance of product

$$\frac{dP}{dt} = q_P\ X - D\ P \tag{5}$$

where:

P = microbial product concentration in the reactor (mgCOD·ℓ$^{-1}$),
q_p = product formation specific rate (mgCOD (mgVSS)$^{-1}$·h^{-1}).

Under steady state Eq. (5) leads to:

$$q_P = \frac{D\ P}{X} \tag{6}$$

In a previous paper, Bertola et al. (1999) reported the formation of soluble compounds (microbial products) due to microbial growth. Additionally, no inert compounds were found in the wastewater model system. Therefore, the measured S_S had two contributions: S and P: (S_S = S + P). Considering the product yield ($Y_{P/S}$) as:

$$Y_{P/S} = \frac{q_P}{q_S} = \frac{P}{(S_0 - S)} \tag{7}$$

the non-degraded substrate concentration in the outlet stream (S) is

given by:

$$S = \frac{S_S - Y_{P/S}\, S_0}{1 - Y_{P/S}} \qquad (8)$$

Besides, biomass yield ($Y_{X/S}$) is defined as:

$$Y_{X/S} = \frac{\mu}{q_S} \qquad (9)$$

The measured substrate consumption specific rate (q) differs from the substrate consumption specific rate (q_S) that was defined in Eq. (2) since q is defined in terms of S_S as follows:

$$q = \frac{D\,(S_o - S_S)}{X} \qquad (10)$$

by combining Eqs. (2), (8), (9) and (10), an equation that relates the measured substrate consumption specific rate with the specific microbial growth rate was obtained:

$$q = \frac{(1 - Y_{P/S})}{Y_{X/S}}\ \mu \qquad (11)$$

and combining Eqs. (4) and (11) the following expression was obtained:

$$q = \frac{(1 - Y_{P/S})}{Y_{X/S}}\ f_M D + \frac{(1 - Y_{P/S})}{Y_{X/S}} kd \qquad (12)$$

Considering a Monod type kinetics (Monod, 1949):

$$\mu = \mu_{\max} \frac{S}{Ks + S} \qquad (13)$$

with:

μ_{max} = maximum specific microbial growth rate,
Ks = saturation constant (substrate concentration corresponding to half of the maximum rate)

Taking into account Eqs. (8), (9), (10) and (13), the following expression was obtained:

$$q = (1 - Y_{P/S})\ q_{S\max}\ \frac{S_S - Y_{P/S}\ S_0}{Ks\,(1 - Y_{P/S}) + S_S - Y_{P/S}\ S_0} \qquad (14)$$

where:

$q_{Smax} = \mu_{max}/Y_{X/S}$

If all quantities were expressed in oxygen units, the oxidation coefficient (Irvine and Bryers, 1985) was calculated as:

$$Y_{O/S} = 1 - Y_{X/S} - Y_{P/S} \qquad (15)$$

The maximum oxygen consumption specific rate (q_{O2max}) was calculated as:

$$q_{O2max} = Y_{O/S}\, q_{Smax} \qquad (16)$$

Respirometric technique

During the endogenous phase of respiration (characterised by the absence of oxidisable substrate) oxygen is utilised at a constant rate. When a pulse of substrate (Sa) is injected into the respirometric cell, oxygen is consumed due to both substrate utilisation and endogenous respiration; an increase in OUR is observed. After a certain time the respiration rate returns to a value equal to, or slightly different from, the original endogenous phase rate (Fig. 1). Considering the specific endogenous rate of oxygen consumption

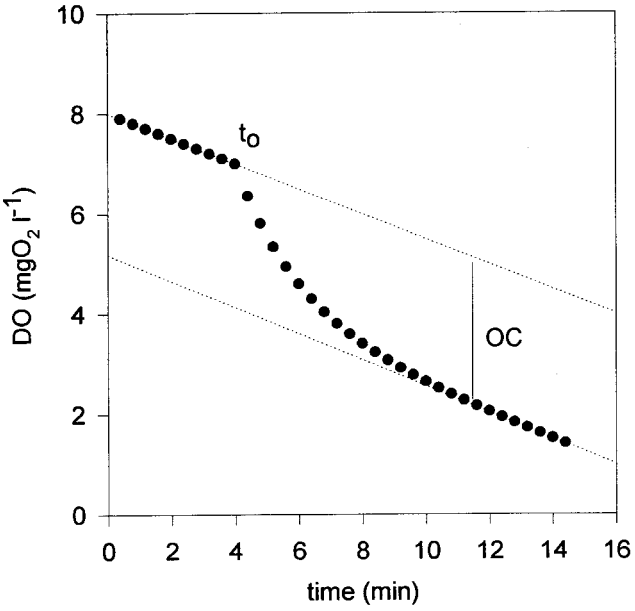

Figure 1
Recorder chart with a typical respirometric curve

(q_{Oe}) and the specific oxygen consumption rate measured after a substrate pulse (q_{Ot}), the specific oxygen consumption rate due exclusively to substrate utilisation (q_{O2}) was calculated, as follows:

$$q_{O2} = q_{O_t} - q_{O_e} = q_{O2\max}\ \frac{Sa}{Ks + Sa} \qquad (17)$$

Sigmoidal curves of DO as a function of time (Fig. 1) can be expressed according to the following equations:

$$DO^{<} = a + r_{Oe}\, t \qquad t < t_o$$
$$DO^{>} = DO^{<} + OC\ [\ \exp(-K(t - t_0) - 1] \qquad t \geq t_o \qquad (18)$$

where:

a and r_{Oe} are the intercept and the slope of the straight line corresponding to oxygen consumption under endogenous respiration, that is, before substrate pulse addition;
t_O is the time at which the substrate enters into the respirometer; and
OC is the oxygen utilised to consume the substrate added with the pulse.

From the derivative of Eq. (18) with respect to time (t) at $t = t_O$, the maximum oxygen consumption rate (r_{Ot}) was obtained:

$$r_{Ot} = -OC\ \ K + r_{Oe} \qquad (19)$$

Eq. (19) was divided by the concentration of biomass in the respirometer (Xr) leading to the following expression:

$$q_{Ot} = -\frac{OC\ \ K}{X_r} + q_{Oe} \qquad (20)$$

where

$q_{Ot} = r_{Ot}/\ Xr;$
$q_{O2} = r_{O2}/\ Xr.$

The specific rate of oxygen consumption for the added substrate concentration (q_{O2}) can be obtained from Eq. (20) as OC K/ Xr.

TABLE 1
Basic data obtained from the continuous-feed method and the respirometric technique

D (h^{-1})	Continuous-feed method			Respirometric technique		
	Ss ($mgCOD \cdot \ell^{-1}$)	X ($mgVSS \cdot \ell^{-1}$)	DO ($mgO_2 \cdot \ell^{-1}$)	q_{O2max} (mgO_2 $(mgVSS)^{-1} \cdot h^{-1}$)	Ks ($mgCOD \cdot \ell^{-1}$)	$Y_{O/S}$ (mgO_2 $mgCOD^{-1}$)
0.01	99	460	8.4	0.054	2.0	0.24
0.02	104	546	—	—	—	—
0.03	103	552	8.0	0.052	5.7	0.27
0.03	104	562	8.0	0.087	2.0	0.27
0.06	118	896	4.8	0.088	3.7	0.27
0.09	156	1148	1.0	0.085	9.3	0.27
0.11	268	1025	0.5	0.114	4.2	0.22
0.13	668	930	0.3	0.132	4.3	0.25
0.14	424	1260	0.3	0.104	25.2	0.23
0.15	854	1030	0.1	0.112	25.7	0.27

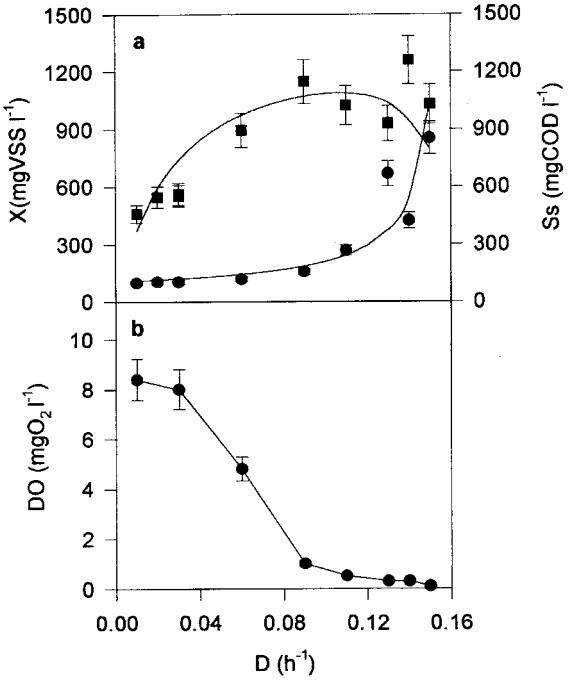

Figure 2
Effect of dilution rate D on: a) biomass concentration X (■) and soluble organic matter in the reactor Ss (●) and b) dissolved oxygen DO. Solid lines correspond to the developed model.

The oxidation coefficient ($Y_{O/S}$) was calculated as the required amount of oxygen (OC) to consume the added substrate (Sa):

$$Y_{O/S} = \frac{OC}{Sa} = \frac{q_{O2}}{q_S} = \frac{q_{O2\max}}{q_{S\max}} \qquad (21)$$

Materials and methods

Wastewater

A model system corresponding to the wastewater of a potato-processing plant was used. The stock wastewater was obtained dipping 1 cm^3 cubes of potato in water at room temperature for 48 h (Bertola et al., 1999) and diluted to obtain 2 000 mg $COD \cdot \ell^{-1}$.

Measurement techniques

DO was measured with an electrode YSI model 58 (YSI Inc., Ohio, USA) with a polarographic membrane YSI 5775. COD and phosphorus determinations were performed by spectrophotometric techniques (COD Reactor Model 45600 and Spectrophotometer DR/2000, Hach Cop., Loveland, USA). Nitrogen was determined by Kjeldahl with a Büchi digestor (Büchi Laboratoriums-Technik, Flawil, Switzerland). Biomass concentration was expressed as VSS (Metcalf and Eddy, 1977). Microscopic observations of the microbial flora in the reactor were carried out by phase contrast using a Leitz Ortholux II microscope (Wetzlar, Germany).

Biological reactors

Two reactors of volume (V) 4.8 ℓ were fed with dosing pumps. Air was supplied by aerators that also assisted in agitating the reactor. Ten experiments were carried out under continuous flow (Q) using dilution rates (D = Q/V) ranging between 0.01 and 0.15 h^{-1}. Once steady state had been reached, microscope observations of the activated sludge were performed and the following parameters were determined in the mixed liquor: DO, biomass concentration as VSS, and organic matter content as soluble COD.

Respirometric technique

Respirometric measurements were assessed on an open respirometer; the respirometric cell is described in detail elsewhere (Chudoba et al., 1985; Drtil et al., 1993; Roš, 1993).

Activated sludges were obtained from the biological reactors operated at different dilution rates under steady state conditions.

A culture volume of 400 mℓ was removed from the continuous reactor and was placed in the respirometric cell in absence of substrate. A nitrification inhibitor (Hach formulation 2533) was also added. The system was then aerated until a constant OUR was found, which corresponded to the endogenous respiration phase. Respirometric tests were carried out by adding different substrate amounts (wastewater of approximately 10 000 mgCOD·ℓ$^{-1}$), that led to substrate concentrations (Sa) of between 1 and 100 mgCOD·ℓ$^{-1}$ in the respirometer. Biomass concentration was considered constant since added substrate pulses (0.1 to 5 mℓ) corresponded to less than 5% culture volume change.

Results and discussion

Wastewater characterisation

The model wastewater system of the potato processing plant was standardised at 2 000 mgCOD·ℓ$^{-1}$ for all experiments. Nitrogen and phosphorus content were 150 and 21 mg·ℓ$^{-1}$, respectively and the COD/N/P ratio was 100/7.5/1.1; this ratio is commonly accepted as being necessary for biomass growth (Winkler, 1996). These results are in accordance with a previous paper (Contreras et al., 2000 b). Wijbenga et al. (1984) reported a COD/N/P ratio of 100/8.8/0.9 and 100/8.1/1.4 for deproteinised and protein-containing wastewater from the potato starch industry respectively.

Continuous feed method

Soluble organic matter content as COD (S_S), biomass as VSS (X) and DO were measured in the continuously-stirred reactor as a function of D. Each run was carried out at different dilution rates. Ss and X were measured until obtained values differed by less than 10% to ensure a steady state (Table 1). Average values were plotted and are shown in Figs. 2a, b; it can be observed that when D increased, DO decreased while Ss and X increased.

Microbial kinetic parameters were determined from the obtained results in a continuously-stirred tank reactor (see Appendix I). Using the value S_0 = 2 000 mgCOD·ℓ$^{-1}$, data of soluble organic matter content as COD (Ss) and volatile suspended solids (X), obtained from the continuous operation of the aerobic reactor (Fig. 2a), the measured substrate consumption specific rate (q) was calculated for different dilution rates applying Eq. (10); the Eq. (14) was fitted to q vs S_S data and the following parameters were estimated by non-linear regression analysis: q_{Smax} = 0.19 mgCOD (mgVSS)$^{-1}$·h^{-1}, Ks = 8.3 mgCOD·ℓ$^{-1}$ and product yield $Y_{P/S}$ = 0.05 mgCOD (mgCOD)$^{-1}$ (R^2 = 0.930). The goodness of fit is shown in Fig. 3 where experimental data of q were plotted as a function of S_S and the curve corresponds to Eq. (14).

Considering the calculated value $Y_{P/S}$ = 0.05 mgCOD (mgCOD)$^{-1}$ and the inlet concentration So = 2 000 mgCOD·ℓ$^{-1}$, Eq.(7) yields a residual COD due to microbial product concentration P = 100 mgCOD·ℓ$^{-1}$ for a totally degraded substrate (S = 0); thus, a concentration of 100 mgCOD·ℓ$^{-1}$ was the minimum value reached in the reactor (Fig. 3).

Parameters kd and $Y_{X/S}$ were obtained from Eq. (12); a linear regression of q vs. D data, allowed determination of $Y_{X/S}$ = 0.61 mgVSS (mgCOD)$^{-1}$ and kd = 0.04 h^{-1} with R^2 = 0.863. By combining the obtained values of $Y_{X/S}$ and q_{Smax}, a value of μ_{max} = 0.12 h^{-1} was estimated (Eq. 9).

The adopted factor to convert the activated sludge biomass from VSS to oxygen units was 1.29 mgCOD (mgVSS)$^{-1}$ (Contreras et al., 2000 a). This value was used to determine $Y_{X/S}$ = 0.79 mgCOD (mgCOD)$^{-1}$ (expressed in oxygen units). Eqs. (15) and (16) allow calculation of $Y_{O/S}$ = 0.16 mgO_2 (mgCOD)$^{-1}$ and q_{O2max} = 0.03 mgCOD (mgVSS)$^{-1}$ h^{-1} respectively. Table 2 shows the obtained parameters using the continuous-feed method.

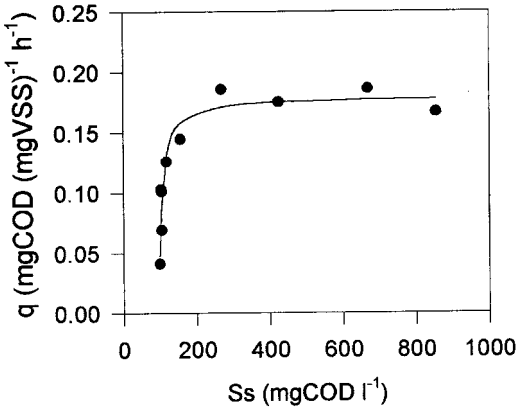

Figure 3
Measured substrate consumption specific rate (q) as a function of organic matter concentration in the reactor (Ss). (•) Experimental values. (-) Eq. (15)

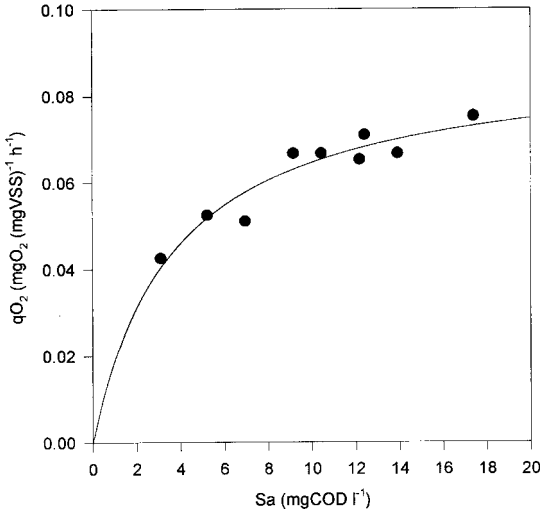

Figure 4
A typical relationship between q_{O2} and Sa. (•) Experimental values. (-) Eq. (17): q_{O2max} = 0.088 mgO_2 (mgVSS)$^{-1}$ h^{-1}; Ks = 3.7 mgCOD ℓ$^{-1}$; in this case D = 0.06 h^{-1}.

Respirometric technique

A respirometric technique was also applied to assess the kinetic parameters on the sludges obtained from the continuously-stirred reactor operating at different D values (see Appendix II). Parameters q_{O2max} and Ks from Eq. (17) were calculated by measuring q_{O2} at different added substrate concentrations (Sa); an example is shown in Fig. 4. The values of q_{O2max} and Ks obtained at different D values are shown in Table 1. Values of q_{O2max} and Ks increased with D; q_{O2max} ranged between 0.05 and 0.13 mgO_2 (mgVSS)$^{-1}$·h^{-1} and Ks between 2 and 26 mgCOD·ℓ$^{-1}$ for D values varying between 0.01 and 0.15 h^{-1} (Fig. 5a, b). For all the experiments, the mean value of $Y_{O/S}$ was 0.25 mgO_2 (mgCOD)$^{-1}$; q_{Smax} was calculated for each D value using Eq. (16); and the Ks values ranged between 0.20 and 0.52 mgCOD (mgVSS)$^{-1}$·h^{-1}. Table 2 shows the obtained parameters using the respirometric technique.

TABLE 2
A comparison between the estimated parameters using the continuous-feed method and the respirometric technique

	Continuous-feed method	Respirometric technique
q_{smax} (mgCOD (mgVSS)$^{-1}$·h^{-1})	0.19 (0.01)	0.20 - 0.52
Ks (mgCOD·ℓ$^{-1}$)	8.4 (3.2)	2.0 - 26.0
$Y_{P/S}$ (mgCOD (mgCOD)$^{-1}$)	0.05 (0.001)	--
$Y_{X/S}$ (mgVSS (mgCOD)$^{-1}$)	0.61 (0.09)	--
kd (h^{-1})	0.04 (0.01)	--
μ_{max} (h^{-1})	0.12 (0.02)	--
$Y_{X/S}$ (mgCOD (mgCOD)$^{-1}$)	0.79 (0.11)	--
$Y_{O/S}$ (mgO_2 (mgCOD)$^{-1}$)	0.16 (0.11)	0.25 (0.02)
q_{O2max} (mgO_2 (mgVSS)$^{-1}$·h^{-1})	0.03 (0.02)	0.05 - 0.13

Standard error between parentheses

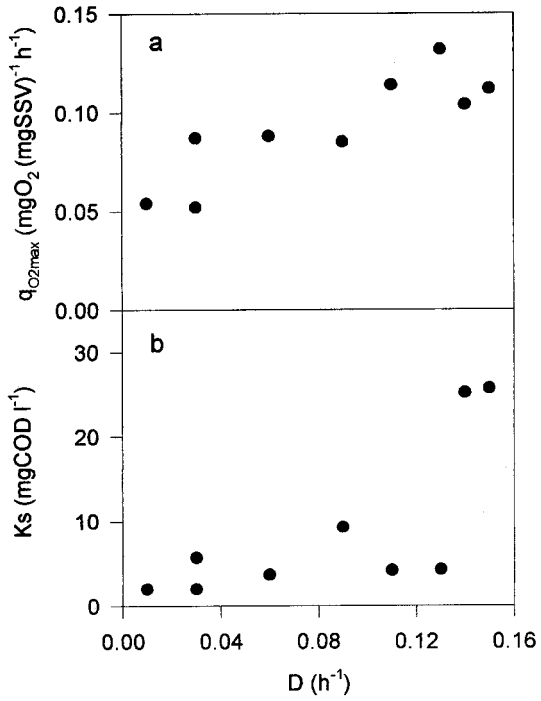

Figure 5
Kinetic parameters obtained by the respirometric method as a function of the dilution rate (D) at which sludges were obtained. (a) maximum oxygen consumption specific rate (q_{O2max}) (b) saturation constant (Ks)

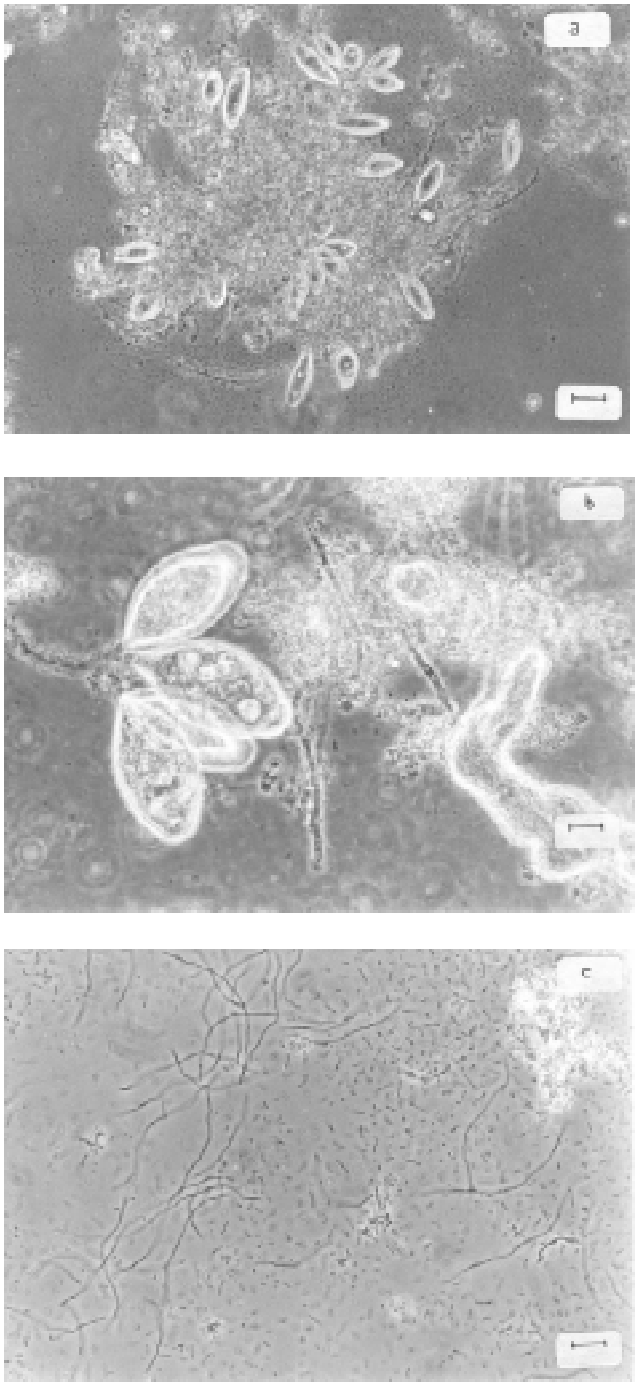

Figure 6
Micrographs of sludge samples obtained with different dilution rates (D)
(a) D = 0.03 h^{-1} (125X, Bar 50 mm)
(b) D = 0.09 h^{-1} (300X, Bar 30 mm)
(c) D = 0.15 h^{-1} (300X, Bar 30 mm)

Comparison between both applied methods

Obtained values of Ks and $Y_{O/S}$ using both methods reflected no significant differences; however, values of q_{Smax} and q_{O2max} using the continuous feed method were lower than those determined by the respirometric technique (Table 2).

The use of respirometric data to assess kinetic parameters is based on an energy balance (Dang et al., 1989): electrons involved in substrate oxidation should be transferred either to the terminal acceptor of electrons (oxygen in aerobic systems) or incorporated into new biomass or into microbial products. Considering that the consumed oxygen is used for the oxidation of the substrate; the formation of biomass; and microbial products, the sum of the yields ($Y_{O/S}$, $Y_{P/S}$ and $Y_{X/S}$) expressed in oxygen units must be 1 (Irvine and Bryers, 1985). In this work, the sum of the experimentally obtained yields using the different techniques was very close to 1 ($Y_{O/S}$ + $Y_{P/S}$

+ $Y_{X/S}$ = 0.25 + 0.05 + 0.79 = 1.09); this result indicates that stoichiometric parameters ($Y_{O/S}$, $Y_{P/S}$ and $Y_{X/S}$) can be accurately estimated by both methods.

Microscope observations

Changes in microbial composition in the continuous reactor was determined by microscope observation. For D < 0.10 h^{-1}, sludge contained mainly floc-forming bacteria, a low number of filamentous micro-organisms and abundant protozoa (Fig. 6a, b). As D increased, protozoa almost disappeared and filamentous micro-organisms increased (Fig. 6c). Filamentous sludges, which appeared at high D values and low DO concentration, showed a higher q_{O2max} and higher Ks values than sludges produced by floc-forming bacteria (Fig. 6a, b).

The observed changes of microbial flora could be associated with the decrease in DO content and/or the increase in organic loading. Taguchi et al. (1978) also observed the filamentous bulking phenomenon with increasing dilution rates. These authors studied two strains of *Flavobacterium*; one was a floc-forming and the other a filamentous strain reporting an increase of filamentous micro-organisms as D increased. Besides, Tanaka and coworkers (1985) studied mixed cultures of floc-forming bacteria (*Pseudomonas* sp.) and filamentous bacteria (*Sphaerotilus* sp.); they observed that when DO was above 1 mg·ℓ^{-1} floc formation and settling properties were good; besides, when DO was below 0.8 mg·ℓ^{-1} filamentous bacteria were the predominant micro-organisms.

These results show the relevance of the previous history of the culture to determine kinetic parameters by the respirometric method. The low substrate/biomass ratios used in the respirometric method limit multiplication and growth of micro-organisms maintaining the initial physiological state of the original biomass (Grady et al., 1996). Consequently, the observed differences in the respirometric parameters reflect the physiological state and/or the microbial composition.

Conclusions

Data from continuous-feed experiments allowed the calculation of kinetic parameters using a Monod type equation and mass balances of substrate, biomass and microbial product. In addition, the respirometric technique allowed also for the calculation of kinetic parameters. However, these parameters depended on D and/or on DO.

Non-significant differences between Ks and $Y_{O/S}$ determined by both applied methods were observed.

Microscope observations showed that sludge composition was a function of dilution rate. Low D values (low Ss and high DO concentrations) yielded sludges mainly formed by floc-forming bacteria. On the contrary, high D values (high Ss and low DO concentrations) yielded sludges mainly formed by filamentous micro-organisms. Since the low substrate/biomass ratios used in the respirometric method limit multiplication and growth of micro-organisms maintaining the initial physiological state of the original biomass, the observed differences in the respirometric parameters reflected the actual microbial composition.

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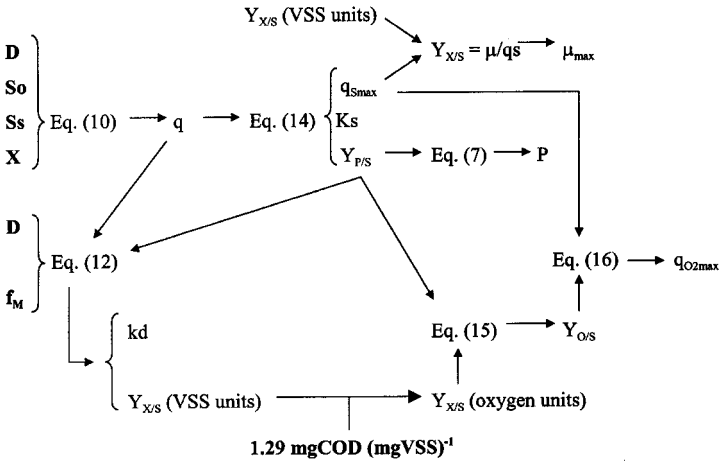
Appendix I

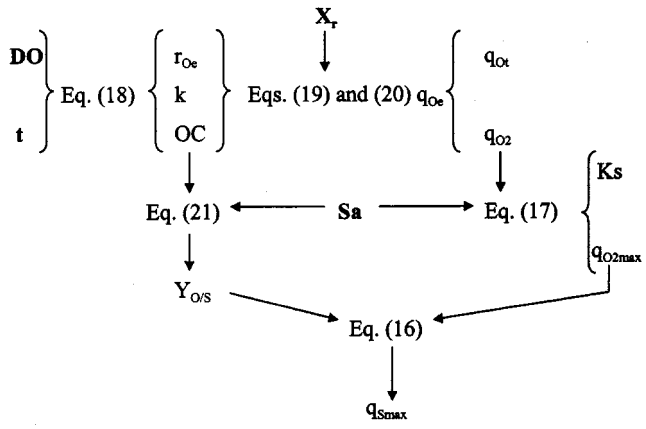
Diagram showing the calculations to determine the kinetics and stoichiometric parameters in the continuous feed system (classical technique)



Basic data: D, S_0, Ss, X, f_M

Appendix II

Diagram showing the calculations to determine the parameters using the respirometric technique.



Basic data: DO, t, X_r, Sa