A modified method to determine biomass concentration as COD in pure cultures and in activated sludge systems

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Abstract

A simple technique to determine biomass concentration as chemical oxygen demand (COD) was developed as an alternative to the standard volatile suspended solid (VSS) method. The proposed technique for biomass measurement as COD is based on the determination of the biomass COD (COD_B) as the difference between total COD (COD_T) and the soluble COD (COD_S) of the sample. The obtained results show that this technique was quicker and simpler than the traditional VSS method.

The validity of the proposed methods was tested with pure cultures of a filamentous micro-organisms (*Sphaerotilus natans*), a floc-forming bacteria and activated sludges. The method was also used for estimating the conversion factor (f_{CV}) from VSS to COD units.

A modification of the standard VSS technique was also proposed using two membranes in the filtration device; this technique allowed the biomass determination in 1 μm size bacteria cultures that cannot be detected by the standard VSS method because cells are not retained by the 1.5 μm diameter pore glass-fibre filter.

Notation

BOD	=	biochemical oxygen demand ($mg \cdot \ell^{-1}$)
COD	=	chemical oxygen demand ($mg \cdot \ell^{-1}$)
VSS	=	volatile suspended solid ($mg \cdot \ell^{-1}$)
f_{CV}	=	conversion factor = ratio between COD_B and VSS
COD_B	=	biomass as COD ($mg \cdot \ell^{-1}$)
COD_T	=	total COD of the sample containing the biomass ($mg \cdot \ell^{-1}$)
COD_S	=	soluble COD ($mg \cdot \ell^{-1}$)
CV	=	coefficient of variation

Introduction

The most commonly used collective parameters in wastewater characterisation are BOD and COD (Wanner, 1994). BOD test indicates consumption of oxygen in receiving water bodies for the biochemical oxidation of organic matter and ammonia remaining in the effluent. Although important, BOD is a test of very little practical use as results are obtained at least in 5 d. In the COD test, organic compounds are not oxidised with molecular oxygen as in the BOD test; a much more aggressive oxidising agent is used. The electrons from organic matter are transferred to dichromate; the reaction is performed in hot sulphuric acid solution and catalysed by silver cations. Only carbonaceous compounds are completely oxidised, so that the COD value does not include ammonia. A few types of organic materials, such as aromatic hydrocarbons and pyridines, are resistant to the oxidising conditions of the test. However, the COD test is relatively easy to perform and the results are obtained in a few hours through the use of commercially available kits.

There are several methods to determine biomass concentration based on different types of measurements, such as mass, volume or linear extent, metabolic rates, light scattering, cell or organelle count (Pirt, 1975). However, the simplicity of the VSS technique has established this method is one of the key parameters used in modelling activated sludge systems (Metcalf and Eddy, 1979). Volatile suspended solids are determined by measuring the mass of oven-dry solids retained by a 1.5 μm glass-fibre filter, volatilised at 550 °C (*Standard Methods,* 1992). In spite of its simplicity, VSS determination has, in some cases, several problems related to the filtration stage such as the presence of filamentous micro-organisms that obstruct the filters or micro-organisms with a size smaller than 1 mm that cannot be retained by the filter.

In most of the mathematical models describing activated sludge systems, equations are expressed in oxygen units (Henze et al., 1987; Kappeler and Gujer, 1992; Henze et al., 1995; Keesman et al., 1998). Since biomass is usually determined as VSS, a conversion factor from VSS to COD units is needed. This conversion factor (f_{CV}) is the COD per unit mass of VSS and it depends on the biomass composition. Due to the diversity of micro-organisms present in activated sludges, the assumption of a given f_{CV} constant could potentially lead to errors in estimation of the COD fraction for the particulate fraction and therefore, in the evaluation of the kinetic and stoichiometric growth parameters characterising the biodegradation of organic compounds in the residual water.

The objectives of this work were:

- to develop and evaluate a simple technique to determine biomass concentration as COD as an alternative to the standard VSS method;
- to propose a modification of the standard VSS method to allow the biomass determination in smaller than 1 μm size bacteria cultures, that cannot be detected by the standard VSS method;
- to determine the conversion factor f_{CV} from VSS to COD units using the developed techniques.

Materials and methods

Micro-organisms

The evaluation of the technique was performed on:

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- pure cultures of two micro-organisms frequently found in activated sludges of wastewater treatment plants namely, the filamentous bacterium *Sphaerotilus natans* and the floc-forming one *Acinetobacter anitratus*; and
- sludges obtained from a laboratory-scale activated sludge treatment plant.

Sphaerotilus natans ATCC #29329 was obtained from the American Type Culture Collection. Cultures were maintained on CGY slants at 4° C and subcultured periodically (Dondero et al., 1961). *Sphaerotilus natans* is a filamentous bacterium frequently found in activated sludge wastewater treatment plants with problems related to filamentous bulking (Richard et al., 1985; Jenkins, 1992; Jenkins et al., 1993; Wanner, 1994).

Strain E932 is a floc-forming bacterium isolated from a laboratory-scale activated sludge treatment plant from CIDCA (Centre of Research and Development in Food Cryotechnology, UNLP - CONICET, Argentina), fed with a model wastewater system of the potato-processing industry (Contreras et al., 2000a). Strain E932 was identified as *Acinetobacter anitratus* using the biochemical test system Sensident-E (Merck).

The activated sludges were obtained from a laboratory-scale activated sludge plant fed with a model wastewater system of a dairy factory with a COD: TKN: P ratio of 100:7:0.6 (Bertola et al., 2001).

Culture conditions

Pure cultures of *S. natans* and strain E932 were prepared in continuous-flow aerobic bioreactor operated at dilution rates (D) of 0.02 to 0.30 h^{-1}. The culture were fed a synthetic influent based on a potato processing industry waste (Contreras et al., 2000a).

Activated sludge samples were obtained from a continuous-flow aerobic laboratory system fed with wastewater model system of a dairy plant. Hydraulic residence time ranged between 15 to 83 h and mixed liquor suspended solids concentration of aproximately 200 to 3000 mg·ℓ^{-1} (Bertola et al., 2001). Operating temperature was 20 ± 1 °C and DO concentrations ranged between 0.5 and 6.5 mg·ℓ^{-1}.

Analytical methods

Biomass measurement by VSS (standard technique)

For activated sludges and pure cultures of *S. natans*, VSS determination was performed according to the standard methods (S*tandard Methods,* 1992). This technique consists of heating a glass-fibre filter of 1.5 µm at 550°C to eliminate any adsorbed organic matter, filtering a known sample volume and drying it at 105°C until it reaches a constant mass. Then the sample is volatilised at 550°C and weighed again; VSS are calculated as the difference between both measurements. In spite of its simplicity, VSS measurement in activated sludge samples may present many problems on some occasions. Some sludges have problems in the filtering step, making this process very slow; in addition, about 24 h are needed for the drying step and subsequent sample combustion; another problem is that bacteria with sizes smaller than 1 µm are not retained by these glass-fibre filters.

Modification of the standard VSS technique (adapted to bacteria smaller than 1 µm)

It was observed that strain E932 was not retained by the glass-fibre filters recommended in the standard method due to its small cell size (approximately 1 µm diameter). Therefore, to determine the biomass corresponding to strain E932, as VSS, a modification of the standard technique was proposed. This modification consisted of the simultaneous use in the filtration device, of a glass-fibre filter of 1.5 µm (Millipore AP40) as a pre-filter, and a polycarbonate membrane (Millipore HTTP, 0.4 µm pore) that retained the cells. These membranes are most appropriate, since they are only slightly hygroscopic, they do not retain proteins and they are completely volatilised at 550 °C. The proposed procedure includes the following steps:

- Heating the glass-fibre filter at 550 °C to volatilise the present organic matter;
- Weighing the polycarbonate membrane (m_1);
- Filtering a culture volume (V) through the glass-fibre filter and the polycarbonate membrane placed together;
- Drying the filter and the membrane with the sample at 105°C to steady mass (m_2);
- Heating the filter and the membrane with the sample at 550°C for 15 min and weigh again (m_3). At this stage, the organic matter and the polycarbonate membrane are volatilised.

The mass was expressed in mg and the volume filtered in mℓ; VSS was calculated as follows:

$$SSV = 1000 \frac{m_2 - m_3 - m_1}{V} \quad (1)$$

Proposed technique to determine biomass as COD

The proposed technique for biomass determination as COD (COD_B) is based on the difference between total COD (COD_T) and soluble COD (COD_S) of the sample. COD_S determination was performed by filtering an aliquot of each sample through a Millipore HA membrane of 0.45 µm pore size that retained the cells.

Samples of the different cultures were divided into two aliquots. One aliquot was reserved for the measurement of VSS and COD_T; the another portion was immediately filtered through a 0.45 µm membrane (Millipore HA) to separate the cells and COD_S was measured; COD_T and COD_S measurements were performed with Hach equipment (method 435 COD-HR).

For comparative purposes, the technique of Bullock et al. (1996) was also used. Bullock's technique consists of the calcination of a glass fibre filter (the same filter used for VSS determination in terms of *Standard Methods* (1992)) to eliminate the organic matter, to filter a known sample volume and to determine the COD corresponding to the filter together with the retained solids and the COD supplied by the filter alone. In both cases the filter was folded and put into the COD digestion tubes (Hach Method) .The COD corresponding to the retained solids is calculated by difference.

Results and discussion

Comparison of the different biomass determination methods

Biomass concentration by VSS

The standard method for biomass determination as VSS and the modified method proposed in the present work (using two filters) were compared. Since the biomass of bacteria smaller than 1 µm (such as strain E932) could not be determined by means of the standard VSS method, comparisons were made using *S. natans* pure cultures. VSS determination by the standard method *(Standard Methods*, 1992) showed a mean value of 805 mg·ℓ^{-1} (N = 5

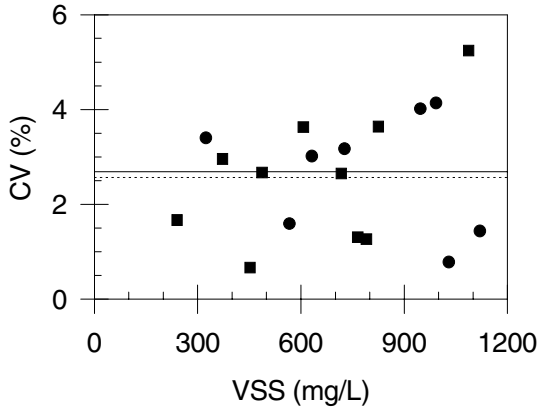

Figure 1
Comparison between the modified method (●) and the standard method (■) to determine biomass concentration as VSS for S. natans. *(—) Average CV (%) for the modified method. (----) Average CV for the standard method (CV = coefficient of variation = standard deviation/mean value)*

determinations) with a standard deviation (σ) of 33 mg·ℓ$^{-1}$. Applying the modified technique proposed in this work, a value of 793 mg·ℓ$^{-1}$ with σ = 14 mg·ℓ$^{-1}$ was obtained. As Fig. 1 shows, the coefficient of variation (CV = σ/mean) was aproximately similar for both techniques. Student t-test shows non significant differences between both methods ($P < 0.05$).

Biomass measurement as COD (COD_B)

COD_B of different samples was determined by the following methods:

- the method proposed in the present work ($COD_B = COD_T - COD_S$) and
- the method developed by Bullock et al. (1996), with the purpose of assessing the accuracy and precision of the proposed technique.

The obtained results with two activated sludge samples and *S. natans* pure cultures are shown in Table 1; in all cases non significant differences ($P < 0.05$) between both methods for COD_B measurement were observed.

The value of COD_B includes different substances:

- viable biomass;
- slowly biodegradable particulate solids;
- non-biodegradable particulate substances from residual water to be treated;
- particulate organic matter generated by the metabolic activity of the micro-organisms in endogenous phase (Orhon and Artan, 1994).

Likewise, VSS determination conducted according to the standard method (S*tandard Methods, 1992)* quantifies this spectrum of substances; in the related literature VSS measurement is associated with the quantity of micro-organisms present (Metcalf and Eddy, 1979) and a relationship can be found between the VSS and the measurement of COD_B (Bullock et al., 1996).

The method proposed in the present study to determine COD_B requires two COD determinations (COD_T and COD_S). These measurements are performed with a commercial kit, and the obtained information is also useful to calculate for example, oxygen consumption, activated sludge model calibration, and to control the performance of the treatment plant (Dang et al., 1989; Aichinger et al., 1992).

TABLE 1
Comparison between the methods applied to estimate the biomass as COD

Sample	Biomass concentration (mgCOD/ℓ)	
	Proposed method (this paper)	Bullock method **
activated sludge 1	1309 (197)*	1463 (44)
activated sludge 2	1104 (48)	1200 (147)
S. natans	1499 (72)	1315 (30)

* Standard deviation is between parentheses.
** Bullock et al. (1996)
Reported data correspond to average values from determinations done in triplicate.

Estimation of the conversion factor f_{CV} relating COD_B and VSS

Once the validity of the proposed method for biomass measurement had been tested, it was used to estimate the conversion factor f_{CV}. This factor was measured for different micro-organisms (*Sphaerotilus natans*, strain E932 and activated sludges) in a continuous reactor.

COD_B was correlated to VSS, for *S. natans* and strain E932 growing in a continuous system operating at different dilution rates. In these experiments, VSS values for *S. natans* were determined using the standard technique; for strain E932, the determination of VSS was performed applying the modified technique proposed in the present work. The f_{CV} factor was estimated as the slope of the straight line corresponding to COD_B vs. VSS data (Fig. 2 a, b). The obtained f_{CV} factors are shown in Table 2; for *S. natans* f_{CV} value was similar to that previously found in batch experiments (Contreras et al., 2000 b).

The applicability of the biomass estimation method as COD, in an activated sludge system, was also tested. A linear relationship between VSS values and COD_B was observed for samples obtained from the laboratory-scale activated sludge treatment system. As may be seen in Fig. 2 c, a satisfactory correlation among data was found. The obtained f_{CV} factor corresponding to activated sludge samples is shown in Table 2.

It should be stressed that obtained f_{CV} values for the different samples did not depend on the tested dilution rates. Similar findings were reported by Marais and Ekama (1976).

Comparison of f_{CV} values

The experimental f_{CV} values obtained in the present work, were compared to data from literature (Table 2). Reported values ranged between 1.14 and 1.66 $mgCOD_B\ mgVSS^{-1}$ for pure cultures and activated sludge systems. The results obtained in the present work are comparable to those reported by other authors. Differences observed in the f_{CV} values of Table 2 might be attributed to various factors:

- the different techniques applied to estimate f_{CV} (experimental determination or calculated from the oxidation equation of the reported biomass cell formula);
- the inherent differences among the micro-organisms studied and/or culture conditions.

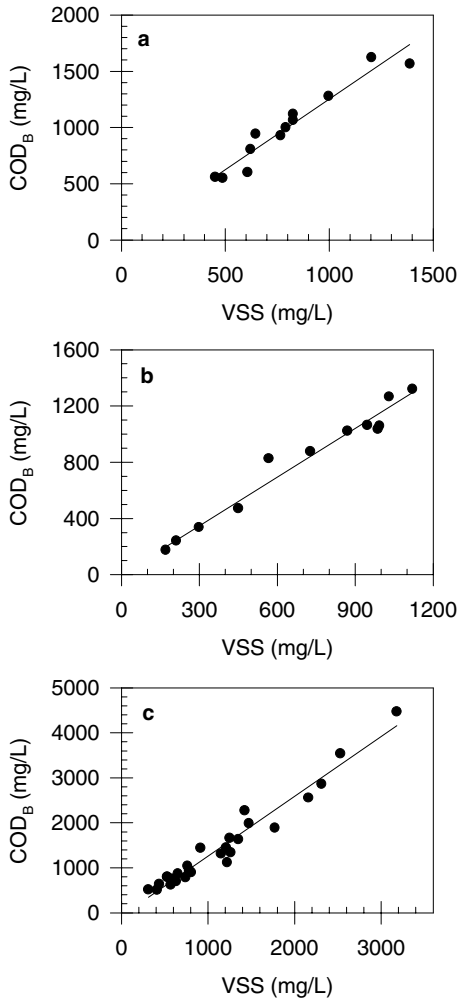

Figure 2
Linear relationship between COD_B (proposed technique) and VSS to determine f_{CV} values for (a) S. natans *(r^2 = 0.9278, n = 12), (b) strain E932 (r^2 = 0.9657, n = 12) and (c) activated sludge (r^2 = 0.9541, n = 27).*

For these reasons, it is very important either to determine a correct f_{CV} value for each individual system or to measure the biomass directly as COD_B , thus eliminating the need to have the f_{CV} value.

Conclusions

A method for biomass determination as COD was proposed, it is quicker and simpler than the traditional VSS method. Additionally, the former needs small volume samples and uses commercially available kits for COD determination, which facilitates the handling of a great number of samples and shortens the test time to 2 h.

A satisfactory agreement between the proposed method of biomass measurement as COD (corresponding to the difference between COD_T and COD_S) and the method developed by Bullock et al. (1996) was observed. However, in the latter a previous calcination step is necessary resulting in the determination lasting at least an hour longer than the proposed method. Additionally, the method proposed in the present paper can provide additional useful information because COD_T and COD_S measurements (necessary to calculate COD_B) can also be used to determine oxygen consumption and activated sludge model calibration and/or to control the wastewater treatment plant performance.

A modification of the standard method for VSS measurements was also proposed. This method allowed for the biomass determination in 1 μm size bacteria cultures (such as strain E932) that cannot be detected by the standard method. VSS values obtained through the modified VSS method and standard method did not show significant differences ($P < 0.05$).

The conversion factor f_{CV} (relating COD_B to VSS) that was determined using the described technique falls within the range reported by other authors, who based their calculations on different cell formula. Factor f_{CV} for *S. natans* was independent from the dilution rate, being similar to the value obtained for activated sludge samples.

According to the obtained results, the proposed method to determine biomass concentration as COD can be used for both laboratory and industrial wastewater treatment plants.

TABLE 2
Values of f_{CV} (mgCOD/mgVSS) corresponding to pure cultures and activated sludges. Comparison between experimental data obtained in the present work with those reported in literature.

	Cell formula	f_{CV} ($mgCOD_B$/mgVSS)	Reference
Pure cultures	variable	1.14 - 1.49*	Roels, 1983
Pure cultures	$C_{44}H_{80}O_{13}N_{11}P$	1.61**	Stanier et al., 1970
Pure cultures	$C_6H_{10}O_3N$	1.39*	Pitter and Chudoba, 1990
Activated sludge	$C_{60}H_{87}O_{23}N_{12}P$	1.45*	Hoover and Porges, 1952
Activated sludge	$C_{118}H_{170}O_{57}N_{17}P$	1.39*	Sawyer, 1956
Activated sludge	$C_8H_{14}O_4N$	1.49*	Pitter and Chudoba, 1990
Activated sludge	$C_5H_7O_2N$	1.42*	Irvine and Bryers, 1985
Activated sludge	n.d.	1.20 - 1.66 **	Bullock et al., 1996
S.natans ATCC #29329	n.d.	1.25 **	Present work
Strain E932	n.d.	1.16 **	Present work
Activated sludge	n.d.	1.29 **	Present work

* Calculated value from oxidation equation of the reported biomass formula.
** Experimentally determined value.

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